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Nutrients and trace organic contaminants removal by an anoxic-aerobic membrane bioreactor (mbr): lab-, pilot- and full-scale investigations

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NUTRIENTS AND TRACE ORGANIC CONTAMINANTS REMOVAL BY AN ANOXIC-AEROBIC MEMBRANE BIOREACTOR (MBR): LAB-, PILOT- AND FULL-SCALE INVESTIGATIONS

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by

Hop Van Phan

School of Civil, Mining and Environmental Engineering
Faculty of Engineering and Information Sciences

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Certification

I, Hop V. Phan, declare that this submission in partial fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Civil, Mining and Environmental Engineering, Faculty of Engineering and Information Sciences, University of Wollongong, is my original work. It contains no materials previously published or written by any other person, unless where due acknowledgement has been made. No part of this thesis has been submitted for qualification in University of Wollongong or any other academic institution.

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Abstract

Increasingly stringent environmental regulations and freshwater shortage are key drivers for a worldwide trend of introducing advanced technologies for wastewater treatment, particularly in removing nutrients (*i.e.*, nitrogen and phosphorous) and trace organic contaminants (TrOCs). Membrane bioreactor (MBR) is a compact process that employs membranes for effective solid-liquid separation, which in turn brings about additional advantages such as decoupling of hydraulic retention time (HRT) and sludge retention time (SRT), maintenance of higher mixed liquor solids concentration (MLSS) than the conventional activated sludge (CAS) process and potentially better removal of resistant contaminants in a single step. The anoxic-aerobic MBR process combines bioreactors harbouring different redox conditions and thus facilitates efficient removal of nutrients, and potentially that of TrOCs. This thesis aims to evaluate the performance of an anoxic-aerobic MBR in terms of nutrient and TrOC removal at lab-, pilot- and full-scale installations. The dynamics of bacterial communities in the MBR system and the corresponding removal performance under different operating conditions have been assessed. The robustness of the anoxic-aerobic MBR during simulated 'hazardous events' *i.e.*, deviations in operating conditions is also evaluated.

Simultaneous nitrogen and TrOC removal (a set of 30 TrOCs) by a laboratory scale anoxic-aerobic MBR was demonstrated. In this study, biodegradation was demonstrated as the main TrOC removal mechanism, with aerobic degradation playing a major role. Low oxidation reduction potential (ORP) regimes (*i.e.*, anoxic) were conducive to biodegradation of some TrOCs, but it may only aid in biosorption in absence of internal recirculation between the anoxic-aerobic zones. Metagenomic approach using pyrosequencing of 16S rRNA genes revealed the bacterial communities developed in the anoxic-aerobic MBR system. Internal recirculation between the aerobic and anoxic bioreactors was observed to be a key driving force shaping the bacterial communities in the anoxic-aerobic MBR. Insights into the shifts in bacterial communities along with the changes in removal efficiencies under different operating conditions have been provided. A more diverse bacterial community was noted during operation without sludge withdrawal ('infinite' SRT) than during an SRT of 25d. However, with a few exceptions, the bulk organic, nitrogen and TrOC removal performance were similar

under the SRTs investigated, suggesting that the shorter SRT investigated in this study (25 d) was adequate for the development of functional bacterial groups in the MBR. Potential bacterial groups participating in TrOC degradation were identified.

During comparison of bulk organics, nutrients and TrOC removal performance by a full- and a pilot-scale MBR from real wastewater originating from a resort town, the pilot-scale MBR demonstrated a very similar COD reduction as the full-scale MBR. However, given the significantly higher MLVSS concentration and presence of additional anoxic and aerobic bioreactors in the full-scale plant, the removal of nutrients, particularly that of phosphorous, and a few resistant TrOCs by the full-scale MBR was significantly higher. Both TN and TrOC removals were facilitated by a delicate combination of multiple redox zones in the bioreactors.

Simulated hazardous events, namely, aeration and power failure, and chemical shock (ammonia and bleach) were found to alter pH and/or ORP of the mixed liquor and inhibit biomass growth, thus affecting the removal of bulk organics, nutrients and TrOCs. Chemical shocks generally exerted greater impact on MBR performance than aeration/power failure events, with ammonia shock exerting the greatest impact. The removal of hydrophilic TrOCs that are resistant and/or occur at high concentrations in wastewater were notably affected by the hazardous events. MBR treatment effectively reduced estrogenic activity in wastewater; however, chemical shocks were observed to temporarily increase the endocrine activity of the effluent. Depending on the chemical shock-dose and the applied membrane flux, hazardous events can exacerbate membrane fouling. Except for ammonia shock, recovery of the MBR performance was achieved within 72 h of hazardous events.

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List of publications

Journal papers

1. **Hop V. Phan**, Faisal I. Hai, Jinguo Kang, Hoa K. Dam, Ren Zhang, William E. Price, Andreas Broeckmann, Long D. Nghiem (2014). Simultaneous nitrification/denitrification and trace organic contaminant (TrOC) removal by an anoxic-aerobic membrane bioreactor (MBR). *Bioresource Technology*. 165: 96-104.
2. **Hop V. Phan**, Faisal I. Hai, James A. McDonald, Stuart J. Khan, Ren Zhang, William E. Price, Andreas Broeckmann, Long D. Nghiem (2015). Nutrients and trace organic contaminants removal from wastewater of a resort town: comparison between a pilot and a full scale membrane bioreactor. *International Biodeterioration & Biodegradation*. 102 (2015): 40-48.
3. **Hop V. Phan**, Faisal I. Hai, James A. McDonald, Stuart J. Khan, Ren Zhang, William E. Price, Andreas Broeckmann, Long D. Nghiem (2015). Impact of hazardous events on the removal of bulk organics, nutrients and trace organic contaminants by a pilot-scale anoxic-aerobic membrane bioreactor. *Bioresource Technology*. 192 (2015): 192-201.
4. **Hop Van Phan**, Faisal I. Hai, Ren Zhang, Jinguo Kang, William E. Price, Long D. Nghiem. Bacterial community dynamics in an anoxic-aerobic membrane bioreactor – Impact on nutrient and trace organic contaminant removal. *Submitted to International Biodeterioration & Biodegradation* (2015).

Conference presentations/publications

1. **Hop V. Phan**, Faisal I. Hai, Jinguo Kang, Hoa K. Dam, Ren Zhang, William E. Price, Andreas Broeckmann, Long D. Nghiem, Simultaneous nitrification and denitrification and trace organic contaminant removal by an anoxic-aerobic membrane bioreactor (MBR). Oral presentation at *the 6th International Conference on the "Challenges in Environmental Science and Engineering (CESE)", Daegu, Korea, 29th October – 2nd November, 2013*.
2. **Hop Van Phan**, Faisal I. Hai, Long D. Nghiem, Ren Zhang, Bacterial communities under different redox conditions of an integrated anoxic-aerobic

Membrane Bioreactor (MBR). Poster presentation at *the 42nd Australian Society for Microbiology (ASM) Annual Meeting, Melbourne, Australia, 6th- 9th July, 2014*.

3. **Hop Van Phan**, Faisal I. Hai, Long D. Nghiem, Ren Zhang, Bacterial communities under two different redox conditions of an integrated anoxic-aerobic membrane bioreactor (MBR). Oral presentation *at the International Conference on Environmental Protection and Human Health (EPHH), Wuhan, China, 13th – 14th December, 2014.* "Published in EPHH 2014 Conference Proceeding, Pennsylvania, USA (ISBN: 978-1-60595-230-7), p: 311-317".
4. **Hop Van Phan,** Faisal I. Hai, William E. Price, Long D. Nghiem, Impacts of redox conditions on microbial community dynamics and trace organic contaminant removal performance of activated sludge-based processes – A review. Oral presentation at *the 8th International Conference on the "Challenges in Environmental Science and Engineering (CESE)", Sydney, Australia, 28th September – 2nd October, 2015*.

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CHAPTER 1. Introduction

1.1 BACKGROUND

Increasingly stringent environmental regulations and freshwater shortages are key drivers for a worldwide trend to introduce advanced sewage treatment infrastructure for removing nutrients (i.e., nitrogen and phosphorus) and trace organic contaminants (TrOCs) along with bulk organics. In particular, discharge of high nutrient flux from untreated wastewater can lead to eutrophication that is detrimental to aquatic ecosystem (Smith, 2003). Level of nitrogen and phosphorus in treated effluent is strictly regulated in many countries. For example, European Union guidelines stipulate effluent TN < 10 mg/L and TP < 1 mg/L in sensitive areas. In the United State, a more stringent effluent standard is applied for 14 selected ecoregions (most estuaries and sensitive rivers and lakes): TN <1.5-3 mg/L and TP < 0.07 mg/L (Oleszkiewicz and Barnard, 2006). Additionally, due to the ineffectiveness of conventional secondary wastewater treatment processes, TrOCs such as pharmaceuticals and personal care products (PPCPs), industrial chemicals, steroid hormones and pesticides are ubiquitous in wastewater treatment plant (WWTP) effluents. Ineffective wastewater treatment is a major conduit by which TrOCs reach natural water bodies. This raises considerable concern regarding their effects on the aquatic organisms and even humans after chronic ingestion (Luo et al., 2014). Therefore, removal of nutrients and TrOCs from wastewater is important for wastewater disposal to sensitive areas and also for safe reuse.

Membrane bioreactors (MBRs) that combine biodegradation by activated sludge with direct solid-liquid separation using membrane filtration are an advancement over the conventional activated sludge (CAS) process to efficiently remove bulk organics and nutrients in a compact format (Hai et al., 2014). In term of TrOC removal, the typical characteristics of MBRs such as long sludge retention time (SRT) and high biomass concentration (relative to the CAS process) may be advantageous. Although a number of studies have reported better and more stable removal of the moderately biodegradable TrOCs by MBR than CAS, little improvement is generally reported in case of hydrophilic and resistant TrOCs (Radjenović et al., 2009; Sui et al., 2011; Tadkaew et al., 2011).

Biotransformation processes can possibly be induced under aerobic (in the presence of molecular oxygen), anoxic (in the absence of molecular oxygen but in the presence of nitrate) or anaerobic conditions (in the absence of both molecular oxygen and nitrate)

(Dorival-García et al., 2013; Joss et al., 2004). The anoxic-aerobic MBR configuration is specifically designed for nutrients removal. Given the recent reports on possible relationships between nutrient and TrOC biotransformation (Fernandez-Fontaina et al., 2014; Helbling et al., 2012), anoxic-aerobic MBRs, which can combine a range of redox conditions (*i.e.*, aerobic/anoxic/anaerobic), may be also effective for TrOC removal. Only a few studies have examined the TrOC removal by a combination of different redox conditions (Sui et al., 2011; Xue et al., 2010). Different redox conditions can induce distinct impacts on biomass growth and the properties of sludge, which may govern TrOC biotransformation and biosorption. The mechanisms of biological nutrient (*i.e.*, nitrogen and phosphorous) removal under different redox conditions are well understood and have been successfully applied in full-scale WWTPs. However, the same cannot be claimed in the case of TrOC removal.

The efficiency of wastewater treatment is ultimately determined by the composition and function of microbial communities (Nielsen and Seviour, 2010). Effective solid liquid separation by the membrane as well as high SRT and biomass concentrations are selective forces that may shape the bacterial communities in MBR. The use of anoxic and aerobic reactors connected via internal sludge recirculation may also lead to a unique niche for the development of specific bacterial communities. These selective factors in anoxic-aerobic MBRs can potentially affect bacterial community structure as well as their growth and enzymatic profiles that decide the biotransformation potential of activated sludge. To date some bacterial groups have been assigned for removal of nitrogen and phosphorus (Lücker, 2010; Nielsen and Seviour, 2010), although the information available is too little compared with the vast diversity of bacterial communities in activated sludge. Furthermore, the diversity and low concentration (ng/L to μg/L) of TrOCs in wastewater environment are challenges for identifying functional bacterial groups responsible for TrOC biodegradation. Only a few studies have investigated TrOC-degrading bacteria in activated sludge community (Boonnorat et al., 2014; Kurisu et al., 2015; Thayanukul et al., 2010), and current understanding regarding functional bacterial groups capable of TrOC degradation remains limited.

Laboratory-scale investigations using synthetic wastewater facilitates focused investigation of selected factors while ensuring that other factors remain unchanged. However, the importance of the validation of observed data via pilot- and full-scale

investigations cannot be overlooked. For example, several reports have shown the correlation of TrOC removal with nitrogen removal, but most of these studies were conducted at small scale with synthetic wastewater (Fernandez-Fontaina et al., 2014; Helbling et al., 2012) and a validation of this phenomenon at full-scale level remains scarce to date. Only a few Australian studies to date have investigated TrOC removal from real sewage (Coleman et al., 2008; Le-Minh et al., 2010; Trinh et al., 2012). Assessment of nutrient and TrOC removal at pilot- and full-scales may provide unique insights into MBR performance at realistic conditions.

Finally, the efficiency of wastewater treatment plants including MBRs can be affected by the deviations in operating conditions, termed as "hazardous events". These deviations can be caused by changes in source wastewater composition, extreme weather events, human error and mechanical malfunctions (Trinh et al., 2014). Previous studies have demonstrated detrimental impact of hazardous events on bulk organics and nutrient removal by CAS processes (Bodík et al., 2008; Panswad and Anan, 1999). CAS performance under hazardous event circumstances may provide useful insights but may not completely represent impacts to MBR performance. MBRs combine membrane separation with biodegradation, and as such the potential impact of hazardous events on membrane hydraulic performance must be additionally considered. However, only one study (Trinh et al., 2015) to date has reported the impact of selected hazardous events on MBR performance. A further notable omission is that except for a limited coverage in the study of Trinh (2013), the impact of hazardous events on TrOC removal remains largely unexplored. Therefore, it is important to validate the robustness of the anoxic-aerobic MBR to the impact of hazardous events.

1.2 RESEACH OBJECTIVES

The overarching aim of this study is to evaluate the performance of an anoxic-aerobic MBR in terms of nutrient and trace organic contaminant removal. The specific objectives include:

- To evaluate nutrient and trace organic contaminant removal by an anoxic-aerobic MBR.
- To analyse the microbial community under different operational conditions in a lab-scale anoxic-aerobic MBR.

- To compare the removal performance of a pilot anoxic-aerobic MBR with a full-scale MBR.
- To assess the impact of a range of hazardous events on anoxic-aerobic MBR performance.

1.3 THESIS STRUCTURE

The structure of the thesis is illustrated in **Figure 1.1**. Chapter 2 provides a comprehensive literature review on the current knowledge of nutrient and TrOC removal by MBR, specifically by anoxic- aerobic MBR system, as well as dynamics of bacterial communities in MBRs. Follows next four main chapters (Chapter 3, 4, 5, and 6) which describe the materials and methods, experimental setups as well as results and discussion in detail. Chapter 3 investigates simultaneous nutrient and TrOC removal by a laboratory-scale anoxic- aerobic MBR under different operating conditions including different internal recirculation (IR) ratio between the anoxic/aerobic bioreactors and SRT. This chapter reveals the role of IR on nutrient and TrOC removal efficiency and the contributions of different redox conditions on TrOC removal. Chapter 4 systematically investigates the dynamics of bacterial communities in the lab-scale anoxic-aerobic MBR system. The shift of bacterial communities due to changes in operating conditions (*e.g.*, SRT, TrOC addition and IR) and its correlation with removal performance was assessed, and the potential functional bacterial groups responsible for degradation of contaminants including TrOCs were revealed. Chapter 5 carries out an evaluation of the performance of a pilot-scale anoxic- aerobic MBR for treating real wastewater originating from a small resort town in comparison with a full-scale MBR. This chapter provides evidence of the impact of combinations of redox regimes on nutrient and TrOC removal as well as the relation between TrOC and nitrogen removal. Chapter 6 assesses the robustness of the anoxic-aerobic MBR system to simulated common hazardous events, namely aeration failure, power failure, ammonia shock and bleach shock. Chapter 7 summarizes the conclusions of this study and the recommendations for future research. Finally, an appendix contains supplementary information regarding materials and methods and data analysis.

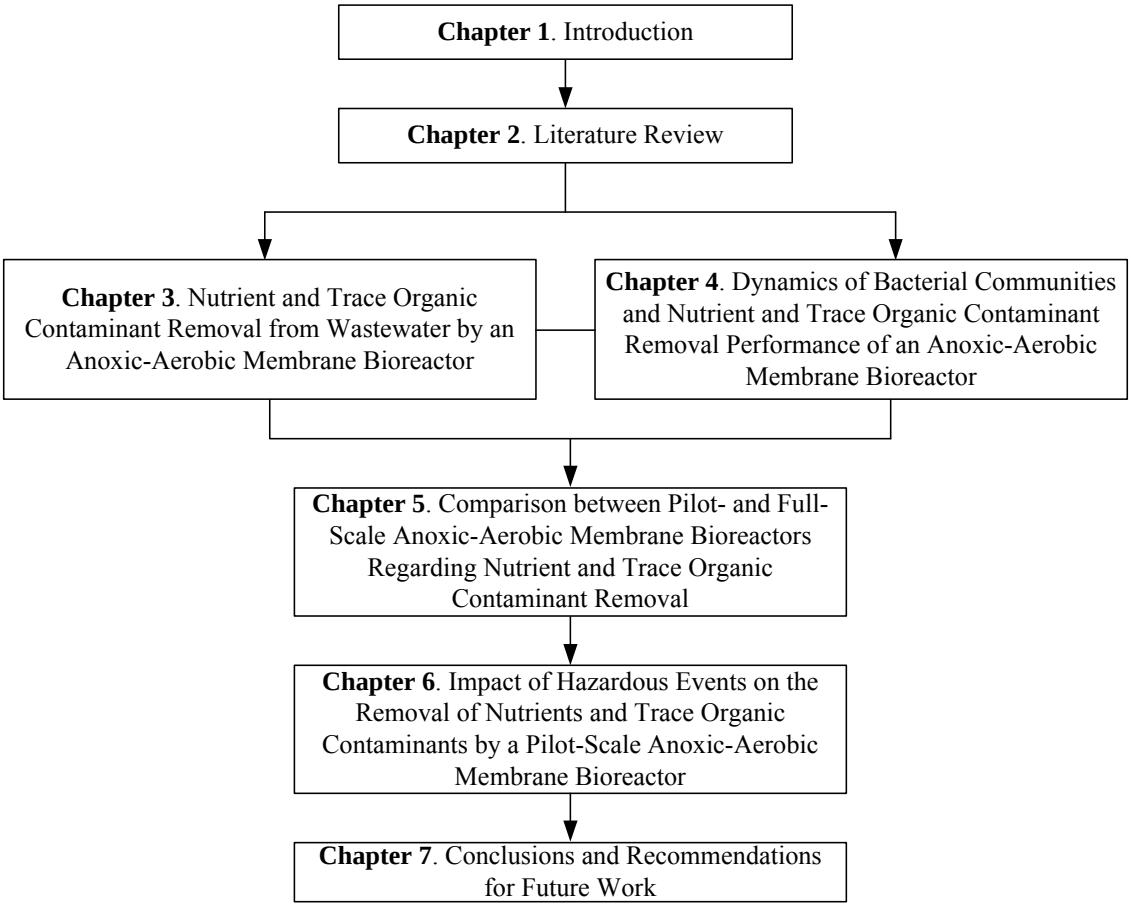

Figure 1.1: Thesis outline

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CHAPTER 2. Literature Review

2.1 INTRODUCTION

Population increase and global warming have led to increased water shortage in many parts of the world. Thus treated wastewater is being considered as an alternative water source. To date treated wastewater reuse has been mainly conducted for industrial, urban, agricultural and environmental purposes (USEPA, 2012). Removal of nutrients (nitrogen and phosphorus) from wastewater is necessary to avoid eutrophication and other detrimental impacts on the aquatic ecosystem. Along with nutrients, the occurrence of the emerging trace organic contaminants (TrOCs) in wastewater and wastewater-impacted natural water bodies is a major human and environmental health concern. Recent studies have paid significant attention to efficient removal of TrOCs from wastewater (Hai et al., 2014b). Over the last two decades, worldwide application of membrane bioreactors (MBRs) has increased tremendously, particularly when wastewater recycling is considered relevant (Meng et al., 2012). The combined anoxic-aerobic MBR processes have been proposed to achieve effluent total nitrogen (TN) concentration below 4 mg/L (Water Environment Federation, 2012).

As outlined in Chapter 1, this thesis focuses on nutrient and TrOC removal via anoxic-aerobic MBRs. Given the dearth of the related knowledge, the dynamics of bacterial community in the anoxic-aerobic MBR under different operating conditions have been also investigated. Furthermore, the performance of the anoxic-aerobic MBR system was compared at pilot- and full-scales. Finally, a validation of the robustness of the anoxic-aerobic MBR system under simulated hazardous events was conducted. This chapter provides an up-to-date review of all the components of this research, namely, environmental concerns with and removal of nutrient and TrOC, performance of the anoxic-aerobic MBR configuration, microbial community dynamics in anoxic-aerobic MBR and hazardous events in wastewater treatment plants (WWTPs). Given the strong focus of this thesis on TrOCs, the literature review commences with the aspect of TrOCs.

2.2 TRACE ORGANIC CONTAMINANTS (TrOCs) IN AQUATIC ENVIRONMENT

2.2.1 Occurrence of TrOCs in the aquatic environment

TrOCs are chemicals that occur in the environment at concentrations from a few ng/L to μg/L. They belong to many chemical groups including pharmaceuticals and personal care products (PPCPs), pesticides, steroid hormones, industrial chemicals, phytoestrogens and many other chemicals. The low concentrations and the diversity of the chemical groups in TrOCs form challenges for risk assessment and formulating treatment solutions. Both anthropogenic activities and natural processes can lead to the occurrences of TrOCs in the environment. Current WWTPs are not specifically designed for TrOC treatment and hence WWTP effluent is considered as a major conduit for the occurrence of TrOCs in the aquatic environment (**Figure 2.1**). A wide variation in TrOC concentrations in WWTP influent and effluent can be noticed (**Table 2.1**). Some pharmaceuticals such as paracetamol, caffeine, ibuprofen, and naproxen can be detected at high concentrations (100 μg/L) while estrogenic compounds appear to occur at lower concentrations (<1μg/L) (**Table 2.1**). Luo et al. (2014) reviewed the occurrence of 39 TrOCs and found that most TrOCs in raw wastewater had a concentration range of 0.1 to 10 μg/L, while their concentration in effluent was mostly in the range of 0.001-1 μg/L. Many factors are thought to affect TrOC occurrence in the environment including mode of production, consumption and disposal of TrOCs, chemical resistance, climatic conditions and particularly removal efficiency of WWTPs (Luo et al., 2014). The frequent discharge of inadequately treated wastewater and other sources (**Figure 2.1**) may lead to a widespread contamination of freshwater supplies with TrOCs. Many TrOCs have been detected in surface water, groundwater and even drinking water (Lapworth et al., 2012; Luo et al., 2014; Pal et al., 2010).

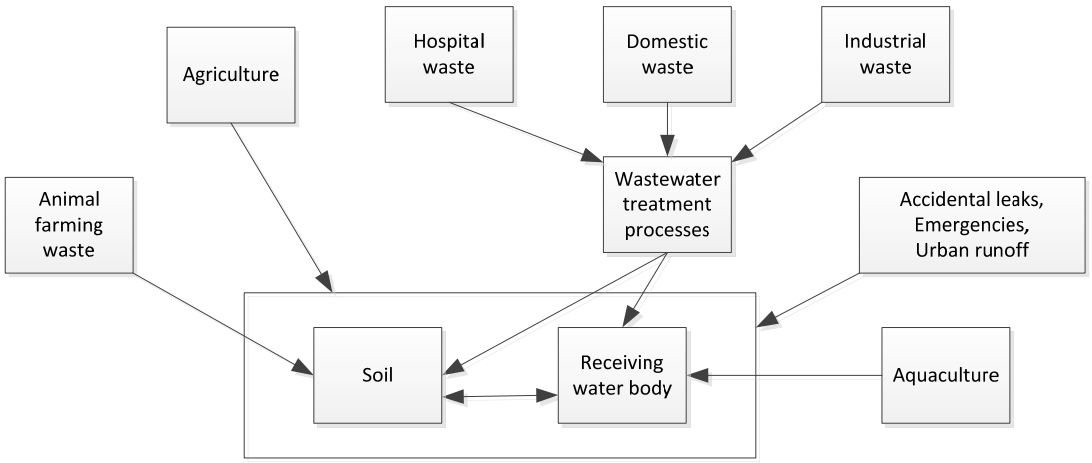

Figure 2.1: Sources and pathways of TrOC occurrence in aquatic environment (Boxall, 2004; Lapworth et al., 2012).

Table 2.1: Reported concentrations of selected trace organic contaminants (TrOCs) in influent and effluent of WWTPs in different countries

Categories	Compounds	Sampling sites	Influent (µg/L)	Effluent (µg/L)	References
Pharmaceuticals and personal care products	Diclofenac	EU-wide, Greece, Korea, Sweden, Switzerland, UK, WB	<0.001–94.2	<0.001–0.69	(Luo et al., 2014)
	Carbamazepine	China, EU-wide, Greece, Korea, Spain, UK,WB	<0.04–3.78	<0.005–4.60	(Luo et al., 2014)
	Naproxen	Greece, Korea, Spain, Sweden, UK, WB	<0.002–52.9	<0.002–5.09	(Luo et al., 2014)
	Gemfibrozil	EU-wide, Greece, Korea, Spain,WB	0.10–17.1	<0.0025–5.24	(Luo et al., 2014)
	Primidone	Germany, Korea, US	N.D - 0.42	0.1 - 0.25	(Bisceglia et al., 2010; Krasner et al., 2006; Ryu et al., 2014; Wick et al., 2009)
	Ketoprofen	China, EU-wide, Korea, Spain, UK,WB	<0.004–8.56	<0.003–3.92	(Luo et al., 2014)
	Metronidazole	China, EU-wide, Italy, Spain, Hongkong,	0.028 - 0.96	0.016 -0.9	(Al Aukidy et al., 2014; Gros et al., 2013; Qi et al., 2015; Verlicchi et al., 2014; Yu et al., 2012)
	Ibuprofen	China, EU-wide, Greece, Korea, Sweden, UK, US, WB	<0.004–603	ND–55	(Luo et al., 2014)
	Triclosan	Spain, UK, US, Greece, Korea, France, EU-wide	0.03–23.9	0.01–6.88	(Luo et al., 2014)
	Triclocarban	Canada, Korea, US	0.09 - 6.7	0.033 - 0.243	(Halden and Paull, 2005; Kim et al., 2014; Li et al., 2013; Lozano et al., 2013; Ryu et al., 2014)

	Amitriptyline	UK	0.341 - 5.143	0.053-0.357	(Kasprzyk-Hordern et al., 2009)
	Salicylic acid	Greece, Spain, UK	0.58–63.7	ND–0.50	(Luo et al., 2014)
	Caffeine	China, EU-wide, Greek, Korea, Spain, UK	0.22–209	ND–43.50	(Luo et al., 2014)
	Omeprazole	Spain	0.057-2.134	ND-0.922	(Rosal et al., 2010)
	Paracetamol	Spain, UK, Korea	1.571 - 482.687	ND - 24.225	(Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Rosal et al., 2010)
	Sulfamethoxazole	EU-wide, France, Korea, Spain, Sweden, Switzerland, UK,WB	<0.003–0.98	<0.003–1.15	(Luo et al., 2014)
	Trimethoprim	China, EU-wide, Korea, Spain, UK	0.06–6.80	<0.01–3.05	(Luo et al., 2014)
	Fluoxetine	Spain, UK	0.086 - 2.3	<0.029 - 0.43	(Baker and Kasprzyk-Hordern, 2011; Radjenović et al., 2009)
	Atenolol	Korea, Spain, Switzerland, UK,WB	0.1–33.1	0.13–7.60	(Luo et al., 2014)
	Verapamil	Czech Republic	0.011 - 0.72	0.006 - 0.055	(Golovko et al., 2014)
	Clozapine	China	0.017-12.783	0.015 - 8.183	(Yuan et al., 2013)
	Meprobamate	US	0.589-1.040	0.200-0.494	(Gerrity et al., 2011)
	Diazepam	Spain, UK, Austria, Czech Republic	ND - 0.008	ND -0.005	(Baker and Kasprzyk-Hordern, 2011; Clara et al., 2005b; Rosal et al., 2010)
	DEET	China, EU-wide	2.56–3.19	0.61–15.8	(Luo et al., 2014)
	Dilantin	Saudi Arabia	0.1-1.49	ND - 0.06	(Alidina et al., 2014a; Dickenson et al., 2009)
	Risperidone		ND	ND	(Gracia-Lor et al., 2012)
	Hydroxyzine	EU-wide		<0.0005-0.0096	(Loos et al., 2013)
Pesticides	Atrazine	EU-wide, France, Spain,	0.02–28	0.004–0.73	(Luo et al., 2014)

		Switzerland, WB			
	Clofibric acid	China, EU-wide, Greece, Korea, Spain, Sweden, UK,WB	0–0.74	ND–0.33	(Luo et al., 2014)
	Propoxur	Australia		0.014-0.112	(Allinson et al., 2012)
	Diazinon	EU-wide, Spain	<0.684	0.0007–4.16	(Luo et al., 2014)
	Simazine	Spain, US	<0.00161-0.028	0.011-1.99	(Köck-Schulmeyer et al., 2013; Oppenheimer et al., 2011; Teijon et al., 2010)
	Phenylphenol	China	0.1836-0.1905	0.0194-0.0258	(Yu et al., 2011)
	Diuron	EU-wide, France, Spain, Switzerland	0.03–1.96	0.002–2.53	(Luo et al., 2014)
	Linuron				(Köck-Schulmeyer et al., 2013)
Steroid hormones	17α-Ethinylestradiol (EE2)	China, France, Germany, Italy, Sweden, US	0.001–0.003	<0.001–0.002	(Luo et al., 2014)
	17α-Estradiol	China, Nertherland, US, Greece	0.0007-0.0202	<0.0001-0.005	(Belfroid et al., 1999; Chang et al., 2011; Pothitou and Voutsa, 2008; Ratola et al., 2012; Yu et al., 2011)
	Estriol (E3)	China, Korea	0.125–0.80	ND	(Luo et al., 2014)
	Estrone (E1)	China, France, Germany, Italy, Korea, Sweden, US	0.01–0.17	<0.001–0.08	(Luo et al., 2014)
	Testosterone	China, Korea, UK	0-0.635	0-0.144	(Chang et al., 2011; Kim et al., 2007a; Kirk et al., 2002; Manickum and John, 2014)
	Androsterone	China, UK	2.667 - 14.040	ND-7.7	(Chang et al., 2011; Kirk et al., 2002)
	Androstenedione	China, South Korea	0.14-0.33	<10	(Chang et al., 2011; Kim et al., 2007a)

	17β-Estradiol (E2)	China, France, Germany, Italy, Korea, Sweden, US	0.002–0.05	<0.001–0.007	(Luo et al., 2014)
Industrial chemicals	4-tert-Butylphenol	US	<1-9.6		(Conn et al., 2006)
	Bisphenol A	China, France, Greece, US, WB	<0.013–2.14	<0.03–1.10	(Luo et al., 2014)
	4-tert-Octylphenol	China, France, Germany, Italy, Spain, UK, US	<0.2–8.7	0.004–1.3	(Luo et al., 2014)
	Nonylphenol	China, France, Germany, Greece, Italy, Spain, US,WB	<0.03–101.6	<0.03–7.8	(Luo et al., 2014)
	TCEP	EU-wide, Germany	0.06–0.50	0.06–2.40	(Luo et al., 2014)
Physoestrogens	Enterolactone	Australia, Finland	0.581-2.111	0.1-48	(Kang and Price, 2009; Smeds et al., 2007)
	Formononetin	Australia, Italy	0.0001-0.01	ND-0.6	(Kang and Price, 2009), (Bacaloni et al., 2005)
UV filters	Benzophenone	Korea, Spain	<0.079–0.90	<0.079–0.23	(Luo et al., 2014)
	Oxybenzone	China, Italy, Switzerland, Spain, US	0.006-10.4	<0.005-0.164	(Kim and Choi, 2014; Liu et al., 2012)
	Octocrylene	China, Italy, Switzerland, Australia	0.012-12	<0.01-0.3	(Balmer et al., 2005; Kupper et al., 2006; Li et al., 2007; Liu et al., 2012; Magi et al., 2013)

Note: ND = Not detected

2.2.2 Health and environmental issues concerning TrOCs

TrOCs are chemicals of emerging concern. Their impacts on environment and human health are yet to be completely revealed (Deblonde et al., 2011) and thus, except for a few, effluent discharge guidelines or standards for most TrOCs have not been introduced. Some countries or regions regulate a few types of TrOCs for wastewater disposal and recycling (European, Canada, and Australia) (Canadian Environmental Protection Act, 1999; European Parliament and The Council, 2008; NRMMC/EPHC/NHMRC, 2008).

Limited information is available regarding quantitative assessment of ecotoxicological potency of different groups of TrOCs. In the studies available, several approaches have been applied for toxicological assessment using fish, algae, mussels, and human cell lines in both *in vitro* and *in vivo* bioassays. Toxicological impact of TrOCs on human and aquatic organisms have been reported in a number of studies (Alexander et al., 2012). Endocrine disrupting chemicals (EDC) such as steroid hormones have attracted the greatest attention due to their estrogenic potency. It is unclear as to what constitutes a unsafe level of estrogenic activity, but an estrogenic activity of 1 ng/L estradiol equivalent (EEQ) is commonly accepted as unlikely to cause significant endocrine effects in exposed aquatic biota (Leusch et al., 2014; Scott et al., 2014). However, a special consideration has been given to 17β-ethinylestradiol that is 10 times more potent *in vivo* than 17β-estradiol (Caldwell et al., 2012; Young et al., 2004). A bioassay-based short- and long- term safe EEQ (EEQ-SSE) was employed by Jarošová et al. (2014). With estrogen receptor-mediated, chemical-activated luciferase reporter gene-expression (ER-CALUX) bioassay, EEQ-SSE was proposed to be 0.2 – 0.4 ng/L EEQ for long-term and 0.6 – 2 ng/L EEQ for short-term exposures (Jarošová et al., 2014). In addition, different kinds of pharmaceuticals such as cardiovascular drugs, antibiotics and antineoplastics or drugs used to cure abnormal tissue growth called neoplasms have been shown to pose adverse impact on aquatic organisms (Sanderson et al., 2004). **Table 2.2** provides examples of TrOC toxicity on aquatic organisms. TrOC transformation products originating during wastewater treatment are also drawing increased attention as more data regarding their occurrence in treated wastewater becomes available, but their formation route, toxicity and risks are yet to be elucidated (Evgenidou et al., 2015).

Table 2.2: Examples of TrOC toxicity on aquatic organisms (modified after Pal et al. (2010)).

No. of compounds studied	Compounds causing risks; concentration of exposure (range of dose at which the risk was observed)	Type of risks involved
1	Diclofenac; 0.5–50 µg/l	Affect tissues of gills and kidney of freshwater fish, brown trout (*Salmo trutta f. fario*), suggesting possible risk to fish populations
27	Ibuprofen, diclofenac, E2 and EE2; ~0.01 µg/l	Risk to aquatic environment with chronic toxic effect (such as inhibited polyp regeneration and reduced reproduction)
13	Mixture of atenolol, bezafibrate, carbamazepine, cyclophosphamide, ciprofloxacin, furosemide, hydrochlorothiazide, ibuprofen, lincomycin, ofloxacin, ranitidine, salbutamol and sulfamethoxazole; 10–1000 ng/l	Inhibit the growth of human embryonic kidney cells HEK293, with the highest effect observed as a 30% decrease in cell proliferation compared to controls
10	Diltiazem, acetaminophen and sulfamethoxazole; 8.2–271.3 µg/l	Hazard quotient N1. Diltiazem: most toxic (lethal conc. 8.2 mg/l for freshwater invertebrate *Daphnia magna*)
4	Ethinylestradiol, zearalonol, 17β-trenbolone and melengestrol acetate; b1–68 ng	Freshwater fish fathead minnows experience different levels of hepatic gene expression
1	17α-ethinylestradiol (EE2); 5–50 ng/l	Brain and inter-renal steroidogenic acute regulatory protein and cytochrome P-450-mediated cholesterol side-chain cleavage expressions of juvenile salmon were modulated with time and concentration
3	Chloramphenicol, florfenicol, and thiamphenicol (veterinary and aquaculture); 1.3–158 mg/l	Inhibit the growth of Chlorella pyrenoidosa (freshwater) *Isochrysis galbana* and *Tetraselmis chui* (marine)

2.3 TrOC REMOVAL DURING WASTEWATER TREATMENT PROCESSES (WWTPs)

2.3.1 Mechanism of TrOC removal

In biological wastewater treatment processes, the overall removal of TrOCs may occur by different mechanisms, namely, abiotic transformation (e.g., photolysis and volatilization),

biotic transformation (mineralization and incomplete degradation) and sorption to activated sludge. The removal efficiency varies depending on the physio-chemical properties of TrOCs, environmental conditions and WWTP design (Hai et al., 2015). Photolysis can occur via direct and indirect mechanisms. Direct photolysis occurs when TrOCs absorb sunlight and undergoes a transformation reaction. Indirect photolysis occurs when TrOCs react with reactive intermediates (e.g., hydroxyl radical, organoperoxy radicals, and carbonate radical) produced due to sunlight absorption by other chemicals such as nitrate and coloured dissolved organic matter (Jasper et al., 2013). However, in biological WWTPs, photolysis is not considered as a significant process for TrOC removal. Volatilization is only considered for aerobic treatment i.e., under vigorous aeration. The contribution of volatilization is considered to be significant for a chemical when its dimensionless Henry's gas water partitioning coefficient (H) is higher than 0.005 (Joss et al., 2006).

Biotransformation and sorption are the two major removal mechanisms during biological treatment (Hai et al., 2014a; Verlicchi et al., 2012). TrOC sorption can be predicted based on log *D*, the pH-dependent n-octanol-water distribution ratio. Log *D* simultaneously considers hydrophobicity and ionization, thus it takes into account both transfer of the neutral species between the aqueous phase and the immiscible phase and the transfer of the hydrophobic, ionized, and organic species as free ions or ion pair (Wells, 2006). Sorption to activated sludge was classified as the major removal mechanism for highly hydrophobic TrOCs (defined as log $D > 3$) (Tadkaew et al., 2011b). Adsorbed TrOCs in sludge can be subsequently removed via sludge withdrawal. Sorption of TrOCs to sludge also increases retention time of TrOCs in the bioreactor that may enhance its biodegradation. For compounds having lower log *D* values, sorption may no longer be the major TrOC removal mechanism and their removal may vary depending on the intrinsic properties (Tadkaew et al., 2011a; Wijekoon et al., 2013).

TrOC biodegradation during wastewater treatment is catalysed via metabolism and/or co-metabolism by the relevant microbes. Metabolic degradation of TrOC is the process in which microbes utilize TrOCs as the sole energy and/or carbon source to maintain their biomass and produce the relevant enzymes and cofactors for their oxidation/reduction (Tran

et al., 2013). Co-metabolic degradation is the process of transformation of a non-growth substrate in the obligatory presence of a growth substrate. To date, it is not clear which biodegradation pathway is dominant in the elimination of TrOCs in WWTPs (Tran et al., 2013). During biological treatment process, TrOCs are degraded at various degrees, resulting in mineralization or incomplete degradation. It was demonstrated that the functional groups in the chemical structure governs the biodegradability of TrOCs: the compounds containing electron withdrawing groups (EWGs) are more persistent to biodegradation process than the compounds containing electron donating groups (EDGs) (Tadkaew et al., 2011b). Joss et al. (2006) reported pseudo first-order degradation kinetics (k_{biol}) for 25 pharmaceuticals, hormones and fragrances. These TrOCs were grouped into hardly biodegradable (k_{biol} < 0.1 L/g SS. d), highly biodegradable (k_{biol}> 10 L/g SS. d) and moderate biodegradable (0.1 L/g SS. d < k_{biol} < 10 L/g SS. d). Some studies also found that sorption of TrOC into activated sludge can enhance biodegradation. Therefore, both sorption and biodegradation govern the fate of TrOC during biological treatment process (Tadkaew et al., 2011b; Wijekoon et al., 2013).

2.3.2 Roles of redox conditions

Biodegradation processes can possibly be induced under aerobic (in the presence of molecular oxygen), anoxic (in the absence of molecular oxygen but in the presence of nitrate) or anaerobic conditions (in the absence of both molecular oxygen and nitrate). Different redox conditions may promote the growth of different microbial consortia leading to the excretion of diverse enzymes, and therefore, achieving varying degree of TrOC biodegradation. Additionally, redox conditions can significantly influence the properties of sludge, which govern biosorption of TrOCs (Dorival-García et al., 2013a). The mechanisms of biological nutrient (i.e., nitrogen and phosphorous) removal under different redox conditions are well understood and have been successfully applied in full-scale WWTPs. However, the same cannot be claimed in the case of TrOC removal (Phan et al., 2014).

A number of studies have been conducted on TrOC treatment under aerobic conditions (**Table 2.3**). Aerobic condition appears to be more favorable for the degradation of most TrOCs investigated, such as estrogens (Dytczak et al., 2008; Hashimoto and Murakami,

2009; Joss et al., 2004b; Pholchan et al., 2008), phenolic compounds (Liu et al., 2008a; Sarmah and Northcott, 2008; Ying et al., 2008), UV filters (Liu et al., 2013), and some pharmaceutical residues (Chen et al., 2011; McAvoy et al., 2002; Zwiener et al., 2000). Recent studies highlight the TrOC removal performance of aerobic nitrifying reactors (Dorival-García et al., 2013a; Suarez et al., 2010b). However, compared to aerobic conditions, fewer studies have been conducted on TrOC removal under anaerobic and/or anoxic conditions (**Table 2.3**). Therefore, to date understanding of TrOC degradation under anaerobic and/or anoxic conditions remains rather limited.

Table 2.3: Impact of different redox conditions (aerobic, anoxic or anaerobic) on TrOC removal efficiency

Experimental conditions	Compounds	Impact of redox conditions on TrOC removal	References
Lab-scale MBR and batch test: near-anoxic *vs.* aerobic.	Carbamazepine and sulfamethoxazole	Removal of sulfamethoxazole: both anoxic and aerobic condition (65%). Carbamazepine removal: anoxic condition (68 ± 10%) *vs.* aerobic (12 ± 11%)	(Hai et al., 2011a)
Batch test (MBR sludge): aerobic *vs.* nitrifying (NH_4^+) and anoxic (nitrate).	Six quinolones antibiotics	Sorption potential: nitrifying and anoxic < aerobic. Biodegradation: nitrifying (36 – 60%) < aerobic heterotrophic (15 - 44%) < anoxic (negligible). Removal depended significantly on bacterial composition of the sludge.	(Dorival-García et al., 2013b)
Batch test: aerobic *vs*. (alternating An/O)	Estrogens	Conversion of E2 to E1: aerobic > anoxic. EE2 removal: up to 22% (aerobic), but persistent under anoxic condition. Total removal of estrogens: aerobic ≈ (alternating An/O). Removal of E1 and EE2: higher with higher nitrification rate.	(Dytczak et al., 2008)
Batch test (granular sludge): aerobic *vs.* anaerobic.	4-tert-octylphenol	Degradation rate: aerobic >> anaerobic. Optimal pH: 9 (aerobic) and 7 (anaerobic). Degradation rate decreased with increase of initial 4-tert-octylphenol concentration.	(Liu et al., 2008a)
Batch test: aerobic *vs.* anoxic.	Estrogens	Estrogen removal: aerobic > anoxic.	(Hashimoto and Murakami, 2009)
Batch test: aerobic *vs.* anoxic.	Diuron and its metabolites	Diuron removal: over 95% (anoxic) *vs.* 60% (aerobic). Sequential use of An/O may improve diuron removal.	(Stasinakis et al., 2009)
Batch test: anoxic (nitrate) *vs.* anaerobic (no nitrate).	17α Ethinylestradiol (EE2)	Anaerobic: no biodegradation of EE2, only sorption to sludge (62%). Anoxic: biodegradation was the dominant process with 95% of total 97% removal.	(Zeng et al., 2009)

Batch test: aerobic *vs.* anoxic.	Sulfamethoxazole, tetracycline and ciprofloxacin	Under anoxic conditions, hydrophobicity-independent mechanisms can significantly impact solid-liquid partitioning	(Plósz et al., 2010)
Lab-scale CAS: nitrifying *vs.* denitrifying. Pilot-scale An/O-CAS	16 PPCPs	Biodegradation kinetics: nitrifying > denitrifying. Fluoxetine, E1/E2 and musk fragrances: transformed under both aerobic (>75%) and anoxic (>65%). Naproxen, EE2, roxithromycin and erythromycin: significantly transformed in aerobic (>80%). Other compounds were resistant to biotransformation. Diclofenac removal was positively related to nitrifying biomass.	(Suarez et al., 2010b)
Batch test: aerobic *vs.* anoxic (nitrate) and anaerobic.	Triclosan	Removal: mainly under aerobic, no or low under anoxic/anaerobic.	(Chen et al., 2011)
Batch test: anoxic *vs.* anaerobic.	Bisphenol A and 4-n-nonylphenol	Partitioning coefficients of both chemicals: anaerobic > anoxic > aerobic. Biodegradation of bisphenol A: both conditions, with anoxic > anaerobic. Biodegradation of 4-n-nonylphenol: only anoxic (< bisphenol A).	(Wang et al., 2013)
Batch test: aerobic (NH_4^+) *vs.* anaerobic (nitrate)	Chlorpyrifos and Cypermethrin	No impact on removal occurred with increased ammonia (aerobic) or nitrate (anaerobic), but significantly enhanced via additional carbon source addition for both conditions. Enhancement of both denitrification and chemical removal in the anoxic niche with reed addition.	(Feng et al., 2014)
Lab-scale SBRs: aerobic *vs.* (alternating An/O) and microaerobic.	Nine pharmaceuticals	Overall elimination varied between compounds and redox conditions. Elimination of atenolol and trimethoprim: highest in aerobic reactor. Sulfamethoxazole loss: highest in microaerobic reactor. Phenytoin was recalcitrant in all reactors.	(Stadler et al., 2015)
Batch test (treated effluent) of alternating An/O with different cycle lengths.	Bisphenol A and 17β-estradiol	Biodegradation: bisphenol A only in aerobic *vs.* E2 in both. A lag period (no degradation) when switching redox condition from anoxic to aerobic.	(Kim and Cunningham, 2014)

Batch test (lake sediment): anaerobic (nitrate/sulfate/iron-reducing or methanogenic conditions).	Estrogens	No EE2 degradation. E2 to E1: all anaerobic conditions, but conversion rate depending on electron acceptor. Inter-conversion of E2 and E1 was observed, depending on electron acceptor. Racemization occurred, E1 transformed to 17-α-estradiol under all but nitrate-reducing condition. Complete degradation of estrogens was minimal under all anaerobic condition.	(Czajka and Londry, 2006)
Batch test (aquifer materials): aerobic *vs.* anoxic.	EDCs: estrogens and phenolic compounds	All EDCs was biodegraded under aerobic conditions *vs.* only E2 degraded under anoxic conditions.	(Ying et al., 2008)
Batch test (river sediment and aquifer material): aerobic *vs.* anaerobic (nitrate/sulfate/iron-reducing conditions).	EDCs: estrogens and phenolic compounds	Rapid degradation of all compounds (>90%) under both conditions in first 2 to 4 d, but were extremely slow after that. Degradation rate under anaerobic (sulfate/nitrate/iron -reducing) conditins were lower than under aerobic conditions.	(Sarmah and Northcott, 2008)
Batch test (wetland sediments): aerobic *vs.* anaerobic.	Ibuprofen, DEET, gemfibrozil, and carbamazepine.	Aerobic half-lives: ~20 d (ibuprofen, DEET and gemfibrozil) or 165 – 264 d (carbamazepine). Anaerobic half-lives: increased by 11-34 times (gemfibrozil and ibuprofen) or 1.5–2.5 times (carbamazepine). No anaerobic degradation of DEET.	(Conkle et al., 2012)
Batch test (aquifer materials): aerobic *vs.* anaerobic (nitrate/sulfate/iron-reducing conditions).	6 UV filters	UV filters were degraded well with biodegradation half-lives depending on redox conditions. Aerobic conditions were more favourable. Nitrate/sulfate-reducing conditions: inhibited biodegradation of three UV filters.	(Liu et al., 2013)

Notes: A/An/O stands for anaerobic/anoxic/oxic, respectively; BFR: biofilm reactor; CAS: conventional activated sludge; SBR: sequencing batch reactor; EDCs: endocrine disrupting chemicals; and PPCPs: pharmaceuticals and personal care products. Activated sludge was used for batch test unless otherwise indicated.

Furthermore, the performance of combined anaerobic and/or anoxic and aerobic reactors has been the focus of only a limited number of recent investigations, and contradictory reports can often be seen in the literature (**Table 2.4**). For example, Li et al. (2011b) reported biodegradation of both natural (17β-estradiol) and synthetic (17α-ethinylestradiol) estrogens under all three redox conditions in a lab-scale anaerobic-anoxic-aerobic activated sludge system. By contrast, estrogens were only degraded under nitrifying conditions in a combined nitrification (aerobic) and denitrification (anoxic) system (Suarez et al., 2012). Differences in results from recent studies may originate from the variation in operating conditions. Systematic studies under controlled operating regimes with a broad set of TrOCs are required to elucidate the contribution of the individual reactors (facilitating different redox conditions) in combined nitrifying and denitrifying systems, but such attempts have been scarce to date.

Table 2.4: Impact of combination of redox conditions on TrOC removal efficiency

Experimental conditions	Compounds	Impact of redox conditions on TrOC removal	References
Full-scale An/O-CASs;	Estrogens	> 90% removal of estrogens. Removal of E1 and EE2: depends on the redox conditions. EE2 degradation correlated with the nitrifying activity. Reduction of E1to E2: maximum under aerobic conditions.	(Joss et al., 2004a)
Full-scale CASs: two for nutrient removal and one for carbon removal only	Eight pharmaceuticals, two polycyclic musk fragrances and nine EDCs	Variation of redox conditions in low loaded CAS promoted higher removal of nonylphenolpolyehtoxylates.	(Clara et al., 2005b)
Full-scale CAS and BNR (A/An/O-CAS)	12 PPCPs	Oxic tank: the most significant contribution.	(Sui et al., 2011)
Full-scale OD Full-scale A/An/O-CAS Full-scale ICEAS.	14 EDCs including progestogens, androgens, estrogens, and phenols	A/An/O-CAS were better than OD and ICEAS. Three CAS processes using A/An/O bioreactor showed different removal rates for 14 EDCs, possibly caused by various factors (HRT, SRT, influent).	(Huang et al., 2014)
Batch BFRs: oxic *vs.* anoxic Pilot-scale An/O-CAS	Clofibric acid, ibuprofen, and diclofenac	Degradation in pilot-scale An/O-CAS: negligible for clofibric acid; 50% for ibuprofen and diclofenac. Elimination in BFRs: negligible for clofibric acid (anoxic and aerobic); 20% (aerobic) and 10% (anoxic) for diclofenac; 90 % (aerobic) and 15% (anoxic) for ibuprofen.	(Zwiener et al., 2000)
Full-scale CAS and trickling filter	Triclosan	Triclosan was readily biodegradable under aerobic conditions, but not under anaerobic conditions	(McAvoy et al., 2002)
Full-scale An/O-CAS	Estrogens	High elimination of natural estrogens under both denitrification and nitrification. EE2 was mainly removed by aerobic biological degradation	(Andersen et al., 2003)

SBRs: aerobic *vs.* (alternating An/A/O)	E1, E2 and EE2	SBR1 (aerobic): ≥ 98% E2 removal. E1 and EE2 removal affected by SRTs. SBR2 (An/A/O): less removal of E2 at the lower MLVSS. No E1 removal in case of poor settling. EE2 removal was mainly by sorption. Binding of estrogens to SBR2 sludge < binding to SBR1 sludge.	(Pholchan et al., 2008)
Full-scale CAS: An/A/O *vs.* An/O	Estrogens and nonylphenolic compounds	Over 90% removals for nonylphenol ethoxylates and estrogens by both systems. Biological activity and biomass sorption: An/O > An/A/O.	(Koh et al., 2009)
Lab-scale CAS: nitrifying *vs.* denitrifying. Pilot-scale An/O-CAS	16 PPCPs	Biodegradation kinetics: nitrifying > denitrifying. Fluoxetine, E1/E2 and musk fragrances: transformed under both aerobic (>75%) and anoxic (>65%). Naproxen, EE2, roxithromycin and erythromycin: only transformed in aerobic (>80%). Other compounds were resistant to biotransformation. Diclofenac removal was positively related to increase nitrifying biomass. Pilot-scale An/O-CAS: poor transformation of E1/E2 under anoxic (<20%) *vs.* moderate transformation of erythromycin under anoxic (60%).	(Suarez et al., 2010a; Suarez et al., 2012)
Full-scale systems: BNR and Symbio (An/O in single tank)	20 PPCPs	Lincomycin, carbamazepine, atenolol, metoprolol, and triclosan: showed better removal in systems with co-existence of An/O conditions (BNR and Symbio).	(Behera et al., 2011)
Full-scale A/An/O-CAS	Glucocorticoids, androgens, progestogens and estrogens	Many glucocorticoids, androgens, and progestogens were eliminated in anaerobic zone, but estrogens were mainly degraded in aerobic zone. Deconjugation led to increased mass of 21α-hydroxyprogesterone and 6α-methylhydroxyprogesterone in anaerobic and anoxic zones.	(Fan et al., 2011)
Lab-scale A/An/O-CAS	E2 and EE2	E2 removal: A, An, and O accounted for 71%, 7%, and 22% of total removal, respectively. 99.99% was biodegraded and 0.01% remained in sludge. EE2 removal (~80%) with A, An, and O accounted for 44%, 8%, 48% of total (79% was degraded, 20 % in effluent, and 1% in waste sludge. Sorption was the dominant mechanism in anaerobic zone.	(Li et al., 2011a)

Batch test Full-scale WWTPs including BNR.	Stereoselectivity of R,S-venlafaxine and its metabolites	Transformation of venlafaxine to O-desmethylvenlafaxine: exclusively under anaerobic *vs.* only a fraction under aerobic conditions. Small stereoisomeric selectivity for degradation of venlafaxine. Remarkable S to R transformation under aerobic conditions but not under anaerobic conditions for degradation of O-desmethylvenlafaxine.	(Gasser et al., 2012)
Pilot-scale step-feed An/O-CAS	E1 and E2	Log K_d of E1 and E2: aerobic > anoxic. Conversion of E1 and E2: anoxic zones (0.38-0.81) > aerobic zones (0.08-0.24).	(Shi et al., 2013)
Full-scale A/An/O-CAS	Six pharmaceuticals	Anaerobic and aerobic zones contributed to biodegradation of caffeine and DEET while anoxic zone had negative effect.	(Wang et al., 2014)
Full- and lab-scale O/An-CAS	Ibuprofen, atenolol, diclofenac, and fluoxetine	Degradation of ibuprofen: mainly in aerobic by cometabolism with biodegradable carbon *vs.* suppressed in anoxic condition. Degradation of atenolol: both conditions (aerobic > anoxic) by cometabolism with biodegradable carbon. Diclofenac and fluoxetine: removed by sorption only	(Pomiès et al., 2015)
Batch and pilot-scale: A/An/O-CAS	Estrogens	96± 5% removal of estrogenecity. Degradation efficiencies in A, An, and O- zones (pilot-scale BNR) were 11±9%, 18±2% and 93±10%, respectively. Biotransformation rate of E2 to E1: 71, 31 and 1 (L/gCOD .d) for O, An, and A, respectively; while corresponding values of E1: 7, 3 and 0.85.	(Ogunlaja and Parker, 2015)

Notes: A/An/O stands for anaerobic/anoxic/oxic, respectively; BFR: biofilm reactor; CAS: conventional activated sludge; SBR: sequencing batch reactor; EDCs: endocrine disrupting chemicals; and PPCPs: pharmaceuticals and personal care products.
Activated sludge was used for batch test, otherwise it is indicated.

2.3.3 TrOC removal by MBR

2.3.3.1 MBR *vs.* conventional activated sludge (CAS) processes

Membrane bioreactor (MBR) is a wastewater treatment technology that combines biological activated sludge process with membrane filtration (microfiltration and ultrafiltration). In comparison with conventional activated sludge (CAS) processes, MBRs possess a number of advantages including high quality product, low sludge production, independent control of sludge retention time (SRT) and hydraulic retention time (HRT), and smaller footprint (Hai et al., 2014b).

Long SRT and high biomass concentration in MBR can promote the development of slow growing microorganism and increase biodiversity of microbial community. It potentially induces a broad range of enzymatic profile and variety of metabolic pathway, so extending the range of degrading substrates including TrOCs. Low food to microorganism (F/M) ratio in MBR can force microbes to scavenge the substrates at low concentration such as TrOCs. High biomass can also enhance TrOC sorption, increasing the retention time of TrOC in bioreactor and subsequently providing more opportunity for biodegradation of TrOCs. Direct rejection of TrOC by micro- and ultrafiltration membranes is limited due to small molecular weight of TrOCs (200-300 Dalton, at least 100 times smaller than the membrane pore size) (Radjenovic et al., 2007; Sahar et al., 2011), but the development of biofilm layer on membrane surface may increase TrOC rejection.

A number of studies have been conducted to compare the removal efficiencies of TrOCs between MBR and CAS or other WWTP technologies (e.g., fixed-bed bioreactors)Error! Reference source not found.. In general, MBRs seem to be superior for elimination of TrOCs that show moderate removal by CAS (15 – 80%), such as diclofenac, sulphonamides, macrolides, trimethoprim, and some pesticides (Bernhard et al., 2006; Göbel et al., 2007; Sui et al., 2011; Zuehlke et al., 2006). MBRs may not show enhancement of removal of easily biodegradable TrOCs (Clara et al., 2005b; Hai et al., 2015; Weiss and Reemtsma, 2008). Also recalcitrant compounds (e.g. carbamazepine, EDTA, hydrochlorothiazide, sulpride) are usually not removed by any treatment technologies (Bernhard et al., 2006; Hai et al., 2015; Radjenovic et al., 2007; Sui et al., 2011). Inconsistent results regarding sorption capacity and biodegradation

rate are also notable (Fatone et al., 2011; Radjenović et al., 2009; Sahar et al., 2011). TrOC removal by WWTPs depends on their intrinsic physical-chemical properties (see Section **2.3.1**) as well as operational conditions. More studies are required to characterize the advantage of MBRs and to optimize their performance as a barrier for TrOC elimination for reuse of treated wastewater.

2.3.3.2 Impact of operation parameters

Sludge retention time (SRT) is one of the most important parameters for WWTP design. SRT relates to the development of microbial community within the bioreactor. A number of studies have focused on the impact of SRT on TrOC removal. Most of these studies found that longer SRT can improve the removal efficiencies of some TrOCs that have slow biodegradation kinetic as classified in Joss et al. (2006), such as diclofenac, ketoprofen, gemfibrozil, erythromycin, trimethoprim, clofibric acid, and 17β-ethinylestradiol (Göbel et al., 2007; Kimura et al., 2007; Suarez et al., 2012; Tambosi et al., 2010). However, no enhancement in removal due to increase in SRT was found for highly degradable TrOCs, such as natural estrogens, acetaminophen, caffeine, and ibuprofen (Maeng et al., 2013). However, some inconsistent findings were also noted. For example, Joss et al. (2005) did not see difference in transformation and sorption of tested compounds (including diclofenanc, ibuprofen, and sulfamethoxazole) for sludge ages between 10 and 60 – 80 d. The variation of SRT (26 – 102 d) did not significantly impact removal of benzotriales, naphthalene disulfonate isomers, naphthalene monosulfonates and benzothiazole-2-sulfonate (Weiss and Reemtsma, 2008). Clara et al. (2005a) found that an SRT of 10 d was a critical value for the removal of bisphenol A, ibuprofen, bezafibrate and the natural estrogens. On the other hand, an SRT of 20 d was reported to be optimal for removal of both estrogen (including synthetic estrogen: 17α-ethinylestradiol) and nutrient removal (Zeng et al., 2013).

No significant effect was found for variation in HRT (5 – 14 h) on the removal efficiencies of benzotriales, naphthalene disulfonate isomers, naphthalene monosulfonates and benzothiazole-2-sulfonate (Huang et al., 2008; Weiss and Reemtsma, 2008). Fernandez-Fontaina et al. (2012) indicated that biodegradation efficiency of TrOCs with slow/intermediate kinetics such as fluoxetine or some antibiotics were reduced at shorter HRTs or increased loading rates. An evidence for a direct impact of HRT on biodegradation of TrOCs is apparently lacking.

Temperature affects microbial growth and activity, solubility and physicochemical properties of organic compounds and hence also the sludge properties. Warmer temperature in spring/summer (18-23^{o}C) resulted in better biodegradation rate of moderate/hardly biodegradable TrOCs such as antibiotics compared to lower winter/autumn temperature (14-18^{o}C) (Suarez et al., 2012). Reduction of 6 ^{o}C in temperature (from 18 to 12^{o}C) did not affect removal of free estrogens, but decreased the removal (from 78% to 59%) of sulfate conjugate of estrone (Koh et al., 2009). Operation of MBR under significantly high temperature (45^{o}C) deteriorated TrOC removal (Hai et al., 2011b). MBR is a compact system, so seasonal variation in temperature in areas of harsh weather may exert a significant impact on its performance.

Mixed liquor pH in bioreactor may not only affect bacterial communities but also TrOC speciation. Urase et al. (2005) found a higher removal of acidic pharmaceuticals (clofibric acid, fenoprofen, naproxen, indomethacin, gemfibrozil, ibuprofen, ketoprofen, and diclofenac) under acidic pH condition (pH of 4.3 - 5). With the pKa of these compounds ranging from 4.15 – 7.3, they were ionized under neutral pH condition. However, they were not ionized under acidic pH condition and thus their hydrophobicity and consequently adsorption to sludge particles increased. A similar observation was reported by Tadkaew et al. (2010). By contrast, Gulde et al. (2014) studied the influence of pH (6, 7 and 8) on the biotransformation of TrOCs with cationic-neutral speciation (pKa of 6.9 -10). They suggested that a pH-dependent removal of polar and ionisable TrOCs in activated sludge is less likely an effect of pH-dependent sorption but rather of pH-dependent biotransformation. It was explained by the qualitative correlation of biodegradation rate constant (k_{biol}) with the neutral fraction of the ionisable TrOCs: the charged compounds are inhibited from permeating through the cell membranes and hence the uptake into the cell is dominated by the neutral species.

2.4 NUTRIENT REMOVAL BY ANOXIC-AEROBIC MEMBRANE BIOREACTOR CONFIGURATION

Along with the removal of bulk organics, removal of nutrients is important to prevent eutrophication in receiving water bodies that causes long-term damage to ecosystems (Smith, 2003). The level of nitrogen and phosphorus in WWTP effluent has been strictly regulated in many countries. For example, European Union guideline proposes

TN < 10 mg/L and TP < 1 mg/L, and North American guideline stipulates TN <1.5-3 mg/L and TP < 0.07 mg/L (Oleszkiewicz and Barnard, 2006). Nutrient removal requires a combination of redox conditions (anoxic and/or anaerobic with aerobic conditions). The biological nutrient removal (BNR) process is applied for removal of nitrogen via the nitrification/denitrification pathways. The removal of phosphorus is a complex process involving the release and subsequent uptake of orthophosphate by phosphorus accumulating microorganisms when subjected to alternating anaerobic and aerobic conditions (Water Environment Federation, 2012).

The anoxic-aerobic configuration is one of the most common process among the BNR technologies. The advantages of this configuration are the ease to retrofit to the existing biological treatment plant, the production of alkalinity before the nitrification step (Metcalf and Eddy, 2003). Compared to other BNRs, this process also optimally utilizes the carbonaceous source in wastewater for heterotrophic denitrifiers (Judd, 2011).

The anoxic-aerobic MBRs have been demonstrated as an efficient process for organic matter and nitrogen removal (Water Environment Federation, 2012). Up to complete nitrogen removal and more than 90% phosphorus removal can be achieved by anoxic-aerobic MBRs (Fu et al., 2009; Shen et al., 2009).

However, the nutrient removal efficiency of anoxic-aerobic MBRs can be significantly impacted by the operation parameters. Firstly, TN removal can be enhanced under long SRTs applied in MBR since this can facilitate the development of slow-growing bacteria such as nitrifiers, and increase biomass concentration. Long SRT also lead to reduction of sludge production and low F/M ratio. However, prolonged SRT can cause negative impacts such as reduced bacterial activity, increased endogenous decay rates, reduced oxygen and substrate transfers, or increased membrane fouling (Ersu et al., 2010; Han et al., 2005; Li et al., 2010). Prolonged SRT may also limit phosphate removal due to low sludge wasting rate. It is critical to determine an optimal SRT that can draw a compromise between these positive and negative impacts. Secondly, high biomass concentration in MBRs may facilitate enrichment of specific microorganisms and increase its diversity that may enhance biodegradation of contaminants (Poyatos et al., 2007). However, high biomass concentration can increase viscosity of sludge properties and hence membrane fouling (Chabaliná et al., 2012; Trussell et al., 2007). Previous studies recommended a critical MLSS of 8-10 g/L for MBR (Chabaliná et al.,

2012; Lousada-Ferreira et al., 2010; Meng et al., 2007), however, this value also depends on other parameters of process design. Nutrient removal may be more impacted than carbon removal by HRT. However, contradictory reports regarding this aspect can be noticed (Cho et al., 2005; Gao et al., 2012; Li et al., 2006; Song et al., 2009). Visvanathan et al. (2005) suggested that HRTs of longer than 12 h only shows negligible impact. However, other factors such as wastewater composition and MBR configuration can change the effect of HRT (Viero and Sant'Anna Jr, 2008).

Controlling DO to maintain aerobic condition (DO > 2 mg/L) and anoxic condition (e.g., DO < 0.5 mg/L) is essential for achieving successful nutrient removal (Hocaoglu et al., 2011; Sarioglu et al., 2009). Furthermore, in the anoxic-aerobic MBR, anIR of sludge between aerobic and anoxic bioreactors is necessary to achieve simultaneous nitrification/denitrification. Increasing IR may lead to increase in total nitrogen (TN) removal (Xing et al., 2006). However, increasing IR can lead to excessive carryover of oxygen from the aerobic reactor to the anoxic reactor that can negatively impact denitrification. High IR also requires higher pumping and aeration energy (Kim et al., 2010). Other factors such as temperature and mixed liquor pH are important parameters for successful biological nutrient removal (Baldwin and Campbell, 2001; Zhang et al., 2006).

2.5 TrOC REMOVAL BY ANOXIC-AEROBIC MEMBRANE BIOREACTOR CONFIGURATION

Along with the nutrients, the removal of emerging TrOCs is important for prevention of aquatic toxicity and enable safe water reuse. The anoxic-aerobic MBR possesses characteristics that are potentially beneficial for TrOC removal. This system is specifically designed for nitrogen removal and recent studies have demonstrated a close relationship between nitrogen and TrOC removal (Fernandez-Fontaina et al., 2012; Helbling et al., 2012). For example, mineralization of some TrOCs such as DEET was found to occur only in the presence of nitrogen (Rivera-Cancel et al., 2007), while the biodegradation of the recalcitrant TrOC diclofenac has been demonstrated to occur only under stable nitrifying conditions (Suarez et al., 2010b; Vieno and Sillanpää, 2014). The better performance of TrOC removal by aerobic nitrifying bioreactor was highlighted in some studies (Dorival-García et al., 2013a; Suarez et al., 2010b). The close relationships between stable NH_4^+-N removal and the removal of a number of TrOCs were reported

(Fernandez-Fontaina et al., 2014; Helbling et al., 2012; Maeng et al., 2013; Vader et al., 2000).

The combination of anoxic and aerobic bioreactors creates multiple micro-niches of different redox conditions (i.e., different DO levels). Better biodegradation of recalcitrant TrOCs under low DO condition or under alternate exposure to different redox conditions can be noted (Stadler et al., 2015; Stasinakis et al., 2009). For example, carbamazepine and sulfamethoxazole underwent better biodegradation under a range of redox conditions, particularly at low DO (Hai et al., 2011a; Stadler et al., 2015). The combination of aerobic and anoxic conditions was found to favour the biodegradation of diclofenac (Vieno and Sillanpää, 2014; Zwiener and Frimmel, 2003). Stasinakis et al. (2009) illustrated an excellent removal of diuron under the combination of different redox conditions. However, most of these studies involved only batch tests. Several studies have investigated TrOC removal performance of this combined process (**Table 2.5**). However, it is not clear whether, like TN, a combination of a number of aerobic and anoxic zones with different levels of DO may be conducive to removal of different TrOC categories.

Table 2.5: Performance of MBR systems combining anoxic and aerobic regimes

WWTPs	TrOCs	Removal performance	References
Pilot-scale A/An/O-MBR	Estrogens	Over 90% removal of estrogens. Removal of E1 and EE2: depends on the redox conditions. EE2 degradation correlated with the nitrifying activity. Reduction of E1to E2: maximum under aerobic conditions.	(Joss et al., 2004a)
Pilot-sale An/O-MBR	Eight pharmaceuticals, two polycyclic musk fragrances and nine EDCs	Carbamazepine was not removed. Bisphenol A, ibuprofen, and bezafibrate : > 90% removal. SRTs suitable for nitrogen removal (SRT > 10 d at 10^{o}C) increased the removal of selected TrOCs. No rejection of TrOCs due to size exclusion by ultrafiltration membrane.	(Clara et al., 2005b)
Pilot-scale A/An/O-MBR	Polar compounds (phenazone-type pharmaceuticals, their metabolites, and carbamazepine) and less polar steroids.	60-70% removal for phenazone, propyphenazone, and formylaminoantipyrine; 95-99% removal for estradiol, estrone and ethinylestradiol	(Zuehlke et al., 2006)
Pilot-scale A/An/O-MBR	Sulphonamides, macrolides and trimethoprim	80% remvoal of sulfamethoxazolel and up to 50% elimination of macrolides and trimethoprim. Up to 90% elimination of trimethoprim, clarithromycin and dehydro-erythromycin at longer SRT (60-80 d *vs.* 16 and 33 d).	(Göbel et al., 2007)
Lab- and pilot-scale An/O-MBR	EDCs	Removal of estrogens: 80-91% of estrone, 49-67% of 17β-estradiol. Alkylphenols: 69-90% of bisphenol A; negative values of 4-nonylphenol.	(Hu et al., 2007)
Lab-scale (alternating A/An)/O-MBR	Bisphenol A and 2,4-dicholorophenol	Removal of COD, TN, TP: > 99%, 74 ± 5% and 79 ± 7%, respectively. Removal of bisphenol A and 2,4-dicholorophenol: 74% and 78%, respectively. Biodegradation was major removal mechanism (>80% of removal).	(Kim et al., 2009)
Full-scale A/An/O-MBR	19 TrOCs including EDCs and PPCPs	Removal: EDCs > 70%; most of PPCPs: 50-100%; carbamazepine, diclofenac and sulpiride < 20%.	(Xue et al., 2010)

		Removal mechanism: Adsorption (significant for hydrophobic chemicals, high under anaerobic) and biodegradation (rate-determining step and positively related to DO level).	
Full-scale CAS – UF for nutrient removal. Pilot-scale A/An/O-MBR	Macrolide, sulphonamide and trimethoprim	~ 20% higher removal of antibiotic by MBR than CAS-UF. Membrane separation may contribute to sorption of antibiotic to biomass and/or membrane biofilm rather than improving biodegradation.	(Sahar et al., 2011)
Full-scale A/An/O-MBR	12 PPCPs	High (up to 100%) and stable removal for easily biodegradable compounds, even during winter season. Moderate removal for diclofenac, trimethoprim, metoprolol, and gemfibrozil. Recalcitrant PPCPs (e.g. carbamazepine and sulpiride) were not eliminated. Oxic tank: the most significant contribution. Membrane filtration: a negligible role to PPCP elimination.	(Sui et al., 2011)
Full-scale An/A/O-MBR	14 EDCs including progestogens, androgens, estrogens, and phenols	Removal of EDCs: major role of secondary treatment. High removal for steroids (>88%), but 70 -90% for phenolic EDCs (except 4-cumylphenol with only ~23%).	(Huang et al., 2014)

Notes: A/An/O stand for anaerobic/anoxic/oxic; UF: ultrafiltration; EDCs: endocrine disrupting chemicals; PPCPs: pharmaceuticals and personal care products.

2.6 BACTERIAL COMMUNITY IN ACTIVATED SLDUGE

Within activated sludge in biological WWTPs, bacterial community are the most predominant and play a major role for biodegradation of wastewater contaminants (Silva et al., 2012; Yu and Zhang, 2012). Changes in WWTP processes including configuration and operational parameters can influence bacterial community structure, their growth rate and enzymatic profiles, and thus the removal of wastewater contaminants. Therefore, understanding the bacterial community and their development during WWTPs is key to solving problems and improving the efficiency of WWTPs.

2.6.1 Bacterial communities in WWTPs

Table 2.6 presents the frequently detected bacterial phyla in various scales of WWTPs including MBR from recent representative studies on bacterial community using advanced molecular technologies such as metagenomics. The most dominant bacterial phylum in WWTPs is *Proteobacteria*, particularly *β-Proteobacteria*, followed by *Bacteroidetes*, *Actinobacteria*, and *Fermicutes*. Many other minor phyla have been also regularly detected, such as *OP10, TM7, Spirochaetes, Gemmatimonadetes, Chlamydiae, WS3, Synergistetes, Cyanobacteria, Deferribacteres,* and *Tenericutes* (Hu et al., 2012; Yu and Zhang, 2012; Zhang et al., 2011).

Table 2.6*:* Summary of microbial diversity in biological wastewater treatment systems (values in parentheses have been described in notes placed at the end of the table)

WWTPs		Bacterial phyla (%)																	Note
		Proteobacteria					*Bacteroidetes*	*Acidobacteria*	*Firmicutes*	*Actinobacteria*	*Nitrospira*	*Verucomicrobia*	*Plantomycetes*	*Chloroflexi*	*Fusobacteria*	*OP10*	*TM 7*	*Unaffiliated*	
		α	β	γ	δ	ε													
Lab-scale EBPR		1.2-2.4	34-54	4.3-19.3	0-0.7	0-0.3	1.1-2.1	N.D	1.3-2.1	1.1-2.8	N.D	N.C	N.D	N.D	N.D	N.D	N.D	5.9-11	[1]
Full-scale WWTP		23	38.5	N.D	N.D	N.D	~3	9.6	N.D	N.C	N.D	N.D	N.D	N.C	N.D	N.D	N.D	9.6	[2]
Full-scale municipal plant		9.1	41	8.2	4.2	0.7	15.4	1.4	N.D	4.9	0.7	2.1	N.D	0.7	0.7	N.D	1.4	N.C	[3]
Full-scale IFAS		3.9 (7.4)	59.3 (9.9)	8.1 (9.2)	3.7 (0.6)	~0.2 (N.D)	5.2 (11.6)	~1.3 (~0.9)	~0.48 (13.6)	3.2 (14.6)	N.D	~1.3 (~0.2)	~0.5 (~1.3)	2.0 (~1.1)	N.D	N.D	N.D	~6.3 (~25)	[4]
Swine WWTP		3.4	24.1	3.4	N.D	N.D	24.1	N.D	3.4	N.D	N.D	N.D	N.D	20.7	N.D	N.D	N.D	17.2	[5]
Anoxic bioreactor		21.3 (17.7)	25 (16.9)	14 (10)	1 (1.5)	N.D	33.1 (37.7)	0.3 (0)	1.6 (0)	18.4 (24.6)	1.3 (0)	0 (2.3)	N.D	0 (5.4)	N.D	N.D	2.4 (6.2)	0 (1.5)	[6]
Full-scale EBPR		44					37	1	3	3	0.2	1	N.D	3	N.D	N.D	N.D	1	[7]
Lab- and pilot-scale MBRs		66 (91.5)					7 (1.2)	N.C	N.C	5.5 (2.8)	N.D	1.3 (1.2)	4.9 (2.8)	N.C (0.4)	N.C	N.D	N.D	N.C	[8]
		(21)	(44.4)	(19.8)	(N.D)	(N.D)													
12 WWTPs	MBR	7.5-42	25-34	12-39	4-27	<1	8.5-55	0-22	1-3	<1	<1	<1	1-6	1-1.1	<1	<1	<1	5-24	[9]
	OD	20-37	28-53	9-25	5-13	<1	6-37	3-10	2-3	1	<1	1-9	1-6	1-14	<1	<1	<1	15-28	
	A/O	7-33	26-43	19-32	5-22	~1	9-38	2-7	2-4	~1	<1	5-12	2-7	3-8	~1	<1	<1	9-18	
Full-scale WWTP		22.35 (49.46)					5.72 (8.06)	<1	3.22 (3.04)	15.03 (6.04)	0.83 (0.91)	0.53 (3.03)	<1	<1	<1	N.D	N.D	N.D	[10]

N.D: not detected; N.C: not calculated; OD: oxidation ditch; IFAS: integrated fixed-film activated sludge system; EBPR: enhanced biological phosphorus removal; values in parentheses (see notes below)

Note for **Table 2.6**

[1]: Classification was based on contigs and scaffolds containing sequences from shot-gun sequencing of EBPR sludges (obtained from USA and Australia) (Martín et al., 2006; McHardy et al., 2007).

[2]Anoxic and aerated basins of a modified Ludzack-Ettinger process receiving pretreated wastewater from an oil refinery industry. Only using rRNA cycle approach (Figuerola and Erijman, 2007).

[3] Taxonomic classification used only 16S rRNA genes from whole metagenomic sequencing (Sanapareddy et al., 2009).

[4] This study relied on pyrosequences of V1-V5 regions of 16S rRNA gene. Bacterial community in suspended samples (without parentheses) and in attached sludge (in parentheses). Other minor phyla were also detected (Kwon et al., 2010).

[5] Metagenomic library approach (Liaw et al., 2010)

[6] Anoxic quinoline-degrading bioreactor. Classified result based on pyrosequencing of V3 region (without parentheses) and cloning approach (numbers in parentheses) (Zhang et al., 2011).

[7] Whole metagenomic sequencing (Albertsen et al., 2012)

[8] Whole metagenomic sequencing, numbers in the parenthesis is percentages by using only 16S rRNA gene (Silva et al., 2012).

[9]Pyrosequences of V4 region of 16S rRNA, confidence threshold 50% of RDP classifier. MBR, OD and A/O: Chlamydiae (<1%), Gemmatimonadetes (<1%), OD1 (<1%), Spirochaetes, WS3, Synergistetes (<1%) and other minor phylum (Hu et al., 2012).

[10]Percentage was calculated from total sequences of three domains (i.e., Archaea, Bacteria, and Eukaryota), viruses as well unassigned sequences using MG-RAST. In parentheses is the percentage of taxonomies based on SSU rRNA reads. Taxonomic results were inferred from both metagenomic and metatranscriptomic data; Cyanobacteria, Deferribacteres, Gemmatimonadetes, Spirochaetes, Tenericutes (Yu and Zhang, 2012)

2.6.2 Functional bacterial groups

2.6.2.1 Bacteria associated with nitrogen removal

The excessive release of ammonia into aquatic system can lead to eutrophication and lack of dissolved oxygen in receiving water bodies. Ammonia and its oxidation products (e.g. nitrite and nitrate) are toxic to aquatic organisms. Therefore, the removal of nitrogen is a critical step in WWTPs in order to protect aquatic environment and human health. **Figure 2.2** illustrates the biogeochemical nitrogen cycle which is governed by microbial catalysis. Ammonia/ammonium is released from organic matter via ammonification processes. In WWTPs, nitrogen removal can be catalysed mainly via nitrification/denitrification pathways and/or anammox (anaerobic ammonium oxidation) pathways. A minor part of nitrogen is removed via assimilation for the growth of microorganisms (Nielsen and Seviour, 2010).

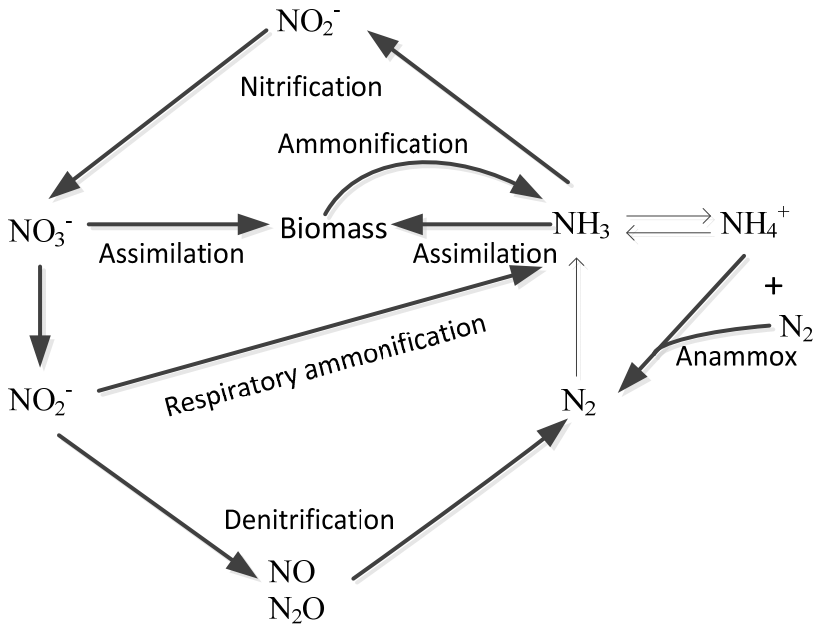

Figure 2.2: Biogeochemical nitrogen cycle. Processes that are relevant to nitrogen removal in wastewater treatment plants are highlighted by bold arrows (Lücker, 2010).

In nitrification/denitrification pathways, autotrophic nitrification is the stepwise oxidation of ammonia to nitrate catalysed by two different functional groups of bacteria (**Figure 2.3**). Under aerobic condition, chemolithotrophic ammonia-oxidizing bacteria (AOB) and archaea-oxidizing bacteria (AOA) facilitate the oxidation of ammonia via hydroxylamine to nitrite (You et al., 2009). It is further oxidized to nitrate by nitrite-

oxidizing bacteria (NOB). To date, all known AOB have been assigned to the *β-proteobacteria* (genera: *Nitrosomonas, Nitrosolobus, Nitrosospira* and *Nitrosovibrio*) and *γ-Proteobacteria* (genus *Nitrosococcus*) (Lücker, 2010; Purkhold et al., 2000). The recent discovery of AOA, *Candidatus* "Nitrosopumilus maritimus", was classified to the phylum *Thaumarchaeota* (Spang et al., 2010). However, the roles of AOA during WWTPs remain unclear.

Chemolithoautotrophic NOB are phylogenetically heterogeneous, belonging to five different phyla: *α-Proteobacteria* (*Nitrobacter*), *γ-Proteobacteria* (*Nitrococcus*), *δ-Proteobacteria* (*Nitrospina*), *β-Proteobacteria* (*Candidatus "Nitrotoga arctica"*), and *Nitrospirae* (*Nitrospira*) (Daims et al., 2001; Lücker et al., 2010). Bacteria affiliated with the genus *Nitrobacter* have the most culture representatives and are the best studied NOB (Starkenburg et al., 2008). *Nitrospira*-like bacteria have been shown to be the main NOB in WWTPs. They have been subdivided into phylogenetic sublineages I-IV (Daims et al., 2001; Juretschko et al., 2002).

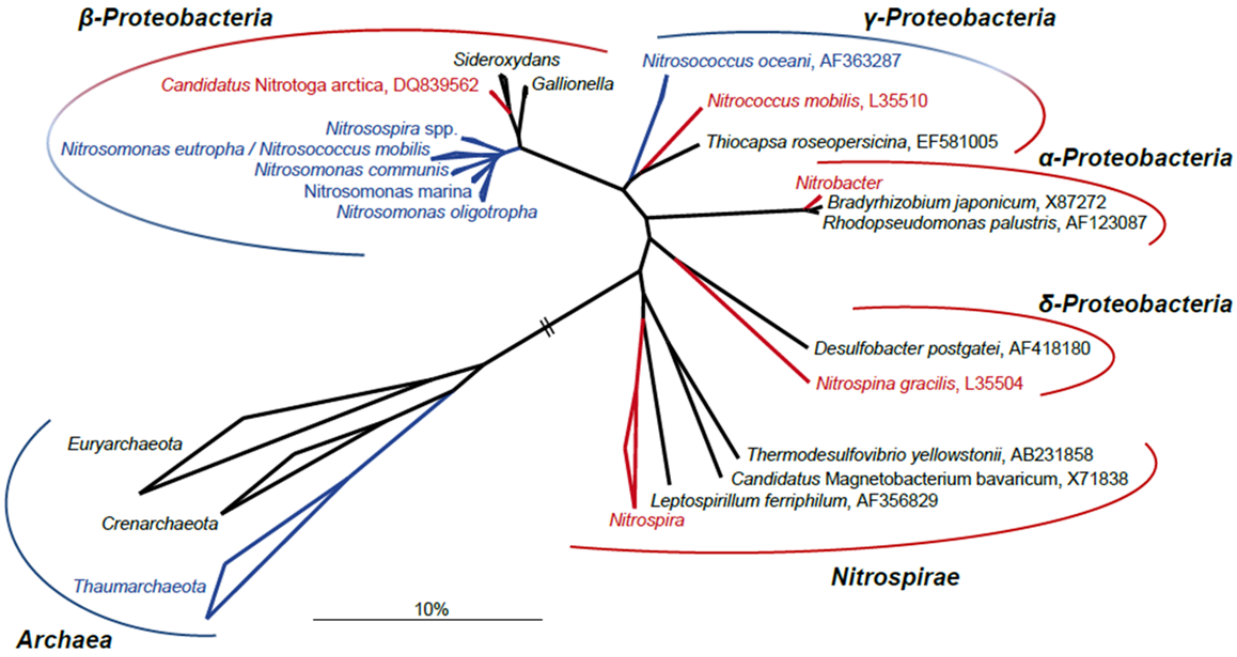

Figure 2.3: 16S rRNA gene-based phylogenetic tree presenting the affiliation of nitrifiers (Blue text: ammonia-oxidizing archea and bacteria ; Red text: nitrite-oxidizing bacteria (Adapted from Lücker (2010)).

Denitrification is a four-step process by which metalloenzymes sequentially reduce nitrate to nitrite, nitric oxide, nitrous oxide and finally nitrogen gas. Denitrification mostly occurs under anaerobic condition. Denitrifying capacity is distributed over many

"prokaryotic" lineages, which contain also non-denitrifying microbes. The link between the denitrifying activity and the phylogenetic affiliation of an organism is limited and hence studying denitrifiers in WWTPs is challenging (Wagner and Loy, 2002). Current studies using cultivation-independent methods (e.g.,16 rRNA gene analysis and FISH-MAR) can provide useful information regarding denitrifying-bacteria in WWTPs. Most of them belong to *β-Proteobacteria* (*Curvibacter, Azoarcus, Thauera, Zoogloea,* and *Accumulibacter*) (Nielsen and Seviour, 2010). In addition to the *β-Proteobacteria* commonly associated with denitrification, Morgan-Sagastume et al. (2008) described a heterogeneous denitrifying community including *α-Proteobacteria, γ-Proteobacteria* and *Actinobacteria*.

Anammox bacteria can oxidize ammonium to nitrogen gas using nitrite as electron acceptor under anaerobic condition. This bacterial group is a member of a deep-branching lineage in the bacterial phylum *Planctomycetes* (Strous et al., 1999). Phylogenetic analysis of the retrieved 16S rDNA sequences reveals diversity in the *Planctomycetales* (**Figure 2.3**), of which *Brocadia* and *Kuenenia* species are the most commonly found organisms in the enrichments from WWTPs and large-scale Anammox reactors (Jetten et al., 2001; Kuenen, 2008).

2.6.2.2 Bacteria associated with phosphorus removal

Phosphorus is an essential growth element of organisms. However, similar to nitrogen, occurrence of phosphorus in excessive concentration can result in eutrophication and hypoxia in receiving water bodies. Enhanced biological phosphorus removal (EBPR) is a specialized process designed for phosphorus removal from wastewater. This process takes the advantage of functional bacteria called polyphosphate accumulating organisms (PAOs) that favourably grow under cyclic anaerobic and aerobic conditions. During the initial anaerobic phase, PAOs degrade stored polyphosphate and glycogen, and synthesize polyhyroxyalkanoaes (PHAs) from short chain volatile fatty acids (VFAs). In the subsequent aerobic stage, PAOs store polyphosphate and glycogen, and degrade PHAs (Wilmes et al., 2008).

The *Candidatus* '*Accumulibacter phosphatis*' (*Rhodocyclales, β-Proteobacteria*) have been identified as the most dominant PAO in EBPR (Albertsen et al., 2012; Kong et al., 2004; Martín et al., 2006). Metagenomic and metaproteomic analysis provides insight

into EBPR metabolic pathways including carbon and phosphorus metabolism as well as other metabolic capabilities of *Accumulibacter* (Martín et al., 2006; Wilmes et al., 2008). Studies also suggest the existence of putative PAOs belonging to *Tetrasphaera* (*Actinobacteria*) (Kong et al., 2005) or *Dechloromonas* (*β-Proteobacteria*) (Kong et al., 2007). Other community members in EBPR include glycogen accumulating (non-polyphosphate) organisms (GAOs) that compete with the PAO for organic carbon substrates and can grow under identical operating conditions (anaerobic followed by aerobic/anoxic conditions) without contributing to phosphorus removal (Nielsen and Seviour, 2010).

2.6.3 Method to study microbial community

Advances in molecular biology have led to the development of many culture-independent methods to study microbial community in environmental samples including activated sludge (**Table 2.7**). Next-generation sequencing and bioinformatics have facilitated the development of "omics" era: metagenomics, metatranscriptomics, metaproteomics. Metagenomics is a powerful approach to studying composition, diversity and potential functions of microbial community. It also provides reference dataset for the application of the other "omic" approaches (metatranscriptomics and metaproteomics). Metagenomics is a versatile approach (**Figure 2.4**) that can be chosen depending on the aims and the budget of the project. Metagenomics have been applied to study microbial community in activated sludge in a number of studies (**Table 2.6**). Comparing the results of these studies with previous studies using other methods (e.g., finger printing methods, cloning, and FISH) (Wagner and Loy, 2002), the dominant bacterial phyla are quite consistent. However, metagenomics have been shown to be more sensitive for studying bacterial diversity than cloning library or finger printing techniques. Many minor phyla were only detected by metagenomics analysis: *OP10, TM7, Spirochaetes, Gemmatimonadetes, Chlamydiae, WS3, Synergistetes, Cyanobacteria, Deferribacteres,* and *Tenericutes* (Hu et al., 2012; Yu and Zhang, 2012; Zhang et al., 2011). However, some phyla have not detected (*Fibrobacteres*) or rarely retrieved (*Chlorobi*) in metagenomics results. There are several explanations for this. Firstly, it is known that bacterial community can change depending on the substrate composition in wastewater (Silva et al., 2012), different stages of treatment processes or supporting environments (attached sludge, suspended sludge or biocake layer) (Kwon et

al., 2010; Lim et al., 2012). Secondly, taxonomic binning was done by different bioinformatic pipelines (RDP, Greengene, MEGAN or MG-RAST) that apply different classified algorithms. Finally, as a result of metagenomics sequencing projects, more sequences were submitted to public database, so it changed the reference data and then may have affected taxonomic assignment.

There are emerging challenges with metagenomics application. The accumulation of metagenomic data, particularly16S rRNA gene sequences, in public databases is vastly outpacing the current ability to classify these sequences and this problem becomes more severe as one moves from the phylum level to genus level (Kwon et al., 2010; Sanapareddy et al., 2009; Silva et al., 2012). This problem can be solved with the improvement of sequencing technology in increasing the read lengths. More, bioinformatics for working with large dataset and optimizing assembly/binning methods will significantly improve the achievement of metagenomics study. Besides, metagenomics only provide the potential function of microbial community. Therefore, the combination with metatranscriptomics, metaproteomics or stable isotope probing may provide a comprehensive understanding of the activity of bacterial community in activated sludge during WWTP operation.

Table 2.7: Methods available for studying microbial community in activated sludge

Methods	Applications	Advantages	Limitations	References
Culture-based methods	To identify and characterize bacteria	Possible to identify bacteria to the level of species or strains. Useful for taxonomical, physiological, and genetic studies.	Time consuming. Inadequate for studying microbial communities since only a small fraction (0.1 to 10%) of environmental microbes can be cultivated.	(Kämpfer et al., 1996)
Genetic finger printing techniques	Using DNA trains to study structure and dynamic of bacterial community	Culture-independent analysis of a wide range of microorganisms	Bias from DNA extraction and PCR; low sensitivity.	(Amer, 2000; Muyzer and Smalla, 1998; Osborn et al., 2000)
Clone library	For investigating phylogenetic diversity in activated sludge	Can clarify biodiversity of community members. Identification of uncultivable bacteria and elucidation of their gene functions. Provides DNA sequences for probing/primer designs.	Expensive; time consuming; labour-extensive; high skill requirement.	(Gao et al., 2013; Juretschko et al., 2002; Silva et al., 2012)
Quantitative real-time PCR	Quantitative monitoring of known microorganisms having a role in a community.	Fast, sensitive, accuracy and automation.	Do not link gene expression with specific microbial activity. Only applicable to known bacterial sequences. PCR-bias.	(Geets et al., 2007; Harms et al., 2003)
Fluorescent In Situ Hybridization (FISH)	To quantify the presence and relative abundance of bacterial population in activated sludge	Fast *in situ* visualization; quantification.	Limits in number of probes. Require a prior knowledge of sample and microorganisms. Testing new probes is complex and time consuming.	(Amann and Fuchs, 2008; Manz et al., 1996; Wagner and Haider, 2012)
FISH-MAR (Microautoradiography)	To describe the functional properties of microorganisms in activated sludge	Link identity of individual bacterial populations to their *in situ* physiology.	High skill requirement, time consuming; expensive; limit of availability of suitable radiolabelled substrates.	(Kong et al., 2004; Lee et al., 1999)

Stable Isotope Probing (SIP)	To track migration of selected substrates into cellular components (biomarkers: DNA, RNA, protein or fatty acids), and then providing phylogenetic information by analysis of biomarkers.	Direct understanding functionality of bacterial community for a specific substrate in activated sludge	High skill requirement, time consuming; expensive; problems of cross feeding.	(Dumont and Murrell, 2005; Ginige et al., 2005; Jehmlich et al., 2008)
Microarrays	To study community structure and function.	High-throughput analysis; taxonomic resolution up to species/strain level; screening larger number of samples	Expensive and time consuming to design and build; require prior knowledge of sequences; DNA/RNA extraction bias; problems with labelling and hybridization with complexity of environmental samples.	(Inoue et al., 2014; Loy et al., 2005; Short et al., 2013; Sun et al., 2014)
Metagenomics	To study composition and diversity of bacterial community; to provide information on potential function of bacterial population.	High-throughput analysis; fast and versatile approach	Need to increase read lengths and sequence assembly/binning methods.	(Albertsen et al., 2012; Martín et al., 2006; Ye et al., 2012; Zhang et al., 2012)
Metatranscriptomics	To measure *in situ* gene expression and also provide taxonomic profile	Focusing on members that are metabolically active; quantification of expression levels if reference genomes or metagenomes available	Challenges with RNA extraction and its instability; bias related to cDNA synthesis and amplification.	(Yu and Zhang, 2012; Zakrzewski et al., 2012)
Metaproteomics	To characterize the protein profile of a microbial community under a given set of conditions at a specific time point.	Potentially provide entire metabolic pathways; detect novel functional proteins.	High skill requirement, no taxonomic information; need to improve protein extraction and sequencing. Currently, feasible only if a comprehensive dataset of proteins or metagenome are available.	(Wilmes and Bond, 2006; Wilmes et al., 2008)

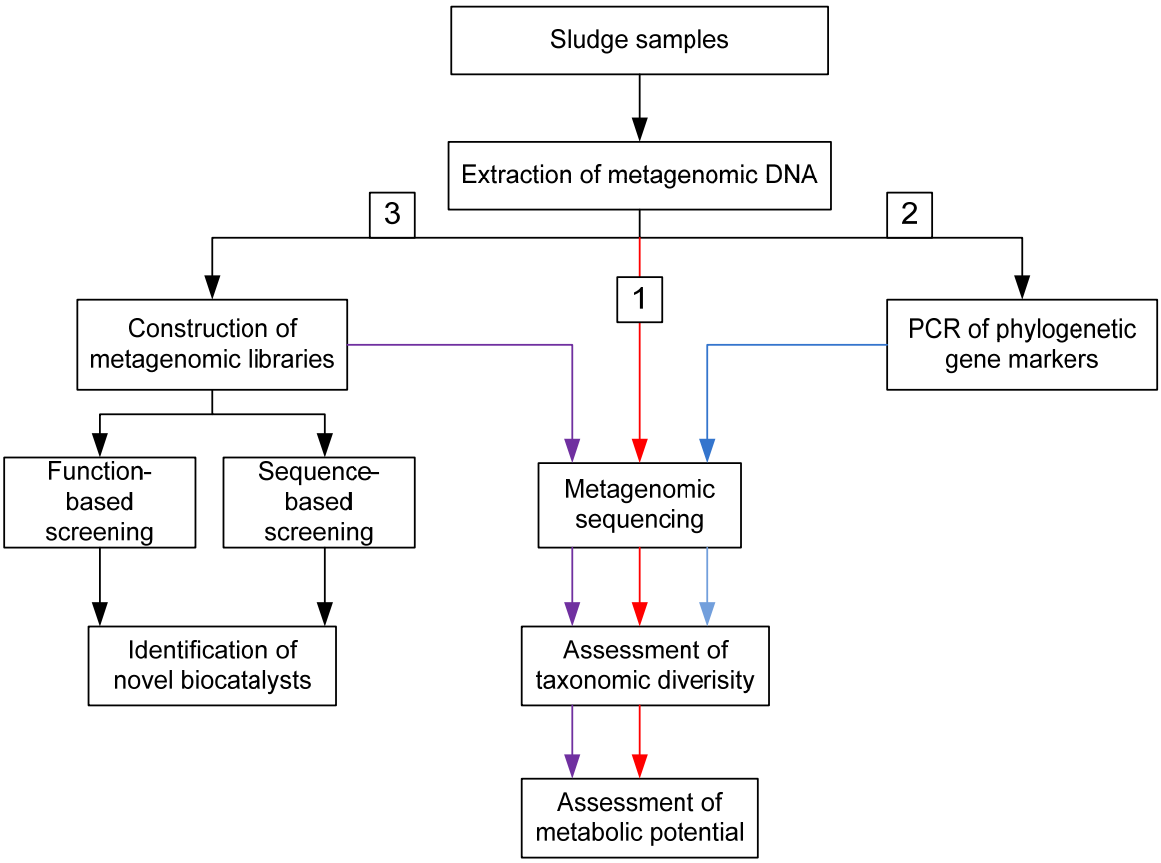

Figure 2.4: Metagenomics approaches for studying microbial community of environmental samples. Numbers (1, 2, and 3) indicate different ways of metagenomics application (Modified from Simon and Daniel (2011)). This study employed the second approach using 16S rRNA gene as phylogenetic markers to profile the bacterial communities in the anoxic-aerobic MBR.

2.7 BACTERIAL COMMUNITY IN ANOXIC-AEROBIC MBR

The application of membrane separation technology in MBRs may exert a different selective pressure on composition and diversity of the microbial community that differs from that of CAS. The retention of bacteria by membrane can potentially enrich organisms that are otherwise washed out with effluent in CAS. MBR operates at higher SRT and MLSS concentration (and hence viscosity), and thus potentially results in bacterial competition for substrate due to low F/M ratio and low mass transfer. Accumulation of microbial products rejected by membrane filtration also increases the 'inert' fraction in MLSS that can be further hydrolysed, so changing the substrate

composition (Ng and Kim, 2007). The hydrodynamic conditions in MBR usually lead to the formation of the smaller size of flocs than that of CAS. It can change the bioavailability of substrate, interaction between organisms and distribution of bacterial species (Manser et al., 2005).

A number of studies have reported distinct bacterial community composition in sludge between MBR and CAS systems (Silva et al., 2010; Wan et al., 2011). For example, the class *β-Proteobacteria* has been usually found predominant in MBR sludge while class *α-Proteobacteria* appears to be more abundant in CAS sludge (Baek and Pagilla, 2009; Silva et al., 2010; Wan et al., 2011). Previous studies revealed that nitrification parameters seem to be significantly better in MBR, but denitrification and phosphorus kinetics are comparable in MBR and CAS (Fenu et al., 2010). Results from the study of Chiellini et al. (2013) demonstrated a higher abundance of the genus *Nitrospira* (phylum: *Nitrospirae*), the most important NOB in WWTPs, in MBR than in CAS plants. Further classification to species level showed that *Nitrospira* in MBR is more closely related to *N. moscoviensis* while *Nitrospira* in CAS belongs to Candidatus "*Nitrospira defluvii*". This difference was explained by the kinetics of NOB that suggests the presence of *K-strategists (high substrate affinities and low maximum growth rate)* in MBR, while *r-strategists(low substrate affinities and high maximum growth rate)* (Schramm et al., 1999) are selected in CAS, possibly because of the lower transient concentration of nitrite in MBR than in CAS. Hu et al. (2012) investigated the bacterial communities from WWTPs employing different treatment technologies: MBR samples showed a lower diversity and evenness of microbial community than samples from other plants. Saunders et al. (2013) found that the majority of the core bacterial groups present in conventional EBPR plants were also present in the full-scale MBR plants. Manser et al. (2005) reported that the membrane separation itself does not affect either the nitrifying community composition or the nitrification performance, however, impact on kinetic parameters are recorded. It is noted that the aforementioned studies comparing microbial communities in MBR and CAS employed different experimental setups, thus making it difficult to resolve the inconsistencies between reports from the studies. For example, Wan et al. (2011) used an MBR with an additional denitrification tank with external carbon source addition when comparing with a CAS. Silva et al. (2010) compared the communities in MBR and CAS with wastewater from petroleum refineries, while the WWTPs studied by Hu et al. (2012) received influent from

different sources. The methods used are also an important factor. For example, Saunders et al. (2013) found that some core groups in CAS EBPR plants were not present in MBR as showed by FISH results, but pyrosequencing results suggested that other groups may fill in the role of these groups. Thus a comprehensive understanding regarding the impact of selective pressures on bacterial community in MBR as compared to CAS have not been reached.

Besides the selective pressure exerted by membrane separation, the anoxic-aerobic MBR configuration can promote the growth of nitrifying and denitrifying bacteria. A few recent (Gómez-Silván et al., 2014b; Xia et al., 2012) studies regarding this type of configuration have indicated that bacterial community profiles in anoxic and aerobic zone may not be significantly different. This is thought to be due to the high recirculation rate between the anoxic/aerobic zones as required to achieve nitrification and denitrification (Gómez-Silván et al., 2014a; Xia et al., 2012). It has been also reported that AOB, NOB and denitrifies are all active in both anoxic and aerobic conditions in the anoxic-aerobic MBR system (Gómez-Silván et al., 2014b). The possible explanation for this is most of the bacteria developed in this system are facultative. Chandran et al. (2011) reported that some AOBs can use nitrite as electron acceptor under oxygen-limited conditions and/or in the presence of high nitrite). Similarly, aerobic denitrification was also recorded in WWTPs. Aerobic denitrification can be regarded as the utilization of nitrite as electron acceptor, but nitrite reduction was not coupled with ammonia oxidation. This process is possibly favoured under high presence of nitrite and frequent switching between aerobic/anaerobic condition (Frette et al., 1997). The activity of the *nitrospira*-like NOBs (which typically oxidize nitrite under aerobic condition) in anoxic conditions is possible as they are *K*-strategists that have high affinity for oxygen, allowing them to sustain respiration in low-oxygen conditions (Downing and Nerenberg, 2008). The bacterial groups for nitrogen removal were also found to respond differently with the accumulation of volatile suspended solids in sludge, BOD, NH_4^+ concentration and C/N ratio of wastewater, suggesting the complexity of the balance among them for successful nitrogen removal (Gómez-Silván et al., 2014b). Possibly, the recirculation ratio between anoxic and aerobic zones greatly impact the development of bacterial communities in the integrated anoxic-aerobic MBR. One way to assess how it impacts the mixed liquor characteristics as well as performance of the system is to observe the bacterial community changes and

corresponding removal performance when recirculation is stopped. Furthermore, it remains to be elucidated how bacterial community in this system react to the trace organic contaminants.

2.8 BACTERIA FOR TrOC REMOVAL

Although a number of bacterial strains/consortia that selectively degrade environmentally common TrOCs have been isolated from various environment samples including activated sludge (**Table 2.8**), due to the diversity of TrOCs and their significantly low concentration (ng/L to µg/L) in the environment, little is known about the functionally important populations that are responsible for TrOC degradation in environment, particularly activated sludge (**Table 2.9**). *Sphaerotilus-leptothrix* related bacteria were demonstrated to be the most important microorganisms involved in estrone degradation (at 200 µg/L) in activated sludge samples (Kurisu et al., 2015; Zang et al., 2008). Thayanukul et al. (2010) proposed that different bacterial groups are responsible for TrOC degradation at different concentrations. Boonnorat et al. (2014) reported better biodegradation of eight toxic TrOCs (at 1000 µg/L) by TrOC-acclimated sludge and the biodegradation was improved over time during MBR treatment. Both removal efficiency and biotransformation capacity potentials revealed by metagenomic analysis did not find any difference between pre- and non-exposed microbial communities to TrOCs (at 300-500 ng/L) in the study of Alidina et al. (2014b). A few other studies have reported that the accumulation of important bacterial populations in activated sludge during treatment that may relate to degradation of specific TrOC groups (Collado et al., 2013; Liu et al., 2008b; Xia et al., 2014). A number of studies have also suggested a strong relationship between TrOC degradation and nitrogen removal, particularly nitrification (Fernandez-Fontaina et al., 2014; Helbling et al., 2012; Roh et al., 2009; Yi and Harper Jr, 2007). Tran et al. (2013) proposed that both heterotrophic and autotrophic microorganisms play important roles in biodegradation of TrOCs. However, information on the microbes responsible for only a limited number of TrOCs is available. Systematic studies regarding functional bacterial groups responsible for biodegradation of specific TrOC groups in continuous flow wastewater treatment systems remain largely unexplored.

Table 2.8: Isolated bacterial strains/consortia that demonstrated degradation capacity of selected TrOCs

Sources	TrOCs	Bacteria	Biodegradation pathways	References
Activated sludge	Triclosan	*Sphingomonas* sp. PH-07	Ether bond cleavage (co-metabolism)	(Kim et al., 2011)
		Sphingopyxis strain KCY1	Via meta-cleavage pathway (co-metabolism)	(Lee et al., 2012)
Activated sludge	Ibuprofen	*Sphingomonas* sp. strain Ibu-2	Removal of the acidic side chain prior to ring cleavage (metabolism)	(Murdoch and Hay, 2005)
Pesticide-contaminated soil	Propoxur	*Pseudomonas* sp.	Hydrolysis to yield 2-isopropoxyphenol and methylamine (metabolism)	(Kamanavalli and Ninnekar, 2000)
Contaminated soil, wastewater	Chloro-s-triazine herbicides (atrazine)	*Nocardioides, Arthrobacter, Agrobacterium, Polaromonas, Sinorhizobium, Klebsiela, chelatobacter, Stenotrophomonas, Pseudaminobacter, Clavibacter, Alcaligenes, Rhizobium, Ralstonia, Pseudomonas*, and some other consortia.	Six successive hydrolysis steps: a dechlorination, two dealkylations, a ring cleavage, a biuret deamination, and an allophanate hydrolysis (cometabolism/metabolism)	(Udikoviç-Koliç et al., 2012)
Contaminated soil	Methylthio-s-triazines (simetryn, ametryn)	*Rhodococcus* sp. FJ1117 YT	Oxidation and hydrolysis of methylthio group (the sole sulfur source)	(Fujii et al., 2007)
Contaminated soil, waste	Pentachlorophenol	*Sphingobium chlorophenolica; Mycobacterium chlorophenolius; Sphingomonas*;	Initiation with *para*-hydroxylation (metabolism)	(Crawford et al., 2007)
Pulp and paper		*Acinetobacter* sp. ISTPCP-3	Ortho ring-cleavage (metabolism)	(Sharma et al.,

mill's waste				2009)
Activated sludge	17β-estradiol and other estrogens	*Sphingomonas* strain KC8	metabolism	(Hu et al., 2011; Roh and Chu, 2010; Yu et al., 2007)
		Bacillus spp.	--	(Jiang et al., 2010)
		Rhodococcus spp.	metabolism	(Yoshimoto et al., 2004)
Soil, sludge, river, sea water and food	Bisphenol A	Gram-negative bacteria (*Sphingomonas, pseudomonas, Achromobacter, cupriavidus* etc.) and gram-positive bacteria (*Bacillus, Streptomyces*)	Different proposed pathways (metabolism): via the formation of phenoniumion intermediate; via rearrangements involving phenonium ion intermediates; or via a type II *ipso* substitution mechanism.	(Zhang et al., 2013)
Environmental water samples	4-tert-butylphenol	*Sphingobium fuliginis* OMI	Meta-cleavage pathway (metabolism)	(Ogata et al., 2013)
Soil, sediment, and activated sludge	Alkylphenols	*Sphingomonads* had the highest degradation capacity. Others including *Nocardia* sp., *Achromobacter* sp., *Thauera aromatica, Desulfobacterium cetonicum, Burkholderia sp., Alcaligenes eutrophus, Bacillus stearothermophilus*, and many *Pseudomonas* species	Oxidation of the ring or the alkyl substituent(s) (metabolism)	(Corvini et al., 2006)
Soil	Benzophenone	*Paenibacillus* sp. KBC101	--	(Sakai et al., 2005)

Table 2.9: Studies on bacterial populations responsible for TrOC degradation in activated sludge

Experimental design	TrOCs	Bacterial community	References
Degradation experiments in serum bottles. Bacterial community was examined by PCR-DGGE.	4-t-octylphenol (220, 120 or 30 µg/L)	Aerobic degradation is much higher than anaerobic degradation. Adding 4-t-OP (as sole carbon source) reduced bacterial diversity. *γ-Proteobacteria* and *Bacillus* (*Firmicutes*) were dominant in sludge where 4-t-OP was added as sole carbon source.	(Liu et al., 2008a)
Activated sludge from full-scale A/A/O -CAS. FISH-MAR was used for examining estrone (E1)-degrading bacteria.	[2,4,6,7-^{3}H(N)]E1 with concentration of 200 µg/L	1-2% of the total cells in the samples contributed to E1 assimilation. Most E1-assimilating cells were associated with *β* and *γ-Proteobacteria*. No E1-assimilating cells were affiliated with *α-Proteobacteria*, *Actinobacteria*, Cytophaga-flavobacterium cluster or *Chloroflexi*, *Nitrospira* and *Planctomycetes*.	(Zang et al., 2008)
		Sphaerotilus and *Leptothrix (β-Proteobacteria)*: ~ 3% of total microbial biomass and 60-80% of E1-degrading bacteria.	(Kurisu et al., 2015)
Activated sludge samples from CAS, A/A/O, and A/O processes. FISH-MAR was used for studying E1-degrading bacteria.	2,4,6,7- ^{3}H (N) E1 (200 µg/L, 4µg/L, 1µg/L, and 540 ng/L)	At 200 µg/L E1: 60% and 40% of MAR (+) cells were *β*- or *γ-Proteobacteria*, respectively. Proportion of *β*- or *γ-Proteobacteria* decreased when E1 concentration decreased, but proportion of *α-Proteobacteria* increased. At 540 ng/L E1, 96% of [^{3}H]E1-incorporating cells were associated with *α-Proteobacteria*. At 1 µg/L E1, 50.4 ± 11% of E1-degrading bacteria were associated with *α-Proteobacteria* . Few MAR (+) cells were *Sphingomonadales*.	(Thayanukul et al., 2010)
Lab-scale bioreactors T-RFLP and clone libraries were used for	A mixture of ibuprofen, naproxen, ketoprofen, diclofenac and clofibric	No effect of adding TrOCs on ammonia removal, but higher NO_2^-- and NO_3^- - N in effluent in reactor receiving 50 µg/L TrOC.	(Kraigher and Mandic-Mulec,

studying microbial community.	acid (0, 5, 50, 200 and 500 µg/L each).	High sublineage II of *Nitrospira*-like NOB in reactors receiving 50 µg/L.	2011)
Lab-scale MBR; Gram staining and dilution count were used for bacterial study	Ametryn (1 mg/L)	Five common bacterial colony types (gram negative and positive bacilli and gram negative cocci) were identified and three of these which were bacilli were resistant to ametryn up to 5 mg/L.	(Navaratna et al., 2012)
Lab-scale SBR; PCR-DGGE was used for studying bacterial community and qPCR for quantification of resistance genes.	Sulfamethoxazole (50 µg/L)	*β-Proteobacteria* and *γ-Proteobacteria* classes were the dominant species. High cell number for *Thiotrix spp*. (*γ-Proteobacteria*). *Sphingobacteria*, *Actinobacteria*, *Chloroflexi* and *Chlorobi* were more vulnerable to the antibiotic load.	(Collado et al., 2013)
Two lab-scale MBRs. PCR-DGGE was employed for studying bacterial community.	8 TrOCs with concentration of 1000 µg/L.	>98% TrOC removal TrOC-acclimated sludge showed better TrOC removal. Biodegradation was improved over time. Phenolic and phthalate-degrading bacteria accumulated along with improved TrOC biodegradation.	(Boonnorat et al., 2014)
Lab-scale MBR. PCR-DGGE was employed for studying bacterial community	Sulfamethoxazole, norfloxacin, prednisolone, ibuprofen, and naproxen at 50 -55 µg/L.	TrOC-degrading and antibiotic-resistant microorganisms, such as *Firmicutes* sp., *Aeromonas* sp. and *Nitrospira* sp., accumulated over time in the reactor.	(Xia et al., 2014)

SBR: sequencing batch reactor. PCR-DGGE: polymerase chain reaction-denaturing gradient gel electrophoresis. FISH-MAR: fluorescent *in situ* hybridization-microautoradioactivity.

2.9 IMPACTS OF HAZARDOUS EVENTS ON THE PERFORMANCE OF WWTPs

In order to adequately evaluate the performance of MBR systems, it is critical to examine their robustness under the risk of deviations in operating conditions during what have been termed "hazardous events" (Trinh, 2013). Hazardous events may include sudden changes in source water composition, extreme weather events, human error and mechanical malfunctions. Examples of common hazardous events are: shock load of organics, nutrient, salinity or some other toxic chemicals (e.g., bleach), feed starvation, and failures of power supply or aeration system. Resistance to such hazardous events may govern treatment reliability and level of risk regarding meeting final water quality objectives. Case studies at water supply systems in Australia, Latin America and the United Kingdom have confirmed that pump breakdown, blower malfunctions and power supply loss are likely to occur a few times a year with durations from minutes to hours (WHO, 2009, Trinh 2013). Ammonia shock may occur once to twice per year, for example, during peak holiday period for WWTPs at tourist destinations. Toxic shocks may also occur more than once per year (Trinh, 2013).

2.9.1 Organic shock loads

Sudden exposures to organic shock loads during WWTP operation may occur due to the discharge from varieties of wastewater sources with different organic compositions. For example, industrial wastewaters usually have a high organic strength with COD > 1000 mg/L, which, in extreme situations, can exceed 200 g/L (Lin et al., 2012). The intermittent discharge of extremely strong wastewater can cause a shock load to WWTPs. As listed in **Table 2.10**, a number of studies have been conducted to date to investigate the resistance of different treatment technologies namely, CAS, SBR, and MBR to organic shock loads with COD concentration ranging from 1.5 – 16 g/L using different organic substrates (glucose, molasses or distinct wastewater such as that originating from petroleum refinery). The CASs were shown to withstand shock loads of up to 1500 mg COD/L. At higher COD loadings, significant impacts were observed on COD removal, biomass concentration and characteristics as well as the microbial community (Manickam and Gaudy Jr, 1985; Saleh and Gaudy Jr, 1978; Therien and Perdrieux, 1981). MBR systems seem to be resistant to higher shock load concentrations –as high as 16 g COD /L (Al-Malack, 2007), while SBR systems were

affected even at a concentration of 500 mg COD/L (Rodríguez Mora et al., 2003). Possibly, a high and stable biomass concentration in MBRs is advantageous for resilience to organic shock loads.

Table 2.10: Studies related to the impacts of organic shock loading on WWTPs

WWTP type and specification	Experimental protocol	Impact on removal efficiency	Impact on other operational parameters	References
Lab-scale MBR: HRT of 12-15 h; SRT of 2-74 d; MLSS of 10-15 g/L.	Shock load of 1.6x, 2.4x, and 3.2x of usual concentration of glucose (usual concentration = 5000 mg COD /L).	COD removal: not significantly affected. COD removal improved with increasing MLSS.	Not reported	(Al-Malack, 2007)
Lab-scale MBR: HRT of 24 h; SRT of 30 d; MLSS of 5 g/L.	Shock load of 15.6x of usual concentration of a mixture of glucose and glutamic acid (usual concentration = 321mg COD /L).	Sharp increase of COD and DOC in permeate. Performance recovery mostly within 72 h.	Slight decrease in mixed liquor pH; increased capillary suction time and membrane fouling rate.	(Trinh et al., 2015)
Lab-scale CAS: MLSS of 2 g/L.	Shock load of 3 x usual concentration of glucose (usual concentration = 500 mg/L) over 17 d.	No significant impact.	Biomass concentration increase.	(Saleh and Gaudy Jr, 1978)
	Shock load of 6x usual concentration of glucose (usual concentration = 500 mg/L) over 15 d.	Deterioration of effluent quality (COD and suspended solids), but rapid recovery.	Mixed liquor colour change; floc size decrease; increase in filamentous bacteria and reduction in protozoan population.	
	Cyclic shock load of 3x usual concentration of glucose (12 h each) over 18 d.	No significant impact.	Slight fluctuation of biomass concentration.	
Lab-scale CAS: MLSS of 2 g/L.	Shock load of 6x usual concentration of glucose (usual concentration = 500 mg/L) over 10 d.	Reduced COD removal; recovery within 4 d.	Changed biomass compositions (protein and carbohydrate)	(Manickam and Gaudy Jr, 1985)
Lab-scale CAS: HRT of 3 h; SRT of 5-15	Shock load (0.5-1 h) of 6x, 22.4x, 32.2x, and 30.7x of	Impacts depended on shock load: not significant (480 mg TOC/L),	Changed in biomass depending on the magnitude	(Therien and

d; MLSS of 1-4 g/L.	usual concentration of glucose (usual concentration = 60 mg TOC /L).	slight (1345 mg TOC/L), and significant (1930 and 1843 mg TOC/L).	of shock load.	Perdrieux, 1981)
Lab-scale SBARs: HRT of 3 h; MLVSS of 4-5 g/L.	Shock load of 2x, 4x, 8x, and 12x of usual concentration of glucose (usual concentration = 600 mg COD /L) over three wk for each shock load.	Over 96%COD removal achieved. Higher the organic loading, higher the suspended solid in effluent.	Shock load >1200 mg COD /L caused washout of biomass and reduced granule size in the reactor using anaerobic granules.	(Thanh et al., 2009)
Lab-scale SBRs (different aeration time): HRT of 24 h; SRT of 70 d; MLVSS of 3-4 g/L.	Shock load of 2.5x of usual concentration of molasses (usual concentration = 200 mg COD /L).	Denitrification rate decreased when aeration time was increased.	Not reported	(Rodríguez Mora et al., 2003)
Lab-scale SBR: MLSS of 2 g/L; HRT of 12.8 h.	Feeding petroleum refinery wastewater with usual organic loading of 0.3 kg COD/kg MLSS d. Shock load of 2x and 3x of usual concentration over 8 and 16 h.	COD removal dropped (over 10%) by threefold shock load for a duration of 16 h. Recovery after 3 d. 70% reduction of COD removal at threefold shock load both for 16 h shock load duration.	Biomass loss with increased suspended solid in effluent.	(Mizzouri and Shaaban, 2013)
Lab-scale biofilm reactors: HRT of 6 h and 12 h; MLSS of 2 g/L.	Shock load of 1.6x, 2.3x, 2.7, and 3.8x of usual concentration of molasses (usual concentration = 500 mg COD/L) over 6 h (each load).	Effluent COD increased from 80 mg/L to 169, 169, 250 and 617 according to shock loads of 808 - 1900 mg/L. Recovery time was proportional to the magnitude of shock loads. Nitrification was impacted when organic loading ≥ 1170 mg COD /L.	Change of bacterial type (gram positive rods → gram negative oval shaped). Autotrophs likely outcompeted by heterotrophs and washed out.	(Seetha et al., 2010)

Note: SBAR: sequencing batch airlift reactor.

2.9.2 Salinity shock loads

Seawater infiltration, and discharge of industrial wastewaters with high salt concentration (petroleum refinery, textile processing, leather processing and food conservation) can lead to suddenly high salinity in the sewage system (Lin et al., 2012; Panswad and Anan, 1999). High salinity conditions could inhibit microbial growth and floc formation because it produces a high osmotic pressure on bacterial cells. Impacts of high salinity shock on activated sludge have been focused in many studies with salt concentration range of 4 – 60 g/L (**Table 2.11**). Generally, CAS and SBR systems have been reported to maintain usual performance at salinity of up to 10 g/L in influent wastewater, but significantly reduced COD removal may be encountered with increasing salt concentrations beyond 20 g/L. High salt concentration may also result in change in sludge properties and microbial population as well as cause cell lysis (Kincannon and Gaudy, 1968; Kincannon and Gaudy Jr, 1966; Ludzack and Noran, 1965; Ng et al., 2005). Panswad and Anan (1999) reported 15- 40% reduction in COD and nitrogen removal over a salt concentration of 0-30 g/L as NaCl. Phosphorus removal was observed to be highly affected under all salt concentrations tested in that study. Available studies on the effect of salinity shock in MBR systems (Reid et al., 2006b; Trinh et al., 2015; Yogalakshmi and Joseph, 2010) observed significant impact on COD removal and nitrification. High salinity can also induce change in sludge properties and, hence, increase the fouling rate in MBR. Recovery time was usually found to depend on the duration and magnitude of the salinity shock on WWTPs (**Table 2.11**).

Table 2.11: Impacts of salinity shock loads on WWTP performance.

WWTP type and specification	Experimental protocol	Impacts on removal efficiency	Impacts on operational parameters	References
Pilot-scale anoxic/Oxic-MBR: HRT of 72 h; SRT of 64 d.	Gradual increase of NaCl from 0.35 to 5 g/L.	COD removal was more impacted by salinity shock than ammonia removal.	Significantimpact on sludge properties: increased SMP and EPS concentrations; decreased membrane permeability.	(Reid et al., 2006a)
Lab-scale MBR: HRT of 8 h; MLSS of 10-15 g/L.	Shock load by 5, 10, 20, 30, 50 and 60 g/L NaCl for duration of 1 d (each).	All shock loads caused reduction of COD and nutrient removal. Poorest removal at highest NaCl (60 g/L) with complete inhibition of nitrification. Recovery time: 4-9 d.	Biomass settleability decreased with increase in NaCl concentration.	(Yogalakshmi and Joseph, 2010)
Lab-scale MBR: HRT of 24 h; SRT of 30 d; MLSS of 5 g/L.	Shock load by 20 g/L NaCl.	Significant reduction of COD removal. Recovery time: 24 h.	Increased fouling rate.	(Trinh et al., 2015)
Pilot-scale CAS: HRT of 1.5 d; infinite SRT; MLVSS of 0.3-3.7 g/L.	Mixture of seawater and synthetic wastewater: up to 20 g NaCl/ L	Temporary reduction of removal efficiencies (BOD and suspended solid).	Impact on protozoa population	(Stewart et al., 1962)
Lab-scale CAS: HRT of 8 h; MLVSS of 2 g/L.	Wastewater made up of fish-meal slurry: 20 g g/L chloride	At < 8 g/L chloride: no impact. At higher dosing: nitrification reduced significantly	Microbial population and activity changed significantly. High chlorides concentration affected respiration and caused poor sludge settling.	(Ludzack and Noran, 1965)
Lab-scale CAS: SRT of 3 d.	Shock load of 30 and 45 g/L NaCL.	30% and 75% reduction of COD removal for fresh and	Cell lysis was observed. The sludge had low carbohydrate and	(Kincannon and Gaudy Jr, 1966)

		acclimated-sludge, respectively. More severe impact at 45 g/L NaCl.	protein contents, but abnormally high lipid and RNA contents.	
Lab-scale CAS: HRT of 8 h; MLVSS of 3.2 g/L.	30 mg/L NaCl	Severe increase of effluent COD (from 120 mg/L to 320 mg/L). Reached a new steady-state after 2 d.	Significan reduction of biomass. Change of predominant species.	(Kincannon and Gaudy, 1968)
Lab-scale anaerobic/ anoxic/oxic-CAS: HRT of 2/2/12 h; SRT of 10 d; MLSS of 2.5 g/L.	Up to 70 g/L NaCl	30 g/L NaCl led to 20-40% and 15-20% reduction in COD and nitrogen removal, respectively. Poor phosphorus removal for all cases.	Significant decrease in biomass concentration	(Panswad and Anan, 1999)
Lab-scaleSBRs: MLVSS of 2.5 g/L.	Up to 60 g/L NaCl	No impact at NaCl ≤ 10 g/L. Deterioration of DOC removal (over 10% reduction) at NaCl > 20 g/L. Significant increase of effluent turbidity at NaCl > 30 g/L.	Loss of protozoa and rotifers with increasing NaCl beyond 5 g/L. Ciliates dominated at NaCl of 5 g/L, but disappeared at NaCl > 10 g/L.	(Ng et al., 2005)

2.9.3 Feed starvation

Feed starvation can occur due to disruption in the sewer network or the WWTP operation (Yogalakshmi et al., 2007). This can also happen due to large fluctuations in the flow and composition inherent to industrial wastewater. In some cases, low activity periods (annual maintenance or seasonal production variations) can lead to complete interruptions of wastewater flows to the WWTPs for weeks or even months (Yilmaz et al., 2007).

A number of studies were conducted to understand the impact of feed starvation on different wastewater treatment technologies (**Table 2.12**). Starvation period of 2-30 d has been investigated in the available studies. In general, starvation leads to the significant reduction of biomass, floc sizes and fractal dimension as well as the decrease of microbial activity (Coello Oviedo et al., 2003; Trinh, 2013; Yogalakshmi et al., 2007). A significant loss of biomass can occur within the first few days (**Table 2.12**). Feed starvation has been found to cause a decline in settleability and dewaterability of activated sludge (Horan and Shanmugan, 1986). Studies have also found a significant change in microbial population in response to feed starvation, with the disappearance of typical activated sludge microbes and occurrence of other opportunistic organisms (Coello Oviedo et al., 2003).

While several studies (Le, 2011; Trinh et al., 2015) observed that MBR was resilient to feed starvation with no significant impact on COD removal over starvation period of 2 – 6 d, other studies reported a significant reduction of organic and nutrient removal (Yogalakshmi et al., 2007). Recovery of removal performance was found to be fast after resuming normal operation, but it took more than a month to recover the loss of biomass.

Table 2.12: Impacts of feed starvation on WWTP performance

Parameter	Treatment process	Impacts of starvation	References
Biomass concentration	Batch test, SBR and MBR	Starvation caused biomass reduction. Biomass decay rate is faster under aerobic condition.	(Coello Oviedo et al., 2003; Trinh, 2013; Yilmaz et al., 2007; Yogalakshmi et al., 2007; Yücesoy et al., 2012)
Floc size and fractal	SBR and MBR	Floc size and fractal dimensions decreased during starvation period	(Kim et al., 2007b; Moon et al., 2011; Yogalakshmi

dimensions		as adaptive responses to starvation stress.	et al., 2007)
Microbial activity	Batch test, SBR and MBR	Microbial activity (SOUR) inside the bioreactor decreased.	(Coello Oviedo et al., 2003; Moon et al., 2011; Torà et al., 2011; Wu and Lee, 2011; Yogalakshmi et al., 2007)
Organic and nutrient removals	MBR	The system appeared to be resilient in terms of COD removals under 2-6 d-feed starvation condition	(Le, 2011; Trinh et al., 2015)
		A 5 d-starvation caused significant reduction in organic and nutrient removals. Recovery may not be achieved before a week	(Yogalakshmi et al., 2007)
	SBR	A 5 d-starvation rapidly decreased the removal efficiencies in the order of COD_{Mn} < TN< TP.	(Kim et al., 2007b)

2.9.4 Power failure and loss of aeration

Malfunction of blowers/ diffusers and power cut-off can lead to temporary failure of the aeration system. Disruption of aeration may adversely affect aerobic treatment performance due to inadequate supply of dissolved oxygen as well as mixing. Previous studies showed that the loss of aeration resulting in a DO < 1 mg/L may lead to disruption of nitrification with increase in effluent NH_3 concentration, occurrence of NO_2^- with a concurrent decrease in effluent NO_3^- concentration (Burgess et al., 2002; Butler et al., 2009). Inadequate aeration can also lead to critical membrane fouling in MBRs. To date only one study has studied the impact of aeration failure on MBR performance, albeit over a short period of 2 h (Trinh, 2013). Nitrification was not monitored in that study, but loss of aeration failure for 2 h did not affect sludge characteristics, COD removal and membrane fouling. Therefore, further investigation is required to clearly understand the impact of aeration failure on the performance of MBR systems, particularly on the nitrification capacity.

Power failure ceases feeding, aeration and mixing of sludge and their recirculation between different reactors in biological nutrient removal processes that combine aerobic, anoxic and/or anaerobic conditions. Besides the consequences of aeration failure, inadequate mixing limits the mass transfer of substrate and dissolved oxygen and hence affects pollutant biodegradation. For example, the cease of sludge

recirculation between reactors can stop the supply of nitrate from aerobic zone to the anoxic zone, hindering nitrogen removal via the nitrification/denitrification pathway. Absence of sludge recirculation between aerobic and anaerobic zones can also lead to the breakdown of phosphorous removal process by the polyphosphate accumulation process. Changes in physico-chemical properties of activated sludge such as deflocculation, change in components of extracellular polymeric substance (EPS), and reduction of oxygen transfer rate throughout different oxygen cut off and starvation times (6-72 h) were observed by Villain et al. (2013) in batch tests. However, a constant COD degradation rate was maintained in that study. To date only one study has studied the impact of power failure on MBR performance (Trinh, 2013). However, as mentioned above, this study did not cover the impact on the biological nutrient removal process.

2.9.5 Ammonia shock

Ammonia in wastewater is responsible for disruption of the ecology of the receiving water body via processes such as eutrophication: at least two possible mechanisms of ammonia toxicity have been postulated: (i) un-ionized ammonia could directly inhibit the activity of cytosolic enzymes; (ii) NH_4^+-N accumulated inside cells might be toxic by its effect on intracellular pH or the concentration of other cations such as K^+. In both cases, high pH and high total ammonia concentration could exert synergistic toxicities synergistically (Kadam and Boone, 1996). However, a study investigating the effects of ammonium on bacteria (*Corynebacterium glutamicum, Escherichia coli* and *Bacillus subtilis*) showed that sodium ions could cause the same retardation of growth as ammonium when present at concentrations of 750 mM (1.5% v/v) or more, implicating that ionic or osmotic effects causes growth inhibition, not the presence of ammonium itself (Müller et al., 2006).

Activated sludge-based systems have been widely used to treat ammonia nitrogen from wastewater; however, the impact of sudden ammonia shock on activated sludge is yet to be clearly elucidated. A number of studies about the impact of ammonia shock on activated sludge have been conducted in different wastewater treatment processes with concentration range of shock load up to 1000 mg/L as NH_4^+-N (**Table 2.13**). For example, increase in ammonia concentration from 50 to 800 mg/L led to the reduction of COD removal (from 95 to 79%) and specific oxygen uptake rate (SOUR) (68 to 45

mg O_2/g MLSS) (Li and Zhao, 1999). Fewer number of studies have investigated the impact in continuous flow reactors. For example, ammonium shock at 70, 190, and 390 mg/L nitrogen (~2, 5 and 10 times of the average ammonia concentration in the influent, respectively) to a bench scale nitrifying sequencing batch reactor (SBR) did not cause any significant effect on any parameters tested (i.e., COD removal, SOUR and sludge volume index) (Henriques et al., 2007). Wu and Chen (2007) observed a minor effect of a shock load of 150 mg/L ammonia on nitrification in an SBR. However, a dose of 700 mg/L ammonia to an aerobic MBR treating raw wastewater resulted to an immediate decrease in COD removal (from 90 - 82 %) (Trinh et al., 2015). The activity of nitrifying bacteria was observed to be more severely inhibited by high ammonia concentration than organic-utilizing bacteria (Ding et al., 2014).

Besides the potential toxicity of ammonia on microorganisms, overload of ammonia may result in loss of available oxygen. Therefore, ammonia shock load can lead to the failure of nitrification/denitrification-based biological nitrogen removal (BNR) system (Dinçer and Kargi, 2000). However, it has not been demonstrated systematically in continuous flow systems, particularly in MBR.

Table 2.13: Impacts of ammonia shock loads on WWTPs

WWTP type and specification	Shock load experiment	Impacts of ammonia shock load	References
Lab-scale MBR: HRT of 24 h; SRT of 30 d and MLSS of 5 g/L.	Raw wastewater. Single dose of 700 mg NH_4^+-N /L as NH_4HCO_3 into bioreactor	Increased pH (6.7 to 8.2) and decreased MLSS. Increased CST and TMP. Decreased COD removal (from 90 to 82%). Fully recovered within 72 h.	(Trinh et al., 2015)
Lab-scale SBR and batch test Synthetic wastewater.	50 (run 1) *vs.* 400 (run 2) (mg/L NH_4^+-N) Sludge from run 1 and 2 were subject to ammonia shock of 50; 200; 350; 600 and 1000 mg/L.	COD removal: 84% (run 1) *vs.* 54% (run 2). Dehydrogenase activity: 13 mg Triphenyl Formazon/g MLSS. h (run 1) *vs.* 5.6 mg Triphenyl Formazon /g MLSS. h (run 2). Ammonia removal: 99.8% (run 1) *vs.* 33% (run 2). High ammonia-acclimated sludge (run 2) showed stronger resistance to ammonia shock inhibition.	(Ding et al., 2014)
Lab-scale SBR: SRT of 14 d; HRT of 12 d; MLSS of 3 g/L; and domestic wastewater.	Loading of 150 mg/L NH_4^+-N by adding NH_4Cl for 3 d.	Nitrification affected due to shortage of alkalinity, but quickly recovered.	(Wu et al., 2007)
Nitrifying SBR (SRT 10 d; ~23^oC) *vs.* Non-nitrifying SBR (SRT of 2 d; 18^oC).	Single dose using NH_4HCO_3. Nitrifying SBR: 70, 90, and 390 mg/L nitrogen (~2, 5, and 10 times). Non-nitrifying: 40, 130, and 280 mg/L nitrogen (~3, 9, and 18 times)	No significant effects on respiration, COD removal, CST, and SVI.	(Henriques et al., 2007)
Lab-scale CAS with MLVSS of 15 g/L.	Increase influent NH_4^+-N from 0.5 g/ m^3.d to 7.5 kg/m^3.d.	pH-decrease and temporary accumulation of nitrite. Lower ammonia conversion rate by biomass (0.5-0.7 g NH_4^+-N/g VSS. d). Performance recovery was quick.	(Campos et al., 1999)
Clay or natural zeolite – based filter media: HRT of 0.95 – 1.43 h**.**	Raw municipal wastewater with NH_4^+-N concentration of 22-42 mg/L. Shock load of 2x of usual concentration of NH_4^+-N as NH_4Cl over 90 d	COD removal: not affected. NH_4^+-N removal: significant impact on expanded clay based filter media, but not the one with zeolite.	(He et al., 2007)

Aerobic reactors: MLSS of 2 g/L and SRT of 3-4 d.	Shock load of 50 to 800 mg/L NH_4^+-N using glucose-based synthetic wastewater or raw leachate.	Ammonia shock decreased COD removal (>95 to <79%), dehydrogenase activity (11.04 to 4.22 µg Triphenyl Formazon/mg MLSS) and SOUR (68 to 45 mg O_2/g MLSS), and increased effluent NH_4^+-N (0.58 to 649 mg/L).	(Li and Zhao, 1999)

2.9.6 Bleach and other toxic chemical shock

Biological wastewater treatment systems are susceptible to many industrial and household chemicals. Chemical shocks can inhibit respiration and bacterial growth and lead to change in microbial population (Henriques et al., 2007; Kelly Ii et al., 2004; Yang and Thavinpipatkul, 1978). Treatment performance including COD removal, nitrification and effluent quality was reported to be significantly impacted by chemical shocks depending on the chemicals and their concentration (**Table 2.14**). The impacts of shock loads of a wide range of chemicals, such as electrophilic chemicals, hydrophobic chemicals, extreme pH levels, heavy metals, respiration inhibitors, and uncouplers of phosphorylation have been studied to date (**Table 2.14**). Organic electrophilic chemicals and heavy metals were found to induce deflocculation of activated sludge (Bott and Love, 2002; Henriques et al., 2007).

Bleach is one of the most common household chemicals and hence its accidental release to municipal wastewater is likely. Bleach, particularly sodium hypochlorite, is a disinfectant that kills microbes via interacting with their heat shock proteins, stimulating their role as intra-cellular chaperone and causing the bacteria to form into a clump that will eventually die off (Winter et al., 2008). Therefore, bleach shock can lead to detrimental impacts on WWTP performance. Only two studies related to the impact of bleach on activated sludge processes are available so far. While Bodík et al. (2008) demonstrated a significant effect of disinfectants containing sodium hypochlorite (0.3 mL/L) on organics removal by activated sludge in batch tests, no discernible effect of a bleach dose of 0.4 mL/L on the performance of bench- or pilot-scale MBRs was observed by Knops (2010).

Table 2.14: Impacts of toxic chemicals on performance and operation of WWTPs

WWTP type and specification	Experimental protocol	Impacts on performance	Impacts on operational parameters	References
Lab-scale MBR: HRT of 12-15 h; SRT of 2-74 d; MLSS of 10-15 g/L.	Phenol shock: single pulse of 400 mg/L *vs*. gradually increased from 50 to 800 mg/L. Chromium shock: by 20, 40 and 50 mg/L (duration of 1d each).	COD and phenol removal decreased as phenol concentration increased. Recovery time: within 24 h. COD removal decreased as chromium concentration increased, but performance recovery was achieved as soon as chromium addition ceased.	Not available	(Al-Malack, 2007)
Lab-scale MBR: HRT of 24 h; SRT of 30 d; MLSS of 5 g/L.	2,4 dinitrophenol shockat 200 mg/L.	Significant impact on COD and DOC removal. Partial recovery after 48 h.	No impact on biomass. Increased capillary suction time and fouling rate	(Trinh et al., 2015)
Lab-scale CAS	pH shock of 4, 3, 9.7 and 10.4. Potassium cyanide shock of 50 mg/L. Phenol shock of 185 mg/L.	pH shock of 4-10.4: no significant impact on COD removal. KCN/phenol shocks deteriorated effluent quality.	No impact of pH shock on biomass, but filamentous bacteria were predominant at pH 4.	(Yang and Thavinpipatkul, 1978)
Pilot-scale CAS	Phenol shock of 0.5, 1 and 2 g/L.	Not available.	No impact at phenol concentration of 0.5-1 g/L, but a drastic change in microbial community at phenol of 1-2 g/L.	(Rozich and Gaudy Jr, 1985)
Lab-scale SBR: HRT of 24 h, SRT of 10 d	Electrophilic chemicals such as chloro-2,4-dinitrobenzene; N-ethylmaleimide; 2,4-dinitrotoluene; benzoquinone;	Not available	Significant potassium efflux from sludge flocs to bulk liquid and deflocculation in response to sublethal shock	(Bott and Love, 2002)

	cadmium; and pentachlorophenol.		loads.	
Lab-scale SBR: HRT of 24 h; SRT of 3-10 d; MLVSS of 1-2 g/L.	Single pulse of 1-chloro-2,4-dinitrobenzene (CDNB) (8.6, 14 and 15 mg/L); Cadmium 17, 27 and 52 mg/L) , Octanol (110, 135 and 209 mg/L); 2,4-dinitrophenol (DNP) (21, 39 and 107 mg/L); pH (5, 9 and 11); and Cyanide (1.7, 3.4 and 8.4 mg/L).	Impacts on nitrification (as nitrate generation rate): CDNB> pH 11> cadmium> cyanide>octanol>DNP. Recovery was slow.	15 – 50 % respiratory inhibition. Inhibition of AOB and NOB.	(Kelly Ii et al., 2004)
Lab-scale SBR: Nitrifying (SRT of 10 d, temperature of 23 ^{o}C)	Single pulse of cadmium, CDNB, DNP, 1-octanol, cyanide and pH.	Impacts on COD removal: Cadmium and pH 11 > CDNB>DNP, cyanide> pH 5, 9 and octanol.	Similar trend as impacts on COD removal for biomass growth. Deflocculation was observed.	(Henriques et al., 2007)
Lab-scale SBR: MLSS of 2 g/L; HRT of 12.8 h.	Potassium dichromate shock of 10 and then 20 mg/L (duration of 8 h and 16 for each concentration).	Cr (VI) shock decreased COD removal and increased effluent TSS, with slightly greater impact at higher concentration.	No impact on MLSS	(Mizzouri and Shaaban, 2013)

2.9.7 Impact of hazardous events on TrOC removal

There has been only one study to date regarding the impact of hazardous events including ammonia shock (700 mg/L) and power failure (2 h) on TrOC removal in MBR. Hazardous events, particularly ammonia shock, affected removal of hydrophilic TrOCs (e.g., ketoprofen and gemfibrozil). A less severe impact by power failure on TrOC removal was observed (Trinh, 2013). More investigation, particularly in membrane-based BNR systems, is critical to improve the understanding regarding the impact of hazardous events on TrOC removal.

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CHAPTER 3. Nutrient and Trace Organic Contaminant Removal from Wastewater by an Anoxic-Aerobic Membrane Bioreactor

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3.1 INTRODUCTION

The widespread occurrence of trace organic contaminants (TrOCs) in the environment raises significant concern regarding the potential detrimental effects on human and other biota. Although a number of studies have reported better and more stable removal of the moderately biodegradable TrOCs by membrane bioreactors (MBR) than conventional activated sludge (CAS) process, little improvement is generally reported in case of hydrophilic and resistant TrOCs (Boonyaroj et al., 2012; Radjenović et al., 2009; Tadkaew et al., 2011). In order to find avenues to enhance TrOC removal by MBR, the effect of different operating parameters such as sludge retention time (SRT) and hydraulic retention time (HRT) (Fernandez-Fontaina et al., 2012; Zeng et al., 2013), mixed liquor pH (Urase et al., 2005) and temperature (Hai et al., 2011c) have been studied. Several studies (e.g., (Dytczak et al., 2008; Hai et al., 2011a; Zwiener et al., 2000)) have investigated the impact of dissolved oxygen concentration (DO) and/or redox conditions (i.e., oxidation reduction potential, ORP). However, a clear consensus has not been reached to date.

Different redox conditions may promote the growth of different microbial consortia leading to the excretion of diverse enzymes, and therefore, achieving varying degree of TrOC biodegradation. Additionally, redox conditions can significantly influence the properties of sludge, which govern biosorption of TrOCs. The mechanisms of biological nutrient (i.e., nitrogen and phosphorous) removal under different redox conditions are well understood and have been successfully applied in full-scale WWTPs. However, the same cannot be claimed in the case of TrOC removal. To date understanding of TrOC degradation under anaerobic and/or anoxic conditions remains rather limited. Furthermore, the performance of combined anaerobic and/or anoxic and aerobic reactors has been the focus of only a limited number of recent investigations, and contradictory reports can often be seen in the literature. Systematic studies under controlled operating regimes with a broad set of TrOCs are required to elucidate the contribution of the individual reactors (facilitating different redox conditions) in combined nitrifying and denitrifying systems, but such attempts have been scarce to date.

In line with the aforementioned research gaps, the aim of this study is to investigate the removal and fate of a set of 30 TrOCs by a laboratory scale anoxic-aerobic MBR. Insights into the influence of anoxic and aerobic conditions on the removal of these compounds from both aqueous and sludge phases along with nitrogen removal are presented.

3.2 MATERIALS AND METHODS

3.2.1 Model TrOCs and synthetic wastewater

A set of 30 compounds representing five major groups of TrOCs, namely pharmaceuticals and personal care products, pesticides, steroid hormones, industrial chemicals, phytoestrogens and UV filters were used in this study. These TrOCs were selected based on their widespread occurrence in domestic sewage and their diverse physicochemical properties (**Appendix Table A-1**). The compounds were purchased from Sigma-Aldrich (Australia) with a purity of 99% or higher. A combined stock solution of TrOCs was prepared in pure methanol and stored at -20 ºC in the dark. Once a stable MBR operation had been achieved (See Section 3.2.3), TrOCs were continuously spiked into the synthetic wastewater to achieve a final concentration of approximately 5 μg/L of each selected compound.

A synthetic wastewater was used to provide a source of carbon, nitrogen, phosphorus and trace metal ions for the growth of the microbes. The synthetic wastewater was prepared fresh each day by dissolving the chemicals into deionized water to obtain a final concentration of 100 mg/L glucose, 100 mg/L peptone, 17.5 mg/L KH_2PO_4, 17.5 mg/L $MgSO_4$, 10 mg/L $FeSO_4$, 225 mg/L CH_3COONa and 35 mg/L urea (Wijekoon et al., 2013).

3.2.2 Laboratory scale MBR set-up

A laboratory scale anoxic-aerobic MBR (**Figure 3.1**) with a 13.8 L anoxic reactor and an 11.7 L aerobic reactor with an immersed membrane module was used. The membrane module used was a hollow fibre ultrafiltration membrane (Zeweed-10) supplied by Zenon Environmental (Ontario, Canada). This membrane had a nominal pore size of 0.04 μm with an effective membrane surface area of 0.93 m^2. Peristaltic pumps (Masterflex L/S, USA) were used for feeding, recirculation and effluent

extraction. The permeate withdrawal pump connected with the membrane was operated using an 8 min on and 2 min off cycle. The on/off time aimed to provide relaxation time to the membrane module. The influent flow rate was adjusted to be the same as the effluent flow rate to maintain a constant water level inside the reactors. A certain volume of the media was constantly recirculated from the aerobic to the anoxic reactor. The ratio of the media recirculation flow rate to the feed flow rate (denoted internal recirculation (IR) henceforth) governed the overflow of media from the anoxic tank to the aerobic tank (See Section 3.2.3). The mixed liquor in the upper quarter of the anoxic tank was intermittently (1 min on and15 min off) mixed by a mixer (200 rpm) to ensure that the sludge transferred from the aerobic tank did not get trapped within the anoxic reactor. An air pump was employed to continuously aerate the (aerobic) reactor via a diffuser located at the bottom of the tank. Another air pump was intermittently operated to provide air flow through the membrane module to reduce cake layer fouling. A high resolution (±0.1 kPa) pressure sensor (SPER scientific, Extech equipment Pty. Ltd, Victoria, Australia) connected to a computer for data recording was utilized to continuously monitor the transmembrane pressure (TMP). The *in-situ* air scrubbing was found adequate to keep the TMP stable at below 5 kPa, and no chemical cleaning was required over the whole operation period. The total hydraulic retention time (HRT) was set at 24 h (i.e.,13 h in anoxic tank and 11 h in aerobic tank), corresponding to a permeate flux of 1.23 $L/m^2.h$. The mixed liquor pH was stable at 7.25±0.75. Dissolved oxygen concentration (DO) was maintained at above 3 mg/L and approximately 0.1 mg/L for the aerobic and the anoxic reactors, respectively. The ORP remained relatively stable at 141 ± 18 mV (n= 55) in the aerobic reactor. In the low DO reactor, the ORP varied from -122 ± 22 mV (n= 40) at an IR ratio of 3 to -230 ± 75 mV (n=15) in absence of IR (See Section 3.2.3).Throughout the period of investigation, the MBR system was covered with aluminium foil to avoid any exposure to sunlight to prevent possible photolysis of the TrOCs.

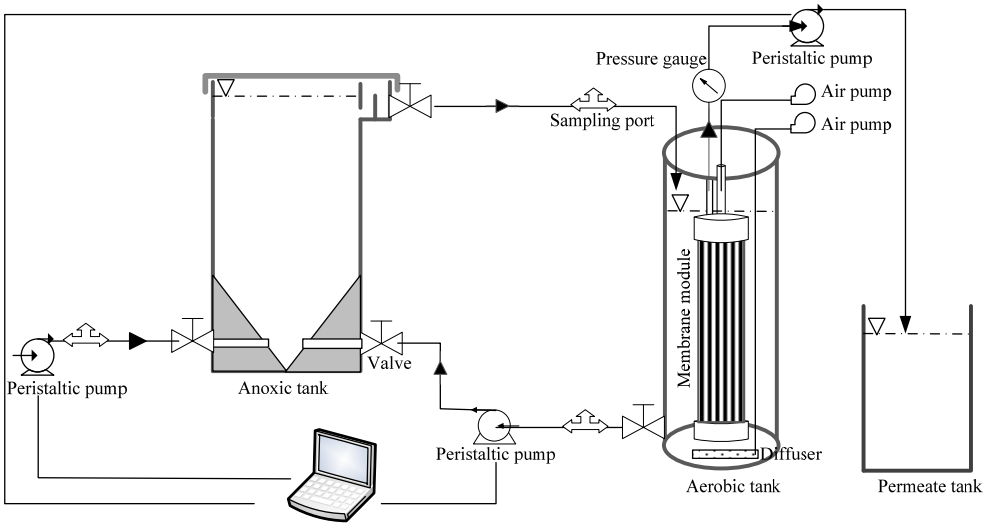

Figure 3.1: A schematic diagram of the laboratory scale anoxic-aerobic MBR

3.2.3 MBR operation protocol

The MBR system was initially seeded with activated sludge from the biological nutrient removal unit of the Wollongong Sewage Treatment Plant (Wollongong, Australia). It was operated for total 305 d (**Table 3.1**). For the initial 180 d, the MBR was operated without any planned sludge withdrawal except for sludge sampling. Under this regime, the MBR was first operated for 125 d for sludge acclimatization and stabilization of TOC and TN removal by fine-tuning the IR ratio (0.5-3) between the anoxic and the aerobic reactor. Following this, TrOCs were introduced to the synthetic wastewater that was continuously fed to the MBR. This part of the study spanned 55 d (Day 126-170) and was conducted with an IR ratio of 3. During this period, the mixed liquor suspended solids (MLSS) concentration increased for both the anoxic (from 8.12 g/L to 10.4 g/L) and the aerobic reactors (from 7.38 g/L to 8.75 g/L). However, MLVSS/MLSS ratios were stable at 0.71 ± 0.02 and 0.70 ± 0.01for the anoxic and the aerobic reactors, respectively (**Figure 3.2**).

The MBR was operated under a fixed SRT of 25 d for the rest of the period (Day 181-305). At the beginning of this trial, the MBR system was operated for a period of 55 d without any addition of TrOCs to the synthetic wastewater. This run was conducted to ensure stable biological performance (e.g., TOC and TN removal) following the change in SRT. TrOC spiking to the synthetic wastewater was resumed from Day 226. The

MBR was hence run for 40 d at an IR ratio of 3 and MLSS concentration of 5.12 ± 0.18 g/L and 3.78 ± 0.23 for the anoxic and the aerobic reactors, respectively. The MBR was operated for further 35 d without IR to assess the impact of recirculation of media from the aerobic to the anoxic reactor. In this paper, the low DO reactor has been generally described as an 'anoxic' reactor except for during the operation without IR when it was described as an 'anaerobic' reactor due to the absence of nitrate.

Table 3.1: Schedule of continuous operation of the anoxic—aerobic MBR

Day	SRT	Internal recirculation (IR) ratio	TrOC added	Operation mode
0-90	Infinite[a]	0.5	No	MBR start- up period (without trace organics in feed)
91-125	Infinite[a]	3	No	
126-170	Infinite[a]	3	Yes	Operation with TrOCs in feed
171-180	Infinite[a]	3	No	MBR run without TrOCs in feed
181-225	25 d	3	No	Stabilization period for SRT of 25 days
226-265	25 d	3	Yes	Operation with TrOCs in feed.
266-305	25 d	0	Yes	Operation with TrOCs in feed.

[a]No sludge withdrawal except sampling, resulting in a theoretical SRT of >1000 d.

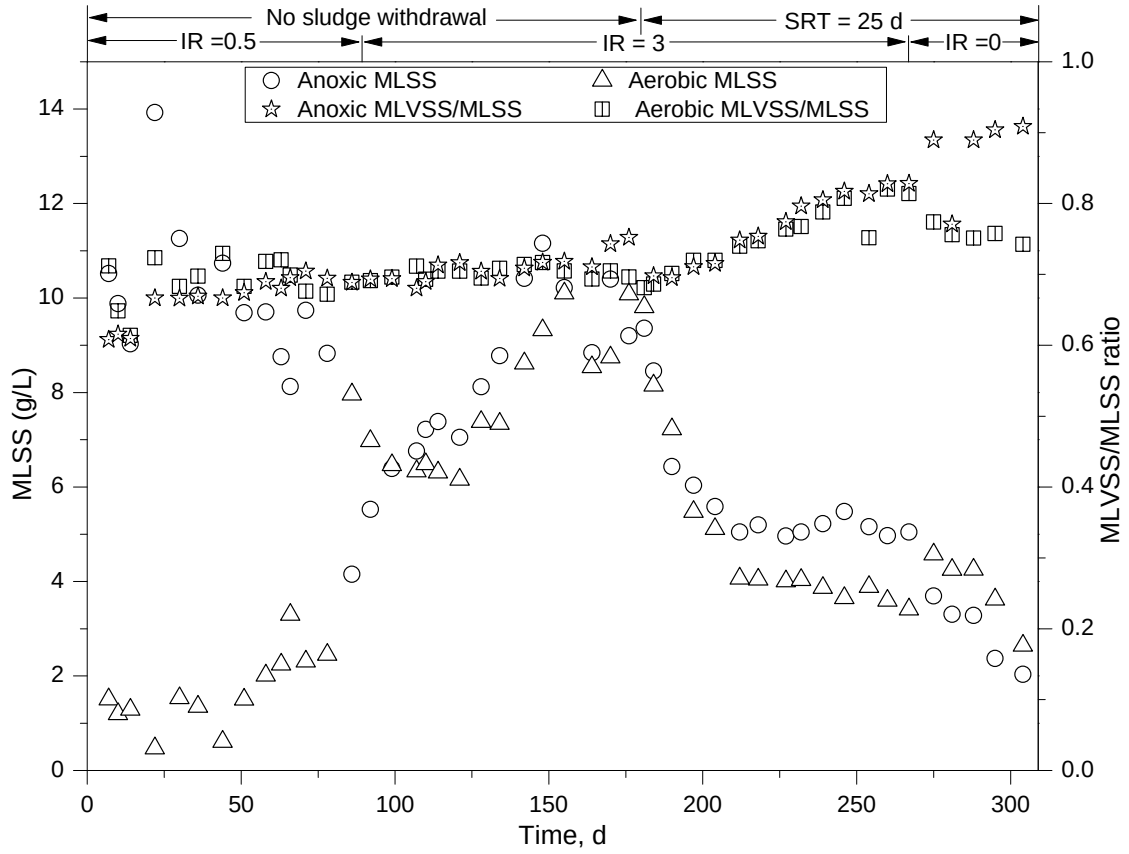

Figure 3.2: MLSS and MLVSS profiles in anoxic and aerobic reactors over the entire operation period.

3.2.4 Analytical methods

3.2.4.1 Basic parameters

Total organic carbon (TOC) and total nitrogen (TN) were analyzed using a TOC/TN-V_{CSH} analyzer (Shimadzu, Japan). Ammonia and orthophosphate concentrations were measured using flow injection analysis (Lachat instruments, Milwaukee, USA) following the standard methods (Eaton et al., 2005). For ammonia, the analysis comprised production of the blue indophenol dye from the Berthelot reaction, intensification of this blue color by the addition of nitroferricyanide and then measurement of absorbance at 630 nm (Standard method: 4500-NH_3 H). In ortho-phosphate analysis, the reaction between ortho-phosphate with ammonium molybdate and antimony potassium tartrate under acidic conditions formed a complex. The reduction of this complex with ascorbic acid led to the formation of a blue complex that absorbs light at 880 nm (Standard method: 4500-P G.) Ion Chromatography (IonPac® AS23 Anion-Exchange Column, Dionex Corporation, USA) was applied to quantify

anions such as nitrate and nitrite. The anions were separated on a strongly basic anion exchanger and converted to their highly conductive acid forms. The separated anions in their acid forms were measured by conductivity. The analysis of other basic parameters was also carried out according to the standard methods (Eaton et al., 2005).

3.2.4.2 TrOC analysis

The concentration of the selected TrOCs in the (i) feed, (ii) supernatant of the anoxic bioreactor and the (iii) aerobic MBR permeate, was determined using a gas chromatography-mass spectrometry (GC-MS) method described by Hai et al. (2011c). Duplicate samples (500 mL) were concentrated and extracted by solid phase extraction (SPE) using Oasis® HLB 6cc cartridges (Water Corporation, Milford, Massachusetts, USA). The TrOCs were eluted and derivatized before being subjected to GC-MS analysis via a Shimadzu GC-MS QP5000 system, equipped with a Shimadzu AOC 20i autosampler, using a PhenomnexZebron ZB-5 (5% diphenyl-95% dimethylpolysiloxane) capillary column (30 m x 0.25 mm ID, d_f = 0.25 μm).

TrOC concentration in sludge was determined using a previously reported method (Wijekoon et al., 2013). The sludge sample was freeze-dried using an Alpha 1-2 LD plus Freeze Dryer (Christ GmbH, Germany). The dried sludge (0.5 g) was extracted successively with 5 mL methanol and 5 mL dichloromethane and methanol (1:1) by ultrasonic solvent extraction. The solvent was then evaporated using nitrogen gas and the extracts were diluted to 500 mL with Milli-Q water for SPE. The samples were then analyzed as described above.

Because a microfiltration membrane was utilized, membrane rejection was not expected to be significant for the TrOCs in this study. Accordingly, the performance of anoxic and aerobic TrOC removal was compared, taking into consideration the TrOC concentration in the supernatant of the anoxic bioreactor and that in MBR permeate.

3.3 RESULTS AND DISCUSSION

The operation of the integrated anoxic-aerobic MBR was initiated with no sludge withdrawal as a reference; however the main focus was on the performance of the system under an SRT of 25d, which is a more realistic value considering the present day full-scale MBRs. Systematic changes in IR ratio were made to verify its effect on bulk

organics, nutrient and TrOC removal and to identify the role of anoxic/aerobic conditions on TrOC degradation. The operation protocol has been detailed in Section 3.2.3 but the important steps are worth reiterating here: (i) fine-tuning IR ratio (0.5-3) during start-up of the MBR; (ii) addition of TrOC to the synthetic wastewater after achievement of high and stable TOC/TN removal at an IR ratio of 3, (iii) change of SRT to 25 d, (iv) operation without IR to identify the impact of anoxic/aerobic conditions on TrOC removal as well as verify the role of IR.

3.3.1 Bulk organics and nutrient removal

A high and stable (up to 99%) overall TOC removal was achieved throughout the operation period (**Figure 3.3**). Notably, irrespective of the level of TOC in the supernatant of the anoxic reactor, the aerobic MBR served as an efficient post treatment step and accordingly a similar level of overall TOC removal was achieved irrespective of the IR ratio (**Figure 3.3** and **Appendix Figure A-2**).

Biological nitrogen removal necessitates an activated sludge system allowing internal sludge recirculation between aerobic and anoxic regimes to facilitate nitrification (oxidation of ammonia and nitrite) and denitrification (reduction of nitrate to nitrogen gas). While nitrification is carried out by autotrophic bacteria under aerobic conditions, denitrification takes place under anoxic conditions. In this study, NH_4^+-N in the supernatant of the aerobic reactor was below the detection limit (0.7 μg N/L as NH_3) (**Figure 3.4**), which implies complete nitrification. The results confirm that an SRT of 25 d (as applied from Day 181 to 305) was adequate to support proliferation of both heterotrophic and slow-growing nitrifying microorganisms that sustain high organics removal, and particularly nitrification. Previous studies also noted that WWTPs operating at SRTs longer than 10 d can induce high removal efficiencies of bulk organics and nutrients (Zeng et al., 2013).

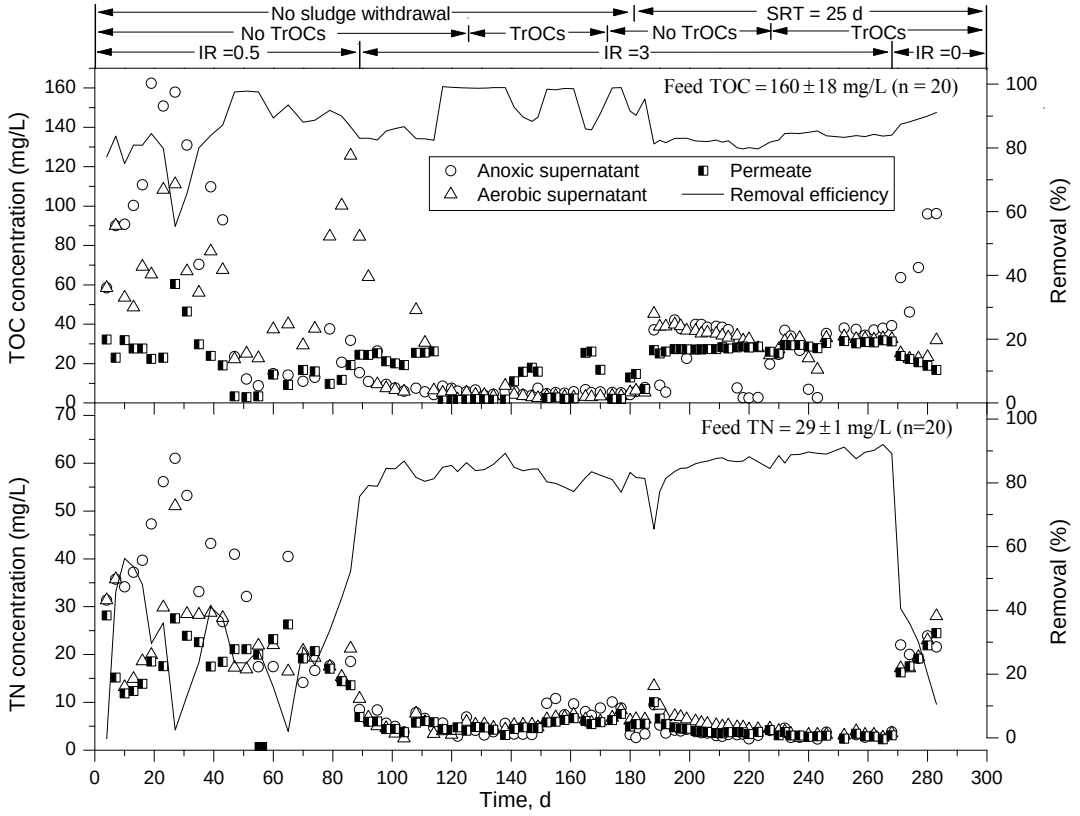

Figure 3.3: TOC/TN concentration and removal efficiency profiles over the entire operation period of the anoxic-aerobic MBR.

In contrast to nitrification, TN removal (which is governed by denitrification) varied depending on the IR, which controlled the supply of nitrate to the anoxic bioreactor (**Figure 3.3** and **Appendix Figure A-2**). High fluctuations in TN removal were observed during the initial 90 d when the MBR system was run under an IR ratio of 0.5. Similarly, during the operation without IR (over the last 35 d), lack of exposure of nitrate to the low ORP environment led to a rapid decline in TN removal (**Figure 3.3**). By contrast, over 80% TN removal (corresponding to a permeate TN concentration of less than 3 mg/L) was achieved consistently at an IR ratio of 3 (Day 91 to 265). A further enhanced TN removal may have been achieved by applying a higher IR ratio, however, that was not attempted because practically a higher IR means requirement of higher pumping and aeration energy (Baeza et al., 2004; Kim et al., 2010).

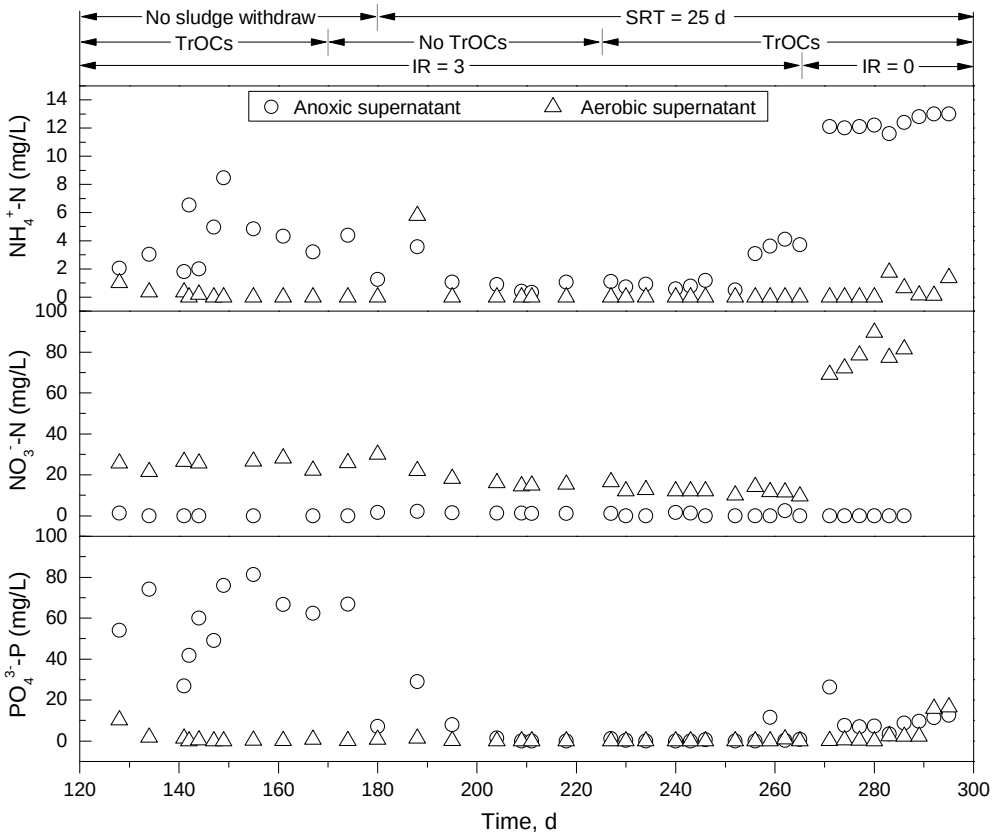

Figure 3.4: NH_4^+ - N / NO_3^- - N / PO_4^{3-} - P concentrations in the supernatant of the anoxic and aerobic reactors. Data has been plotted from Day 120 (start of stable TN removal).

It is also interesting to note that more than 90% phosphate removal (**Figure 3.4**) was achieved during operation at an SRT of 25 d and an IR ratio of 3 although the system was not specifically designed for phosphorous removal (i.e., a strictly anaerobic reactor was not used). This may be attributed to the relatively low phosphorous concentration in the synthetic wastewater (about 4 mg/L as P) as well as the role of the phosphorus accumulating organisms (PAOs). Under anaerobic conditions, PAOs assimilate fermentation products (i.e., volatile fatty acids) into storage products within the cells with the concomitant release of phosphorous from stored polyphosphates. Conversely, in the aerobic zone, energy is produced by the oxidation of storage products and polyphosphate storage within the cell increases. As a portion of the biomass is wasted, the stored phosphorous is removed from the bioreactor for ultimate disposal with the waste sludge (Kim et al., 2010). Therefore, integral to biological phosphorous removal are IR and sludge withdrawal. The role of PAOs in the current study is evident from the

significant accumulation of phosphorus in the anoxic reactor (**Figure 3.4**) in absence of either sludge withdrawal or IR (Day 0-124 and 266-305, respectively).

The introduction of TrOCs to feed wastewater did not show any discernible impact on the basic biological performance of the MBR system including TOC and TN removal (**Figure 3.3**) and the ratio of MLVSS/MLSS (**Figure 3.2**). This observation is consistent with several previous studies (Abegglen et al., 2009; Dorival-García et al., 2013). At trace concentrations, TrOCs may induce impact on oxygen uptake rate of microorganisms but not hinder the overall performance of the system (Hai et al., 2014). Overall removal efficiency of bulk organics and nutrients under different operational conditions is summarized in **Table 3.2**

Table 3.2: Summary of the removal efficiency of bulk organics and nutrients

Parameter	Removal efficiency (%)			
	Condition 1	Condition 2	Condition 3	Condition 4
TOC	94 ± 5 (n =16)	83 ± 4 (n = 18)	84 ± 1 (n = 13)	89 ± 1 (n = 11)
NH_4^+-N	99 ± 1 (n = 11)	100 (n = 10)	100 (n = 11)	100 (n = 9)
TN	83 ± 3 (n = 16)	84 ± 5 (n = 18)	89 ± 2 (n = 13)	27 ± 12 (n = 11)
PO_4^{-3}-P	92 ± 5 (n = 11)	97 ± 3 (n = 10)	98 ± 2 (n = 11)	78 ± 37 (n = 9) (< 30 % after 20 d)

Note: Condition 1: Infinite SRT, IR = 3, with TrOCs; Condition 2: SRT = 25 d, IR = 3, no TrOCs; Condition 3: SRT = 25 d, IR = 3, with TrOCs; Condition 4: SRT = 25 d, IR = 0, with TrOCs.

3.3.2 Overall aqueous phase TrOC removal

It is worth reiterating that, in this study, TrOCs were introduced to the influent continuously over three intervals (**Table 3.1**): (i) Day 126-170 (no sludge withdrawal, IR ratio =3), (ii) Day 226- 265 (SRT=25 d, IR ratio =3), and (iii) Day 266-305 (SRT=25 d, no IR). This section provides an overview of the TrOC removal depending on the compound categories during Stage (i) and (ii) (**Figure 3.5**). Discussion on the comparative removal by the anoxic and aerobic bioreactors along with the critical impact of IR (i.e., Stage (ii) *vs.* Stage (iii)) has been conducted in Section 3.3.3 and

3.3.4, while the relative contribution of biodegradation and biosorption has been elucidated in Sections 3.3.5 and 3.3.6.

Over 90% removals of all five steroid hormones, three industrial compounds and three UV filters were observed in this study (**Figure 3.5**). It is noteworthy that these compounds possess significant hydrophobicity (logD > 3), which may explain the similarities of their aqueous phase removal efficiencies (Joss et al., 2004; Suarez et al., 2012; Wijekoon et al., 2013; Xue et al., 2010). On the other hand, despite low hydrophobicity (logD < 3), significant removal of the phytoestrogens was achieved, possibly due to the presence of –OH (**Appendix Table A-1**), which is a strong electron donating functional group (EDG), in their structure. The presence of EDG increases the biodegradability of TrOCs (Hai et al., 2011b; Tadkaew et al., 2011).

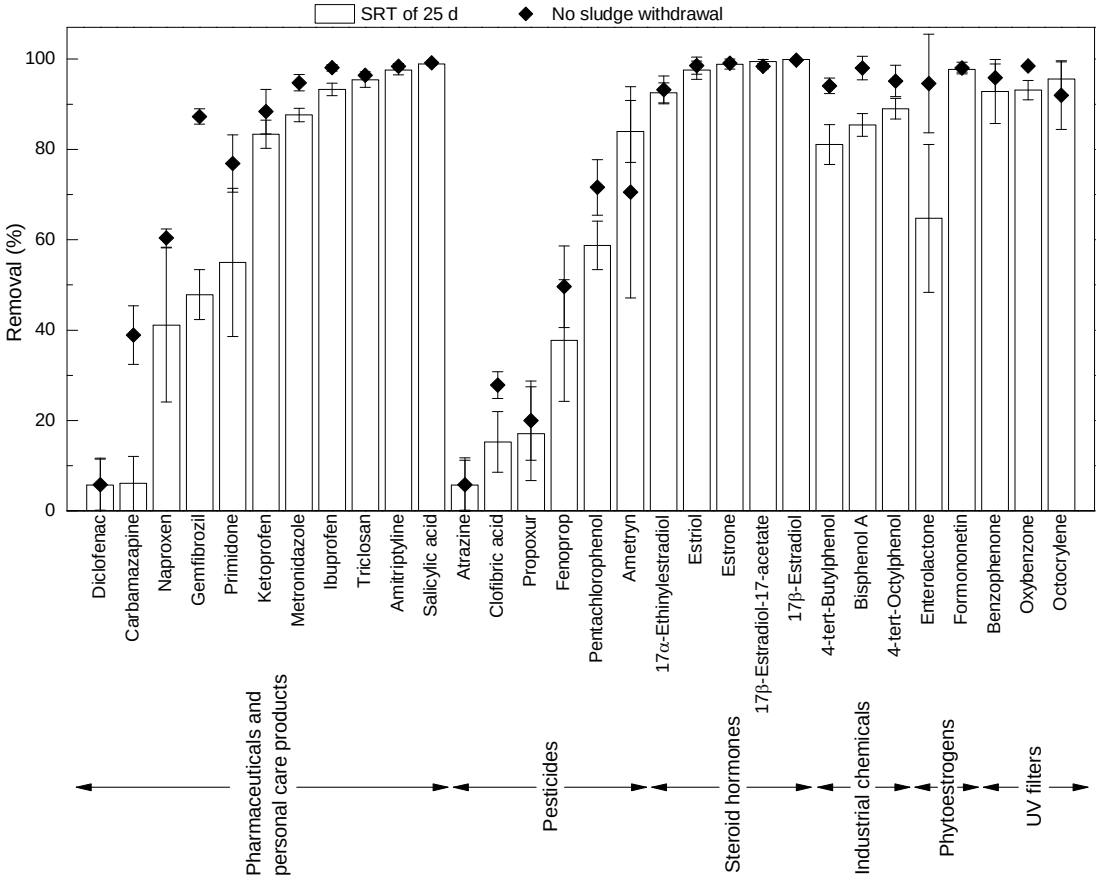

Figure 3.5: TrOC removal by the MBR with no sludge withdrawal and at an SRT of 25 d. Error bars represent the standard deviation of duplicate samples taken once a week over the operation period.

All pharmaceuticals and personal care products (except triclosan and amitriptyline), and all pesticides (except pentachlorophenol) investigated in this study were hydrophilic, and, therefore, no generalizations can be inferred for their aqueous phase removal based on hydrophobicity i.e, log D (**Figure 3.5**). Given the considerable dissimilarity in the molecular structure among these TrOCs (Appendix Table A-1), differences in their removal efficiencies can be expected. Among the pesticides, atrazine, clofibric acid, fenoprop and pentachlorophenol contain one or more –Cl group which is a strong electron withdrawing group (EWG). Of these four pesticides, pentachlorophenol was well removed, possibly because it contains –OH, which is a strong EDG, in addition to being a hydrophobic compound (Methatham et al., 2011). Atrazine and ametryn are both triazine compounds, but only ametryn was well removed (**Figure 3.5**), possibly because of the presence of –Cl (strong EWG) in atrazine but not in ametryn.

Of the 11 pharmaceuticals and personal care products selected in this study, five, namely, diclofenac, carbamazepine, naproxen, gemfibrozil and primidone showed significantly lower removal efficiencies (negligible to 60%), particularly at an SRT of 25 d (**Figure 3.5**). These compounds are hydrophilic and their molecules possess strong EWGs such as $–CONH_2$ and -Cl or are devoid of any strong EDGs (**Appendix Table A-1**). Thus, the low removal efficiency could be attributed to a combined impact of low hydrophobicity and resistance to biodegradation (Tadkaew et al., 2011; Wijekoon et al., 2013).

Assessing the impact of SRT was beyond the scope of this study and the TrOC removal during the operation without sludge withdrawal (Day 126-170) was intended to serve as a reference. Nevertheless, it is worth noting that TrOC removal trend during this period was generally similar to that during the 25 d SRT operation (Day 226- 265), and, furthermore, the removal was significantly better for two compounds, namely, carbamazepine and gemfibrozil (**Figure 3.5**). In a lab-scale anaerobic/anoxic/aerobic-activated sludge treatment study by Zeng et al. (2013), no significant effect of SRT on the removal of natural estrogens over a range of 10-25d was observed, but the removal of synthetic estrogen increased with SRT. Recently, Maeng et al. (2013) achieved effective removal of seven pharmaceutical and personal care products and two natural estrogens (17β-estradiol and estrone) at an SRT of 8 d, while the removal efficiency of gemfibrozil, ketoprofen, clofibric acid and 17α-ethinylestradiol increased when the SRT

was increased from 20 to 80 d. The removal of resistant compounds may improve at comparatively longer SRTs, although controversies regarding this observation exist in the literature (Hai et al., 2014; Luo et al., 2014) . Nevertheless, long term operation of an MBR under an extremely long SRT is associated with operational problems including inefficient mixing and increased aeration demand for the biological metabolism and membrane cleaning. Accordingly, further discussion on TrOC removal focuses on the operation at an SRT of 25 d, which is more relevant to present day MBRs.

3.3.3 TrOC removal by the anoxic bioreactor

As discussed in Section 3.3.1, not only the inclusion of an anoxic bioreactor (low DO and ORP environment) but also the application of an appropriate IR ratio (=3) between the anoxic and the aerobic bioreactors was essential to achieve a significant level of denitrification (**Figure 3.3**). Notably, because of the significant exchange of the mixed liquor between the bioreactors at an IR ratio of 3, the TrOC concentrations in the supernatant of these two reactors were generally similar (**Figure 3.6**). Therefore, with IR between the reactors, the impact of different redox conditions (anoxic or aerobic) *vs.* the impact of exchange of sludge in between the bioreactors could not be demonstrated. Accordingly, TrOC removal in the absence of IR (Day 266-305) was additionally observed.

3.3.3.1 Role of low DO and ORP regimes

Before discussing the impact of additional factors other than redox conditions, it is worth noting that, under both anoxic (IR ratio =3) and anaerobic (no IR) conditions, moderate to high removal (over 50% and up to 90%)was consistently achieved for the following TrOCs: four pharmaceutical and personal care products (primidone, metronidazole, triclosan, and amitriptyline), one steroid hormone (17β-estradiol-17-acetate), one industrial chemical (4-tert-octylphenol) and all selected UV filters (benzophenone, oxybenzone, and octocrylene) (Figure 3). This observation implies that these TrOCs are removed under low DO and ORP conditions.

The observation made here regarding benzophenone, octocrylene and 4-tert-octylphenol removal is consistent with several previous studies. Liu et al. (2013) reported the

degradation of six UV filters including benzophenone and octocrylene under aerobic and anaerobic conditions (nitrate, sulphate or iron as the electron acceptor). Similarly, Liu et al. (2008) reported anaerobic degradation of 4-tert-octylphenol by granular sludge. However, the current study shows for the first time the removal of 17β-estradiol-17-acetate and the pharmaceuticals and personal care products such as primidone, metronidazole, triclosan and amitriptyline under low DO and ORP conditions. As triclosan, amitriptyline and 17β-estradiol-17-acetate are hydrophobic compounds (log D > 3.2, **Appendix Table A-1**), they can be removed by sorption and/or biodegradation (See Section 3.3.5). On the other hand, primidone and metronidazole are hydrophilic, but they were removed under low DO and ORP conditions and then eliminated well overall. No prior work on the assessment of anaerobic biodegradation of primidone could be found for comparison; one possible explanation is that the reducing condition may induce the ring cleavage of primidone (such as an attack of nucleophilic form of hydride at 2-position) to form phenylethylmalonamide. Conversely the data presented here differs from the previous reports on negligible anaerobic/anoxic removal of metronidazole (Ingerslev et al., 2001; Kümmerer et al., 2000). The reason for this discrepancy could not be resolved but it is possible that microbial community composition is an important factor, which can be influenced by other operating parameters in addition to the redox conditions. Dorival-García et al. (2013) reported that the removal of the selected antibiotics under different redox conditions (i.e., aerobic, nitrifying and anoxic conditions) depended significantly on the bacterial composition of the sludge. Assessment of the microbial community is an important research gap; however, this is beyond the scope of this study.

3.3.3.2 Impact of internal sludge recirculation

The similar removal efficiencies under both anoxic (IR ratio =3) and anaerobic (no IR) conditions for the aforementioned nine compounds indicate the suitability of low DO and ORP regimes for their removal. However, IR appeared to exert a significant impact on the anoxic (anaerobic) removal efficiency (**Figure 3.6**) and sorption onto sludge (Section 3.3.5) of the rest of the compounds. Particularly, 11 TrOCs including three pharmaceuticals and personal care products (ketoprofen, ibuprofen, and salicylic acid), all steroid hormones except 17β-estradiol-17-acetate, one pesticide (ametryn), two industrial chemicals (4-tert-butylphenol and bisphenol A), and one phytoestrogen

(formononetin) showed moderate to very high removal under the anoxic regime (IR=3), whereas these compounds had no or very low removal under the anaerobic regime (no IR). The discrepancy between removal in absence and presence of IR in this study suggests that the TrOC removal by an anoxic bioreactor is governed not only by the specific redox conditions (i.e., low DO or ORP) but also by other conditions arising from sludge exchange with the aerobic bioreactor.

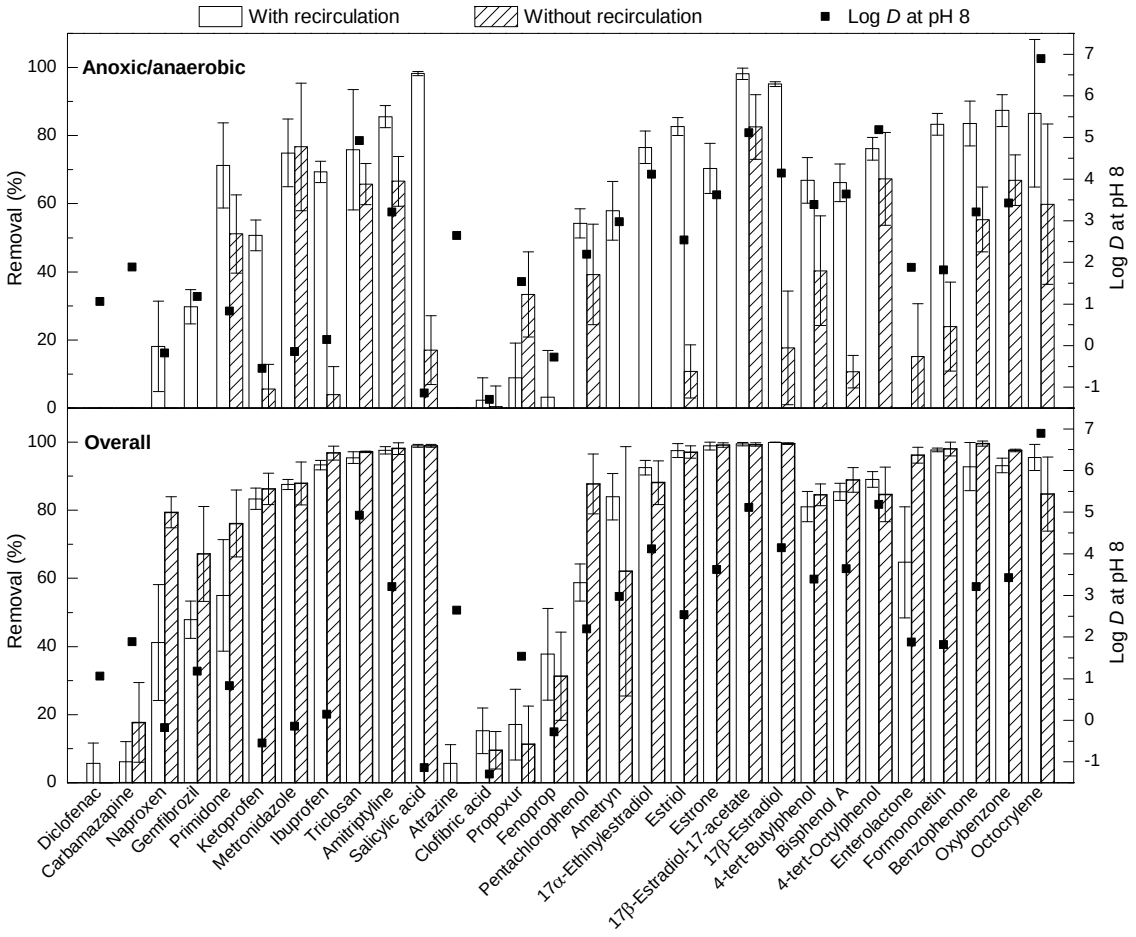

Figure 3.6: TrOC removal by the anoxic reactor as compared to the overall removal (SRT of 25 d; with and withoutIR). Error bars represent the standard deviation of duplicate samples taken once a week for six weeks.

IR from the aerobic to anoxic bioreactor may lead to the following: (i) dilution of the media, (ii) improved mixing/ mass transfer, (iii) supply of nitrate, and (iv) transfer of a portion of DO from the aerobic tank, potentially facilitating some extent of aerobic degradation even within the anoxic reactor (Andersen et al., 2003; Suarez et al., 2010;

Suarez et al., 2012; Xue et al., 2010). Another possible factor is the impact on development of bacterial community. A shared bacterial community that is highly functionalized for contaminant removal may flourish due to sludge exchange between two redox conditions during long-term operation of an MBR. While all these factors may be relevant, there is particularly strong evidence regarding the role of the presence of nitrate in anaerobic TrOC degradation. For example, Zeng et al. (2009) reported two distinct modes of anaerobic 17α-ethinylestradiol removal depending on the presence or absence of nitrate: in the presence of nitrate, biodegradation was the dominant process, while in the absence of nitrate, the removal was simply a result of sorption onto activated sludge. Similarly, Xue et al. (2010) reported that an anaerobic reactor (in absence of nitrate) may achieve significant TrOC removal, but mostly due to enhanced biosorption. Therefore, low ORP corresponding to anoxic/anaerobic regimes may enhance the degradation of certain TrOCs, but the application of IR between the bioreactors (facilitating phenomenon such as presence of nitrate) is an important prerequisite to that. Further discussion in this line is furnished in Section 3.3.5 in relation to biosorption.

3.3.4 The importance of the aerobic bioreactor

Despite the significantly different removal of certain TrOCs in the preceding bioreactor (depending on the IR), the permeate quality of the subsequent aerobic MBR did not vary significantly (**Figure 3.6**), indicating an important role of the aerobic bioreactor for TrOC removal. The crucial role of aerobic conditions in promoting the overall TrOC degradation has been consistently reported in the literature (Andersen et al., 2003; Li et al., 2011; Suarez et al., 2012; Xue et al., 2010). However, to date this aspect has been studied in relation to only a few compounds. For example, Dytczak et al. (2008) reported similar removal of natural (estrone and 17β-estradiol) and synthetic (17α-ethinylestradiol) estrogens under aerobic and alternating anoxic/aerobic conditions. Joss et al. (2004) investigated17α-ethinylestradiol degradation kinetics under different redox conditions, and reported that it was removed at a significant rate only under aerobic conditions. A similar observation regarding 17α-ethinylestradiol degradation was made by Andersen et al. (2003) in combined anoxic/aerobic treatment plants. McAvoy et al. (2002) and Chen et al. (2011) observed better biodegradation of triclosan under aerobic than anoxic or anaerobic conditions. Recently, Dorival-Carcia et al. (2013) reported a

much higher biodegradation of six quinolones under nitrifying than anoxic conditions. The originality of this study is that the data presented here confirms the importance of aerobic biodegradation under same operating conditions with a broader set of TrOCs than the above examples.

3.3.5 TrOC sorption on sludge

In addition to biodegradation, TrOCs can be removed from the aqueous phase by mechanisms such as biosorption, volatilization and photolysis. In this study, photolysis was prevented by covering the bioreactors (Section 3.2.2). Given the vapor pressure or Henry's law constant of the TrOCs investigated (**Appendix Table A-1**), volatilization could also be considered negligible. However, biosorption was monitored to clarify the impact of different operational regimes on the removal of the TrOCs, particularly the impact of IR which was observed to significantly influence the aqueous phase removal by the anoxic (anaerobic) reactor (Section 3.3.3).

Two important observations regarding sludge adsorption were made in this study (**Figure 3.7**): (i) TrOC adsorption on sludge within the anoxic and aerobic reactors was similar due to the significant mixing of the mixed liquor at an IR ratio of 3, however, mostly higher sorption on anaerobic sludge than aerobic sludge was observed in absence of IR, and (ii) For certain TrOCs sorption on sludge in the anaerobic reactor was much higher in the absence of IR than with IR.

The higher sorption within the anaerobic tank is evident by the accumulation of some TrOCs (e.g., amitritypline, benzophenone, triclosan, 4-tert-octylphenol and octocrylene) in the sludge phase and their high removal from the aqueous phase by the anaerobic reactor (no IR). It is hypothesized that the anaerobic/anoxic conditions can facilitate their sorption to sludge (Li et al., 2011; Suarez et al., 2010; Zeng et al., 2009), however, these TrOCs are degraded only if an electron acceptor such as nitrate (with IR) is available. The sludge adsorption data reaffirms the point noted in Section 3.3.3.2 that IR between the anoxic and aerobic bioreactors is an important prerequisite to anoxic biodegradation.

In this study, higher concentration of hydrophobic compounds such as amitriptyline, benzophenone, triclosan, 4-tert-octyphenol and octocrylene in sludge under anaerobic

conditions demonstrated high sorption capacity of anaerobic sludge. Two other hydrophobic compounds, namely, oxybenzone and 17β-estradiol-17-acetate were removed efficiently without significant accumulation in sludge. This can be explained by the presence of EDGs (e.g., -OH and -CH_3) in their structure. Probably, these TrOCs are quickly absorbed to the sludge and subsequently biodegraded/biotransformed under the anaerobic/anoxic regimes.

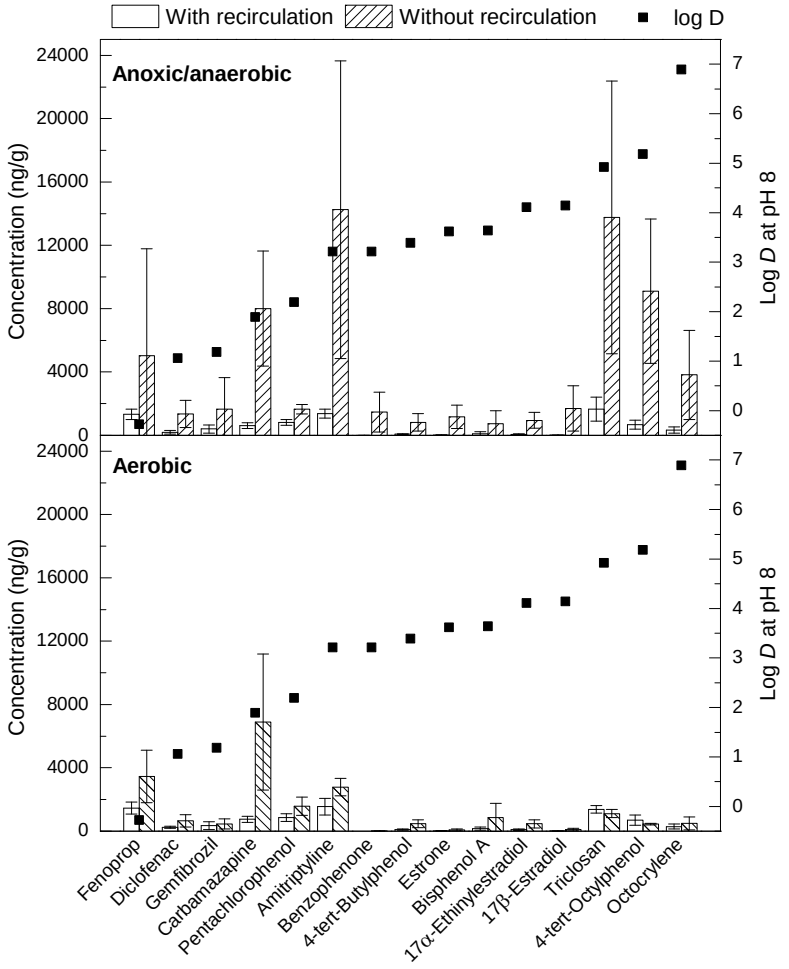

Figure 3.7: Concentration of TrOCs showing significant adsorption on sludge in anoxic and aerobic reactors of the MBR system (SRT of 25 d; with and without IR). Error bars represent the standard deviation of samples taken once a week for six weeks. Large standard deviation in case of some TrOCs is due to their progressive accumulation in sludge.

3.3.6 Overall fate of the TrOCs

In this section, insights into the fate of the TrOCs during MBR treatment is provided focusing on the period of steady state operation at an SRT of 25 d and an IR ratio of 3 (Day 226 to 265, **Table 3.1**). A mass balance based on the total amount of TrOCs in the feed, permeate and sludge during that period was conducted (**Figure 3.8**). TrOC removal from wastewater by bioreactors is the result of a dynamic equilibrium between biosorption and biodegradation, which occur simultaneously. Apart from the poorly removed compounds, stable concentrations of most TrOCs were observed in both liquid and solid phases during the steady state operation at an SRT of 25 d and an IR of 3 (**Figure 3.6** and **Figure 3.7**). For the well removed compounds, in line with contemporary reports (Abegglen et al., 2009; Wijekoon et al., 2013), mass balance (**Figure 3.8**) confirms biodegradation/transformation as the predominant removal mechanism for most TrOCs during MBR treatment.

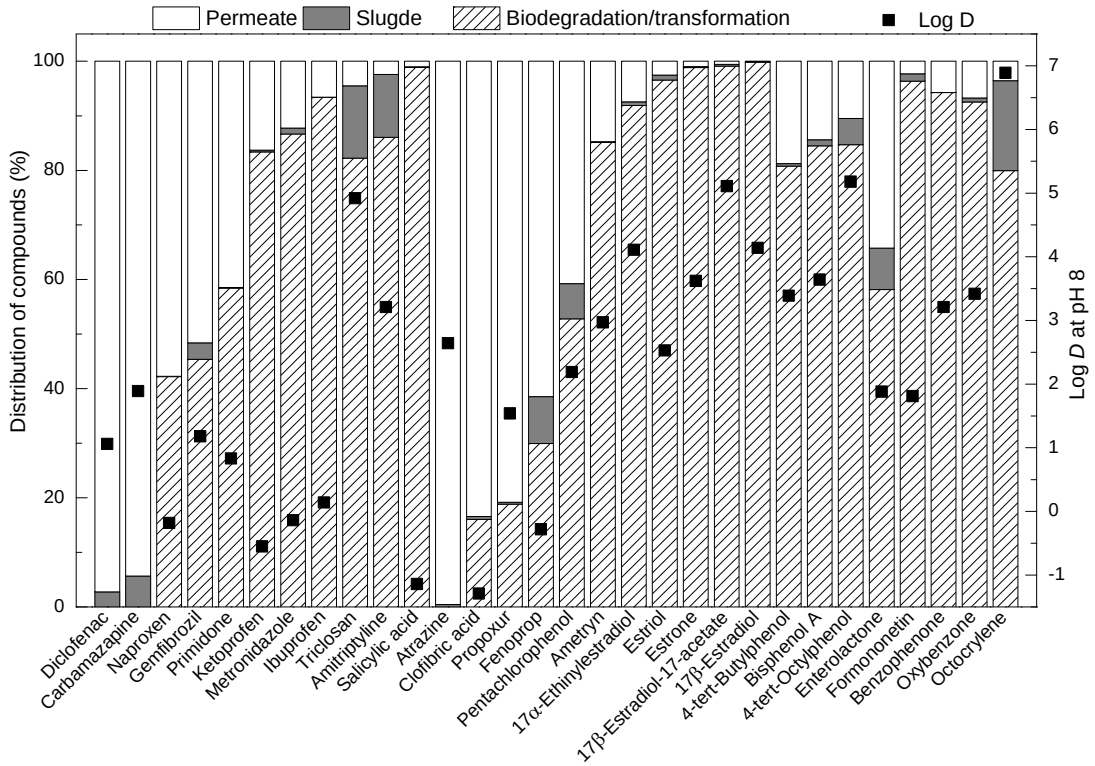

Figure 3.8: Fate of the TrOCs during MBR treatment (SRT of 25 d; with IR).

Among the compounds showing significant sorption (**Figure 3.7** and **Figure 3.8**), octocrylene, amitriptyline, triclosan and 4-tert-octyphenol are hydrophobic compounds, which can explain their high distribution in the solid phase. A low distribution of other hydrophobic compounds in sludge can be attributed to their high biodegradability. The significant distribution in sludge of certain hydrophilic compounds, namely, carbamazapine and fenoprop can be attributed to their recalcitrant structure (Wijekoon et al., 2013). Results presented here highlight the combined influence of intrinsic properties of TrOCs (Section 3.3.2) and operational parameters such as redox conditions and IR (Section 3.3.3).

3.4 CONCLUSIONS

Long-term operation of an integrated anoxic-aerobic MBR revealed that low DO or ORP (i.e., anoxic/anaerobic) regimes are conducive to biodegradation of some TrOCs. However, an important prerequisite to anoxic biodegradaton of TrOCs is internal recirculation (IR) between the anoxic and aerobic bioreactors, in absence of which anoxic/anaerobic regimes alone may only enhance biosorption. Dependence of TN removal on IR that controls the supply of nitrate to the anoxic reactor was also evident. Despite the significantly different removal of certain TrOC by the preceding anoxic bioreactor (depending on the IR), TrOC concentration in effluent from the aerobic MBR was stable.

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CHAPTER 4. Dynamics of Bacterial Communities and Nutrient and Trace Organic Contaminant Removal Performance of an Anoxic-Aerobic Membrane Bioreactor

4.1 INTRODUCTION

Wastewater treatment efficiency is ultimately determined by the composition and function of microbial communities, but which selective factors shape these communities in anoxic-aerobic membrane bioreactors (MBRs)? As compared to a conventional activated sludge (CAS) bioreactor, the special features of an MBR such as membrane retention, lower F/M ratio, lower mass transfer, and other unique hydrodynamic conditions due to the presence of the membrane in the reactor can influence the development of bacterial community (Chiellini et al., 2013; Fenu et al., 2010). In anoxic-aerobic MBRs, internal recirculation (IR) between two bioreactors possibly creates a unique niche for the development of a shared bacterial community. It has been suggested that higher the IR, more similar the bacterial communities in anoxic and aerobic bioreactors is (Gómez-Silván et al., 2014b; Kim et al., 2013; Xia et al., 2012). Nitrifying and denitrifying bacteria were found to be active in both redox conditions (Gómez-Silván et al., 2014a) in such cases. Possibly, the bacterial communities that grow in this combined system are facultative, but this is yet to be systematically verified.

Chapter 3 demonstrated the importance of SRT, MLVSS concentration and the combination of redox conditions in anoxic-aerobic MBRs for enhanced biotransformation of TrOCs. These operational parameters or other environmental factors affect the development of bacterial communities and their functions that ultimately influence TrOC biotransformation in activated sludge. This chapter intends to cast line on this aspect.

The diversity and low concentration (ng/L to µg/L) of TrOCs in environment are challenges for identifying functional bacterial groups of TrOC biodegradation. Controversies related to impact of TrOCs on bacterial community development also exist in the literature. For example, while TrOCs were found to affect oxygen uptake rate of bacterial population, removal of carbon and nitrogen was within the range of stable performance (more than 90% and 93% removal of COD and TN, respectively) (Aubenneau et al., 2010; Delgado et al., 2010; Navaratna et al., 2012). Boonnorat et al. (2014) reported better biodegradation of eight toxic TrOCs following acclimatization of the sludge to a TrOC dose of 1000 µg/L. However, Alidina et al. (2014) did not notice

any significant difference in removal performance or the microbial community following acclimatization to TrOCs at 300-500 ng/L.

Given the research gaps discussed above, this study employed pyrosequencing technology to profile the bacterial communities developed in different redox regimes of the anoxic-aerobic MBR system with and without IR between the anoxic and the aerobic reactors. Insights into the impacts of TrOC addition on the bacterial communities have been provided. Discussed also were the potential impacts of operating SRT on bacterial communities and the removal performance. A systematic monitoring of the shift of bacterial communities under the changes of individual operating parameters combined with removal performance data over long-term period helped shedding light on the functional bacterial groups in the anoxic-aerobic MBR system treating nutrients and TrOCs.

4.2 MATERIALS AND METHODS

4.2.1 Laboratory scale anoxic-aerobic membrane bioreactor

The laboratory scale anoxic-aerobic MBR comprised of an anoxic (13.8 L) and an aerobic (11.7 L) bioreactor. Details of the reactor set-up and operation protocol have been described in Chapter 3. Briefly, a hollow fibre ultrafiltration membrane (Zeweed-10) supplied by Zenon Environmental (Ontario, Canada) was immersed in the aerobic reactor. This membrane had a nominal pore size of 0.04 μm, with an effective membrane surface area of 0.93 m^2. A certain volume of the media was constantly recirculated from the aerobic to the anoxic reactor. The ratio of the media recirculation flow rate to the feed flow rate (denoted internal recirculation (IR) henceforth) governed the overflow of media from the anoxic tank to the aerobic tank.

The MBR system was initially seeded with activated sludge from the biological nutrient removal unit of the Wollongong Sewage Treatment Plant (Wollongong, Australia). It was operated for 305 d with a total hydraulic retention time (HRT) of 24h (i.e., 13 h in anoxic tank and 11 h in aerobic tank).

The system was fed by synthetic wastewater prepared fresh daily by dissolving the following chemicals into deionized water to obtain a final concentration of 100 mg/L glucose, 100 mg/L peptone, 17.5 mg/L KH_2PO_4, 17.5 mg/L MgSO4, 10 mg/L FeSO4,

225 mg/L CH_3COONa and 35 mg/L urea. A set of 30 compounds representing five major groups of TrOCs, namely pharmaceuticals and personal care products, pesticides, steroid hormones, industrial chemicals, and phytoestrogens were used in this study. These TrOCs were selected based on their widespread occurrence in domestic sewage and their diverse physicochemical properties (**Appendix Table A-1**). A combined stock solution of TrOCs was prepared in pure methanol and stored at -20 ^{o}C in the dark. To assess the MBR performance under TrOC dosing, TrOCs were continuously added to the synthetic wastewater at a final concentration of 5 µg/L of each selected compound.

After a start-up period of 125 d (i.e., sludge acclimatization and stabilization of TOC/TN removal), the MBR was run under four main operational regimes as described in Phan et al. (2014) and in Chapter 3 to evaluate the MBR performance and to determine the role of IR and redox conditions on the nutrient and TrOC removal by the system. Briefly, the MBR was first operated without sludge wastage ('infinite' SRT) and with an IR of 3 in presence of TrOC in the influent for 55 d (Days 126-170). This run was conducted to establish a baseline. The SRT of the MBR was changed to 25 d for the rest of the investigation period. At the beginning of the operation under an SRT of 25 d, the MBR system was operated for a period of 55 d without any addition of TrOCs to the synthetic wastewater. This run was conducted to ensure stable biological performance (e.g., TOC and TN removal) following the change in SRT. TrOC spiking to the synthetic wastewater was resumed from Day 226. The MBR was then run for 40 d at an IR ratio of 3. The MBR was operated for further 35 d without IR to assess the impact of recirculation of media from the aerobic to the anoxic reactor.

4.2.2 Sampling, DNA extraction and Pyrosequencing

Activated sludge samples for analysis of bacterial communities were collected from both anoxic and aerobic bioreactors at the end of each operational regime (Day 152, 225, 265 and 304 as shown in **Table 4.1**). The sludge samples were immediately frozen at -20^{o}C for later processing or pelleted immediately by centrifugation at 10,000 xg for 10 minutes. DNA extraction was carried out using the FastDNA spin kit for soil (MP Biomedical, Australia) following the manufacturer's protocol. The genomic DNA extract was analysed to determine its integrity, purity and concentration using 1% electrophoresis agarose gel and Nanodrop® ND-1000 spectrophotometer.

Table 4.1: Activated sludge samples from the anoxic-aerobic MBR for bacterial community analysis

Day	Operational regime	Day of sampling	Sample ID
0 - 125	MBR start-up period: *Infinite SRT, fine-tuning IR ratio (0.5 – 3), without TrOCs	No sequencing samples	
126 - 170	(**Condition1**) *Infinite SRT, IR = 3, with TrOCs	Day 152	$Anoxic_{152}$ $Oxic_{152}$
171 - 180	Stabilizing period: *Infinite SRT, IR = 3, no TrOCs	No sequencing samples	
181 - 225	(**Condition 2**) SRT = 25 d, IR = 3, without TrOCs	Day 225	$Anoxic_{225}$ $Oxic_{225}$
226 - 265	(**Condition 3**) SRT = 25 d, IR = 3, with TrOCs	Day 265	$Anoxic_{265}$ $Oxic_{265}$
266 - 305	(**Condition 4**) SRT = 25 d, IR = 0, with TrOCs	Day 304	$Anoxic_{304}$ $Oxic_{304}$

Note: SRT= sludge retention time; IR= internal recirculation ratio; *No sludge withdrawal except for sampling, resulting in a theoretical SRT of > 1000 d.

DNA samples for 454 pyrosequencing were sent to the Australian Genome Research Facility (AGRF) (Gehrmann Laboratories, University of Queensland, Australia). Amplicon pyrosequencing was performed using a standard Roche 454/GS-FLX Titanium Platform. The Bacterial domain was targeted by selecting the V1-V3 regions of the 16S rRNA gene (Kumar et al., 2011) with the forward primers 27F (5'-AGAGTTTGATCMTGGCTCAG- 3') and the reverse primer 519R (5'-GWATTACCGCGGCKGCTG- 3') (Lane, 1991).

4.2.3 Analysis of sequence data

SSU rRNA gene amplicons generated from pyrosequencing were processed using the Quantitative Insights Into Microbial Ecology (QIIME v1.8.0) software package (Caporaso et al., 2010b). Sequences were binned by barcode and quality filtered using the "split_libararies.py" script. Sequences with errors in the barcode or primer, shorter than 200 nt, longer than 1000 nt, with minimum average quality score less than 25, homopolymer run greater than 6 nt and sequences that contained ambiguous base calls were discarded from downstream analysis. The quality filtered data were then subjected to the following procedures using QIIME scripts with the default settings: (1) sequences were clustered at 97% similarity ; (2) cluster representative were selected, (3) GreenGenes taxonomy was assigned to the cluster using *uclust* (Edgar, 2010); (4) OTU

table with the abundance of different OTUs and their taxonomic assignments were generated; (5) the cluster representatives were aligned against the GreenGenes database (DeSantis et al., 2006) using *PyNast* (Caporaso et al., 2010a) and then alignment were filtered to remove the gap and excessive variable regions; (6) Netwick format phylogenetic tree was generated using *FastTtree* (Price et al., 2010). Chimeric sequences were identified by using *ChimeraSlayer* (Haas et al., 2011) and then filtered out of OTU table.

In order to eliminate heterogeneity related to different numbers of sequences among the samples, the OTU table was subsampled by randomly selecting (n = 10) the lowest number of sequences (1900) found among the samples. The diversity analysis was performed using procedures using QIIME scripts with the default setting. Alpha diversity (diversity within a given community) was characterized using qualitative species-based measure (Chao1) (Hughes et al., 2001), quantitative species-based measure (Shannon index) (Shannon, 2001) and qualitative divergence-based measure (PD_whole_tree/phylogenetic distance) (Faith, 1992). Chao1 determines the asymptote on an accumulative curve, predicting how many OTUs would be present if a high number of sequences had been collected. Shannon index accounts for both species diversity and evenness. A higher value for the Shannon index indicates greater microbial diversity and more even distribution. PD_whole_tree estimates the phylogenetic relationships based on the sum of total branch length in a phylogenetic tree that leads to each member of a community. The Good's coverage was used to estimate the sampling completeness and estimate the probability that a randomly selected amplicon sequence from a sample has already been sequenced. Good's coverage was calculated as $G = 1- n/N$, where n is the number of singleton phylotypes and N is the total number of sequences in the sample.

The community beta diversity (the partitioning of biological diversity among environments or along a gradient, e.g. the number of species shared between two environments) was measured using both unweighted and weighted UniFrac. Unweighted UniFrac (qualitative measurement which use only the presence/absence data) measures the distance between two communities by calculating the fraction of the branch length in a phylogenetic tree that leads to descendants in either, but not both, of the two communities (Lozupone and Knight, 2005). Weighted UniFrac (quantitative

measurement which use the abundance of each taxon) weights the branches of a phylogenetic tree based on the abundance information (Lozupone et al., 2007). The UniFrac distance metrics were interpreted to compare all communities simultaneously using standard multivariate statistical methods: a hierarchical clustering method called unweighted pair group method with arithmetic averages (UPGMA) (Sneath and Sokal, 1973) and principal coordinate analysis (PCoA) (Gower, 1966). UPGMA sequentially joins the least different samples to create a tree structure describing the differences between communities (cluster the community samples to relate the community samples to one another, not used to build the phylogenetic tree that relates the sequences). PCoA uses distance matrix to plot the n samples in (n-1) dimensional space. The vectors in this space or factors that describe as much as variation as possible can be plotted as axes in two dimensions for visualization to determine which environmental factors have the largest impact on community composition (Lozupone et al., 2007).

4.3 RESULTS AND DISCUSSION

The integrated anoxic-aerobic MBR was initially operated with no sludge withdrawal (infinite SRT) as a reference, and then under an SRT of 25 d for the rest of the operation period. The changes in operating conditions, namely SRT (infinite *vs*. 25 d), presence/absence of TrOC in influent, and application of IR affected the bulk organics, nutrient and TrOC removal performance. Changes in operating conditions can lead to changes in bacterial communities and their functions. The aim of this study is to assess the relationship of the changes in bacterial community with system performance.

4.3.1 Removal performance

A detailed discussion on the removal performance has been presented in Chapter 3. The removal efficiency of bulk organic and nutrient has been summarized in Error! Reference source not found. (Chapter 3)**.** Briefly, the MBR achieved high (up to 99%) and stable overall TOC removal throughout the entire operation period. Similarly, effluent ammonia was below detection limit regardless of changes in SRT, the presence/absence of TrOC in influent, and IR. Conversely, the removal of TN and orthophosphate depended on IR: over 80and over 90% removal of TN and orthophosphate, respectively, was achieved under an IR of 3 (condition 1, 2 and 3), which declined significantly when IR was ceased (condition 4).

The introduction of 30 selected TrOCs to feed wastewater did not show discernible impact on the basic biological performance of the MBR including TOC, TN and biomass. More than 90% removal was observed for 21 of the 30 added TrOCs (**Figure 3.5**). In general, a similar TrOC removal performance was noted irrespective of the SRT i.e., during application of infinite SRT (condition 1) and an SRT of 25d (condition 3), except for two recalcitrant compounds (carbamazepine and gemfibrozil) that were better removed during the longer SRT operation (**Figure 3.5**). In presence of IR, due to the well mixed conditions, virtually no difference between the removal performance of the anoxic and aerobic reactors was noted. However, operation without any IR between the bioreactors provided a clearer indication of the TrOC removal capacity of the anoxic reactor. In absence of IR, nine TrOCs, namely, primidone, metronidazole, triclosan, amitriptyline, 17β-estradiol-17-acetate, 4-tert-octylphenol, benzophenone, oxybenzone and octocrylene were removed with efficiencies ranging 50-90% by the anoxic bioreactor (**Figure 3.6**). Among these, however, triclosan, amitriptyline, 4-tert-octylphenol, benzophenol and octocrylene were removed mainly via adsorption to the sludge (**Figure 3.7**), suggesting a low TrOC transformation capacity by anoxic sludge in absence of IR (condition 4). Despite the variation in TrOC concentration in the anoxic reactor (depending on IR), the TrOC in the effluent of the subsequent aerobic MBR remained stable, indicating a major contribution of TrOC biodegradation by the aerobic reactor to the overall removal achieved by the anoxic-aerobic MBR (**Figure 3.6**).

4.3.2 Impact of operating conditions on bacterial diversity and structure

16S rRNA gene amplicons generated from pyrosequencing was binned by barcode and quality filtered using *Quantitative Insights Into Microbial Ecology (Qiime 1.8.0)*. After removing Chimeric sequences using *ChimeraSlayer*, a total of 33116 high quality amplicon reads were obtained with sequence statistics of 1946/5728/4014/1318 (min/max/median/std.dev., respectively). A total of 3422 operational taxonomic units (OTUs; at 97% sequence similarity cut-off level) were observed.

4.3.2.1 Alpha diversity

To verify the changes in the diversity of bacterial communities under different operational conditions in the MBR, the alpha diversity (diversity within a given

community) was characterized using a qualitative species-based measure (i.e., Chao1 index), quantitative species-based measure (i.e., Shannon index) and a qualitative divergence-based measure (PD_whole_tree/phylogenetic distance). Additionally, Good's coverage estimate was used to assess the sampling completeness and estimate the probability that a randomly selected amplicon sequence from a sample has already been sequenced. Although pyrosequencing libraries typically vary in the number of sequences per sample and the diversity estimates may vary with sequencing depth, trends in diversity among samples were less sensitive (Lundin et al., 2012). In order to eliminate the bias due to variation of sequencing depth , alpha diversity indices were measured and compared at an even sequencing depth of 1900 sequences per sample (i.e., the lowest number of sequences per sample noted among the samples) that was sufficient to capture the trend of sample diversity (Gihring et al., 2012; Lundin et al., 2012).

Under an IR of 3, the diversity of bacterial communities (as indicated by Chao1, Observed species, PD_whole_tree and Shannon index) was not significantly different between the anoxic and aerobic bioreactors for each condition (i.e., conditions 1, 2 and 3). Activated sludge samples in Condition 1 (samples $Anoxic_{152}$ and $Oxic_{152}$ at infinite SRT) showed the highest diversity (**Table 4.2**). Previous studies with SRT within the range of 3 to 60 d reported higher diversity of bacterial communities under longer SRTs (Duan et al., 2009; Roh and Chu, 2011; Vuono et al., 2014; Xia et al., 2012). While consistent with that in previous studies, the results in the current study extend the understanding to the case of no sludge withdrawal (i.e., theoretical infinite SRT). Notably, the bacterial diversity between the anoxic and aerobic communities was more similar for samples representing Conditions 1 and 3 (TrOC addition) than Condition 2 (no TrOC addition) (**Table 4.2**), indicating the effect of TrOC addition on the development of bacterial community.

The cessation of recirculation between the anoxic and aerobic bioreactors (i.e., IR=0, in Condition 4) resulted in the formation of two distinct communities in the respective bioreactors, as evidenced by the significantly different alpha diversity indices for the samples $Anoxic_{304}$ and $Oxic_{304}$. All alpha diversity indices (i.e., Chao1, Observed_species; PD_whole_tree; and Shannon index) demonstrated a significantly higher diversity of the aerobic community than that of the anoxic community in

Condition 4 (**Table 4.2**). Without recirculation, the anoxic bioreactor sample ($Anoxic_{304}$) showed an uneven distribution of bacterial community as shown by low Shannon index, indicating the dominance of a few bacterial groups.

Table 4.2: Alpha diversity indices (1900 sequences per sample, with iteration of n = 10).

Samples	No. of Seqs	Chao1	Good's coverage [%]	Observed_ species	PD_ whole_tree	Shannon
$Anoxic_{152}$	5728	1197 ± 154	82	536 ± 9	47.08 ± 0.77	7.42 ± 0.04
$Oxic_{152}$	5331	1272 ± 146	82	545 ± 12	47.43 ± 0.81	7.41 ± 0.04
$Anoxic_{225}$	1946	545 ± 14	91	320 ± 2	32.80 ± 0.09	5.48 ± 0.01
$Oxic_{225}$	3203	793 ± 63	88	372 ± 13	35.07 ± 0.96	6.00 ± 0.03
$Anoxic_{265}$	4654	851 ± 93	87	395 ± 15	36.30 ± 1.22	5.84 ± 0.09
$Oxic_{265}$	3165	852 ± 70	87	396 ± 9	34.25 ± 0.77	6.04 ± 0.05
$Anoxic_{304}$	3375	473 ± 78	93	230 ± 9	25.31 ± 0.85	4.60 ± 0.06
$Oxic_{304}$	5714	959 ± 90	86	408 ± 13	35.07 ± 0.75	6.32 ± 0.04

4.3.2.2 Beta diversity

Beta diversity indicates the partitioning of microbial diversity among environments or along a gradient, e.g., the number of species shared between two environments (Lozupone et al., 2007). In order to identify the impact of different operational factors (i.e., SRT, TrOC, IR and redox conditions) on microbial community in the integrated anoxic-aerobic MBR system, the community beta diversity was measured using both unweighted and weighted UniFrac. It is worth reiterating that unweighted UniFrac is a qualitative measurement based on the presence/absence of bacterial phylotypes, while weighted UniFrac is a quantitative measurement based on the abundance of each bacterial phylotype. The UniFrac distance metrics were interpreted to compare all communities simultaneously using standard multivariate statistical methods (Lozupone et al., 2007), namely, a hierarchical clustering method called Unweighted Pair Group

Method with Arithmetic Averages (UPGMA) (**Figure 4.1 A** and **B**) and Principal Coordinate Analysis (PCoA) (**Figure 4.1 C** and **D**).

UPGMA of both unweighted and weighted UniFrac grouped anoxic and aerobic samples in one cluster for all samples (i.e., Conditions 1, 2 and 3) under IR of 3 (**Figure 4.1 A** and **B**). Data for the anoxic and aerobic samples representing these conditions were also plotted closely by PCoA (**Figure 4.1 C** and **D**), suggesting that the bacterial communities developed in aerobic and anoxic reactors in presence of IR were highly similar in composition, structure and phylogenetic relationship. Previous studies also found no significant difference of bacterial communities between different redox conditions of the combined systems with IR (Gómez-Silván et al., 2014a; Kim et al., 2013; Xia et al., 2012). IR from aerobic to anoxic bioreactor may lead to the following: (i) dilution of the media, (ii) improved mixing/mass transfer, (iii) supply of nitrate, and (iv) transfer of a portion of DO from aerobic tank. Possibly, such sludge recirculation leads to the formation of a distinct ecology that facilitates the development of particular bacterial lineages. Gómez-Silván et al. (2014b) found that bacterial populations in an anoxic-aerobic MBR system including AOB, NOB and denitrifiers were active under both anoxic and aerobic conditions. By contrast, cessation of IR (condition 4) led to the formation of two separate redox zones (anoxic *vs.* aerobic). Accordingly, bacterial communities in anoxic ($Anoxic_{304}$) and aerobic ($Oxic_{304}$) samples were distantly clustered into two separate branches of the UPGMA tree (**Figure 4.1 A** and **B**) and also plotted distantly from each other in PCoA (**Figure 4.1 C** and **D**). It is likely that the separateredox zones (in case of IR=0) sustained the growth of specific bacterial lineages according to their oxygen requirement (further discussed in Section **4.3.3**). These observations suggest that IR is a key driving force for the development of bacterial communities in the integrated anoxic-aerobic MBR system.

In addition to IR, the impacts of SRT and TrOC addition were evident via both unweighted and weighted UniFrac analyses, although the order of importance of the factors according to each analysis appeared to be somewhat different. In unweighted UniFrac analysis, the cluster of samples belonging to IR of 3 was perfectly grouped into two distinct clusters according to the SRT (infinite *vs.* 25 d), and then according to TrOC (**Figure 4.1 A** and **C**). By contrast, weighted UniFrac clustered the bacterial communities principally by capacity of TrOC biotransformation (**Figure 4.1 C** and **D**).

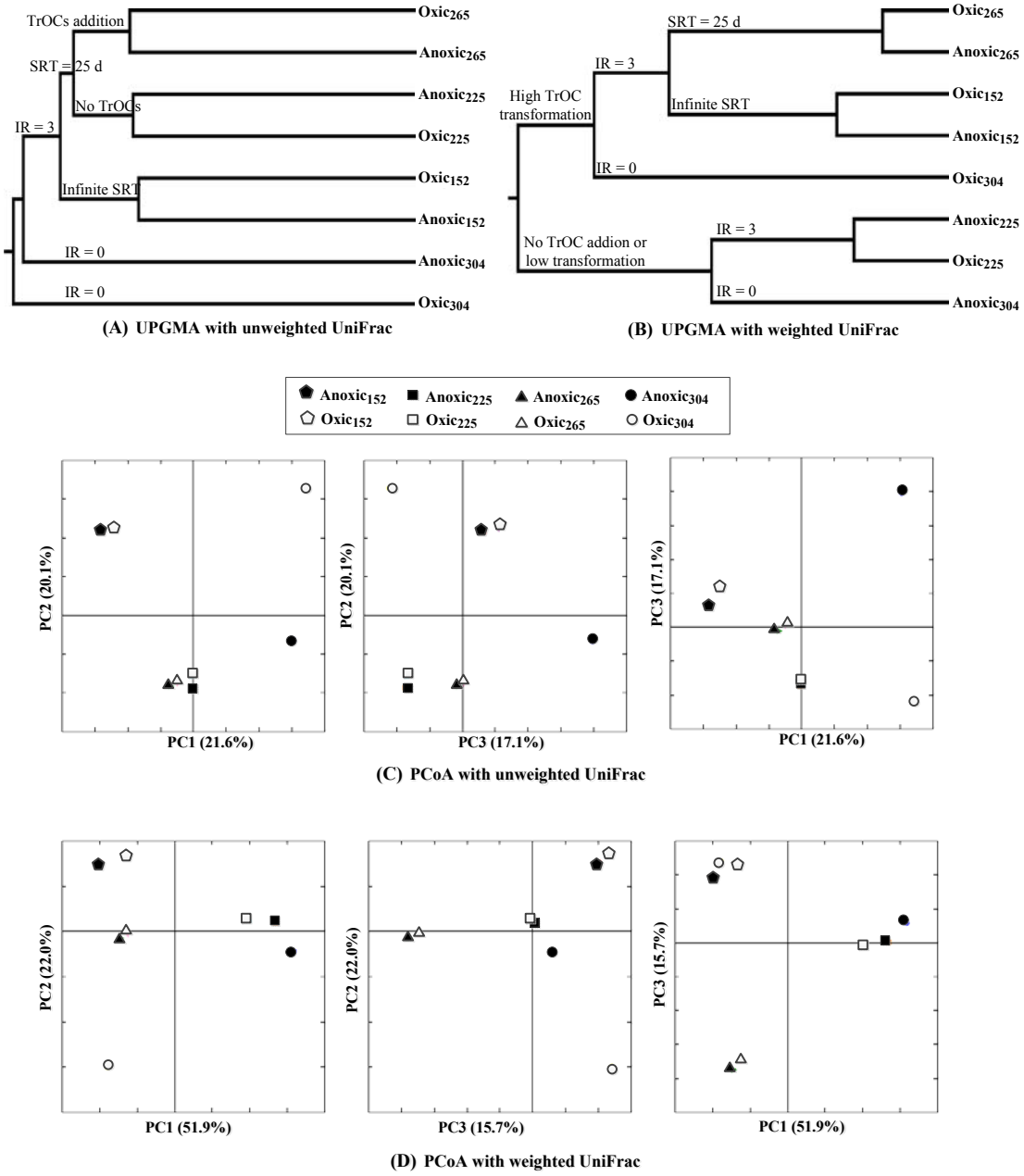

Figure 4.1: Analysis of bacterial communities with unweighted and weighted UniFrac visualized by hierarchical clustering of Pair Group Method with Arithmetic mean (UPGMA) and Principal coordinated analysis (PCoA). (A) and (B) are hierarchical cluster diagram for UPGMA with unweighted UniFrac and weighted UniFrac, respectively. (C) and (D) are PCoA with unweighted and weighted UniFrac, respectively.

4.3.3 Shifts in taxonomic profile

26 phyla were assigned to sequence reads of all eight samples (**Figure 4.2** and **Appendix Table A-3**). *Proteobacteria* and *Bacteroidetes* were observed to be the two dominant phyla in all conditions with a relative abundance of 22.8 – 64.1% and 14.7 - 47.7%, respectively (**Figure 4.2**). This observation is consistent with their previously reported abundance in activated sludge in general (Zhang et al., 2012). Based on the presence/absence of each taxon, the diversity analysis presented IR as the key driving force shaping the microbial communities in the integrated anoxic-aerobic MBR, while based on abundance of each bacterial phylotype the correlation of bacterial communities with TrOC biotransformation capacity was evident (**Figure 4.1)**. Taxonomic breakdown data (**Figure 4.2, Figure 4.3** and **Figure 4.4**) confirmed the difference in bacterial communities between different conditions (i.e., Conditions 1, 2, 3 and 4) and illustrated the impact of the operating parameters (i.e., SRT, TrOCs and IR). Consistent with the alpha (**Table 4.2**) and beta diversity (**Figure 4.1)** indices, the similarity of bacterial communities between the anoxic and aerobic bioreactors in all conditions in presence of IR was also clearly demonstrated by the taxonomic data right from the phylum level (**Figure 4.2)** up to the genus level (**Figure 4.3** and **Figure 4.4**).

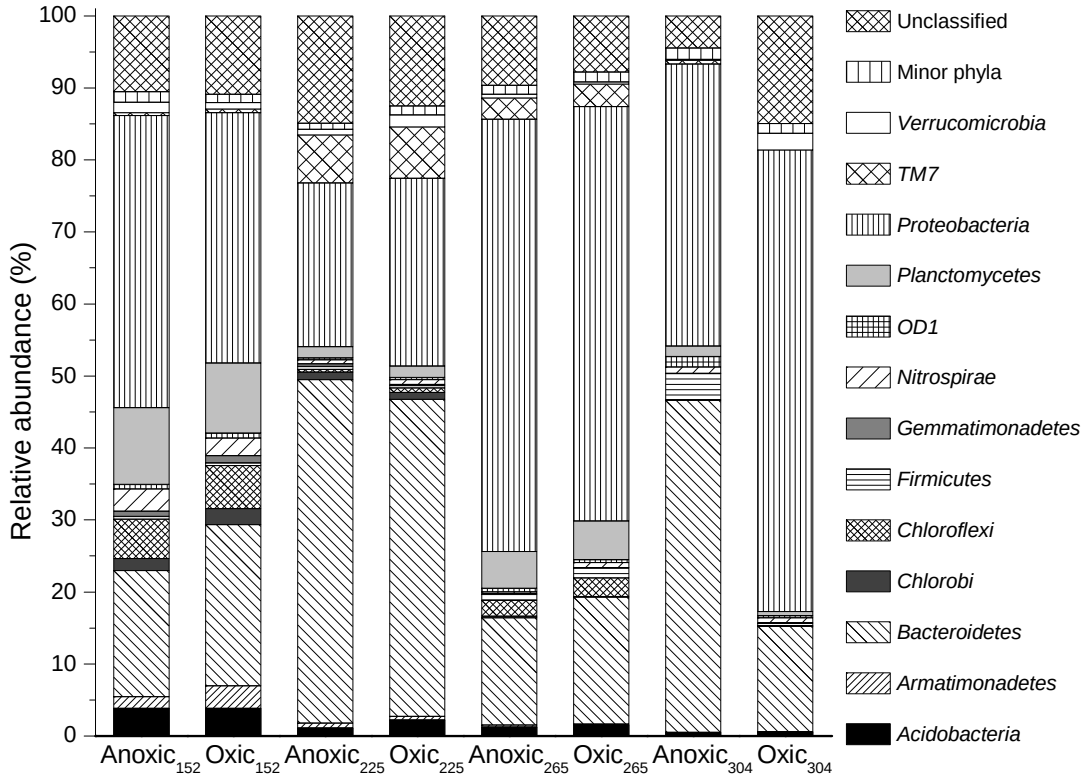

Figure 4.2: Relative abundance of bacterial phyla. 'Unclassified' indicates the sequences that could not be classified up to domain level. Phyla that were observed at less than 1% average abundance were grouped in 'Minor phyla' (see also **Appendix Table A-3**).

4.3.3.1 Impact of SRT

SRT relates to the specific biomass growth rate. Only the microorganisms that have doubling times less than the SRT will grow fast enough in the system to avoid wash out from the system (Vuono et al., 2014). *r-k* selection theory is based on, in addition to maximum growth rate, resource-use efficiency: *k*-strategists that are capable of efficiently utilizing scarce resources will enjoy advantages under long SRT conditions, while *r*-strategists (fast-growing organisms) are adapted for high resource environment under short SRT conditions (Schramm et al., 1999).

In the current study, switching operation from infinite SRT (condition 1) to an SRT of 25 d (conditions 2 and 3) led to disappearance or great reduction in relative abundance of many bacterial phylotypes (**Figure 4.2** and **Figure 4.3**). A significant reduction in relative abundance was clearly observed at the phylum level for *Plantomycetes*, *Chlorofloxi*, *Acidobacteria*, *Nitrospirae* and *Armatimonadetes* (**Figure 4.2**). These

bacterial phyla (except *Armatimonadetes*) were previously found to significantly decrease in proportion when SRT was reduced from 30 d to 12 d and then to 3 d (Vuono et al., 2014).

In order to have more detailed information, the relative abundance of the bacterial phylotypes at deeper levels of classification was scrutinized (**Figure 4.3**). Switching operation from infinite SRT to an SRT of 25 d led to a significant reduction in relative abundance of the taxa affiliated with *Rhodocyclaceae* (from 7.3 ± 1.5%, *n = 2* to 2.8 ± 0.9%, *n = 4*), *Phycisphaerales* (from 5.3 ± 0.24, *n = 2* to 0.9 ± 0.4%, *n = 4*), *Saprospirales* (3.04 ± 0.11%, *n = 2* to 0.10 ± 0.12%, *n = 4*) and *Oxalobacteraceae* (*Burkholderiales*) (from 2.32 ± 0.03%, *n = 2* to 0.84 ± 0.18, *n = 4*) (**Figure 4.3**). The proportion of *Nitrospira* (NOB) was found to significantly decrease from 2.76 ± 0.45% (*n=2*, condition 1: infinite SRT) to 0.54 ± 0.22% (*n=4*, condition 2 and 3: SRT of 25 d). This can be explained by the fact that *Nitrospira* is a *k*-strategist that shows dominance at longer SRT (Schramm et al., 1999; Vuono et al., 2014). The implication of these changes on removal performance will be discussed in Section **4.3.4.1**. The taxa affiliated with the phyla *Chloroflexi* and *Armatimonadetes*, which were detected at a relative abundance of 1-2%, mostly disappeared when the SRT was reduced to 25 d (Conditions 2 and 3) (**Appendix Table A-4**).

A number of taxa were found at higher relative abundance at an SRT of 25d (Conditions 2 and 3) than at infinite SRT (Condition 1) (**Figure 4.3**). For example, a significant increase in relative abundance was noted for the taxa affiliated with *Chitinophagaceae* (*Sphingobacteriales*) with SRT-decrease (SRT of 25 d: 5.59 ± 0.62%, *n = 4 vs*. infinite SRT: 0.42 ± 0.16%, *n = 2*) (**Figure 4.3**). Increase in the relative abundance of the taxa within *Sphingobacteriales* following a decrease in SRT was also reported by Vuono et al. (2014). The relative abundance of *Candidatus Accumulibacter* (*Rhodocyclales*) increased from 1.89 ± 0.03% (*n = 2*) to 5.84 ± 0.76% (*n = 4*) when the SRT was reduced. This increase probably relates to the function of this group – it is known as the most important polyphosphate accumulating organism (PAO) in activated sludge (Nielsen and Seviour, 2010), which requires periodic sludge withdrawal for phosphorous removal. Vuono et al. (2014), also observed an increase in relative abundance of the taxa under the order *Rhodocyclales* following a decrease in SRT. In the current study, *Acinetobacter* (*Pseudomonadales*), another PAO (Kim et al., 1997),

was also found at higher relative abundance at an SRT of 25 d (2.4 ± 0.63%, *n = 4 vs.* 1.14 ± 0.28%, *n = 2,* infinite SRT). The implications of the relative abundance of the PAOs on the phosphorous removal performance have been further discussed in Section **4.3.4.1**.

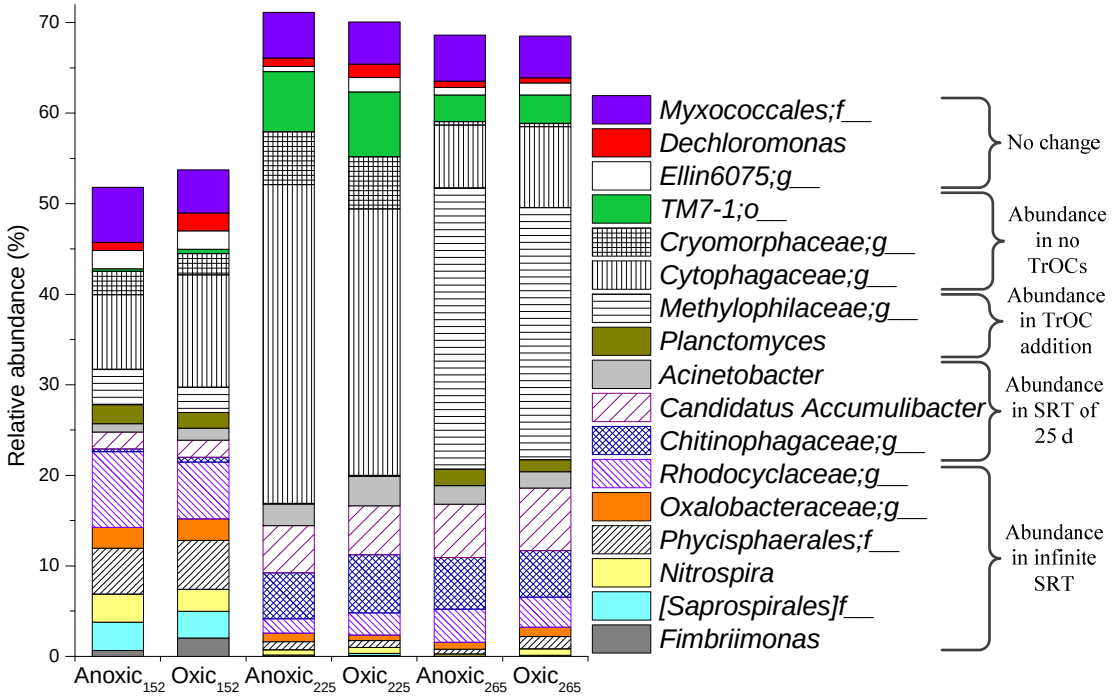

Figure 4.3: Relative abundance of the bacterial phylotypes at the deepest level of classification (order, family or genus) revealed by the data analysed. Anoxic and oxic samples were collected on day 152 (Condition 1: Infinite SRT, with TrOC), 225 (Condition 2: 25 d SRT, no TrOC), and 265 (Condition 3: 25 d SRT, with TrOC). The bacterial phylotypes showing at least an average relative abundance of 2% were arranged from the bottom to top in the following sequence: more relative abundance under infinite SRT, more relative abundance under SRT of 25 d, more relative abundance with TrOC addition, more relative abundance with no TrOC addition and, finally, no significant change. The protocol used in this study could provide information up to the genus level, but in some cases analysed data fell short to reveal the genus ("g__"), family ("f__") or even the order ("o__").

4.3.3.2 Impact of TrOC addition

Weighted UniFrac analysis (**Figure 4.1 C and D**) categorized the bacterial communities into two distinct clusters: i) communities detected in samples representing periods of

effective TrOC biotransformation in both reactors (Conditions 1 and 3) and those representing the aerobic reactor in absence of IR (Condition 4), and ii) communities present in samples when no TrOC was added (Condition 2) or when reduced TrOC biotransformation was observed (in the anoxic reactor in absence of IR, Condition 4). This pattern was also observed in the taxonomic profiles at phylum level (**Figure 4.2)**. *Proteobacteria* was the most abundant phylum (51.41 ± 12.93%, $n = 5$) in sludge with high effective TrOC transformation (Condition 1, 3 and aerobic sludge in condition 4). By contrast, *Bacteroidetes* was found to be the most abundant phylum in sludge with low TrOC transformation (anoxic sludge in Condition 4) or in absence of TrOC (Condition 2) (**Figure 4.2**). Higher relative abundance of *Plantomycetes* and *Chloroflexi* was noted in the presence of TrOC in influent, while TM7 was detected in greater proportions in absence of TrOCs (**Figure 4.2**).

The relative abundance of the bacterial phylotypes at deeper levels of classification was in line with the aforementioned trend observed at the phylum level (**Figure 4.3**). Considering the bacterial groups with at least 0.45% relative abundance, 7 phylotypes were found to occur only when the influent contained TrOC. Additional 11 phylotypes were more abundant in presence of TrOC. Conversely, 6 phylotypes showed higher abundance in absence of TrOC (**Figure 4.3** and **Appendix Table A-4**). The most abundant bacterial phylotypes in sludge samples from the period of effective TrOC removal (condition 3) was affiliated with *Methylophilaceae* which showed a relative abundance of 32.51% and 29.42% in anoxic and aerobic samples, respectively (**Figure 4.3**). However, it was absent in samples collected during Condition 2 with the same SRT of 25 d and the IR of 3, but without TrOC in influent. Without IR (Condition 4), this group showed higher relative abundance in aerobic sludge (6.63%) than in the anoxic sludge (3.94%) (**Figure 4.4**). This observation indicates that *Methylophilaceae* is a potential candidate for TrOC metabolism as discussed further in Section **4.3.4.2**. Some genera such as *Hyphomicrobium*, *Methylosinus* (*Rhizobiales)*, and *Allochromatium* (*Chromatiales)* were also mostly absent in Condition 2 (no TrOC addition in the influent) and anoxic sludge in Condition 4 (low TrOC transformation) (**Appendix Table A-4**).

Bacterial phylotypes associated with *Plantomyces* (*Plantomycetales*), *Gemmataceae* (*Gemmatales*), *Caldilinea* (*Caldilineales*) and *Lactococcus* (*Lactobacillales*) showed

relative abundance of 1 – 2 % during periods when IR was applied and effective TrOC removal was achieved (Condition 3 and/or Condition 1), while they showed very low relative abundance in Condition 2 (0.05 – 0.36%). In absence of IR (Condition 4), these bacterial phylotypes mostly disappeared or occurred at very low relative abundance (< 0.3%) (**Appendix Table A-4**). When detected, these bacterial lineages comprised a small portion of the whole community, but it is likely that they contributed to TrOC degradation. For example, many genera in *Lactobacillales* are thought to include species resistant to a broad range of antibiotic (*Lactobacillus, Enterococcus, Streptococcus, Leuconostoc* and *Pediococcus*) (Rabia and Shah, 2011) or capable of converting estrogens (*Streptococcus*) (Ojanotko-Harri et al., 1991). *Gemmatales* and *Planctomycetales* (*Plantomycetes*) have been reported to be involved in degradation of aromatic compounds including ethylbenzene, aminobenzene, naphthalene, bisphenol A, chloroxyclohexane, and polycyclic aromatic hydrocarbon (Guo et al., 2014; Musat et al., 2010). Larcher and Yargeau (2012) reported sulfamethoxazole degradation by *Rhodopirrellula baltica*, a member of *Plantomycetales*.

In line with the observations at the phylum level, bacterial phylotypes associated with *Cytophagaceae* (*Cytophagales*) formed the most abundant group (34.09 ± 4.21%, $n = 3$) in aerobic/anoxic sludge without TrOC addition (Condition 2) or in anoxic sludge showing low effective TrOC transformation in absence of IR (Condition 4). However, they only accounted for 7 – 12% in sludge with effective TrOC transformation (Conditions 1, 2 and aerobic sludge in Condition 4) (**Figure 4.3** and **Figure 4.4**). A phylotype affiliated with *Cryomorphaceae* (*Flavobacteriales*) showed higher relative abundance during the period of no TrOC presence (5.82 ± 0.06%, $n = 2$, compared to 1.02 ± 1.14% in presence of TrOC) (**Figure 4.3** and **Figure 4.4**). *Chryseobacterium* (*Flavobacteriales*), which was observed in absence of TrOC (0.21-0.44%) (**Appendix Table A-4**) or during low TrOC transformation (3.08%), mostly disappeared in presence of TrOC (**Figure 4.4**). A previous study using microautoradiography-fluorescene hybridization (Zang et al., 2008) found no estrogen-assimilating cells affiliated with the *Cytophaga-Flavobacterium* cluster. This cluster was also reported not to bear pharmaceuticals or s-triazines degradation capacity (Caracciolo et al., 2010). Possibly TrOC addition and their transformation products induced inhibition on the growth of these bacterial phylotypes.

Members belonging to the order *Myxococcales* were found at similar relative abundance (4.6% – 6.1%) in all three conditions (i.e., 1, 2 and 3), meaning that the factors SRT or TrOCs did not exert noticeable impact on their growth (**Figure 4.3**). However, their abundance was significantly changed by stopping IR in Condition 4 as discussed in the next section.

4.3.3.3 Impact of IR

IR between anoxic and aerobic reactors possibly formed a distinct environment that facilitated the development of a particular bacterial community shared between these two redox regimes. This shared community may be responsible for the core function of the integrated anoxic-aerobic MBR system. Taxonomic profile reveals that without IR, anoxic and aerobic reactors developed unique bacterial communities distinct from the shared communities formed under an IR of 3.

Stopping recirculation led to the disappearance or significant reduction in relative abundance of many bacterial phylotypes that were abundant in Condition 3 (i.e., SRT=25 d, IR=3, TrOC in influent). Significant changes in bacterial communities were evident at phylum level (**Figure 4.2** and **Appendix Table A-3**). As discussed in Section **4.3.3.2**, bacterial phylotype affiliated with *Methylophilaceae* was the most predominant group under Condition 3 (30.96 ± 2.19%, n = 2). In absence of IR (condition 4), its abundance decreased significantly to 3.79 and 6.04% for anoxic and aerobic samples, respectively (**Figure 4.4**). "*Candidatus* Accumulibacter" (*Rhodocyclales*), a key PAO, was one of the most predominant genus detected in Condition 3 (6 – 7%), but this bacterial phylotype almost disappeared in Condition 4 (0.4% and 0.1% for anoxic and aerobic sludge, respectively) (**Figure 4.4**). This may be explained by the fact that PAOs rely on alternative anoxic/aerobic regimes to accumulate phosphate and produce energy for growth. A phylotype affiliated with *Chitinophagaceae* (*Saprospirales*) showed a relative abundance of 5.42 ± 0.38% (n =2) in Condition 3, which reduced to 2.90% and 1.45% in anoxic and aerobic samples after stopping recirculation (Condition 4) (**Figure 4.4**). The relative abundance of the phylotypes belonging to the families *Saprospiraceae* and *Gemmataceae*, and genus *Planctomyces* also significantly reduced from 1 – 3% in Condition 3 to less than 0.6% in Condition 4 for both anoxic and aerobic samples (**Figure 4.4**). A number of phylotypes, which were detected in Condition 3 (0.45 - 3 %), were not found in samples representing Condition 4 (i.e, no IR). These include the

genera *Lactococcus* (*Lactobacillales*) *Cadilinea* (*Caldilineales*), *Allochromatium* (*Chromatiales*), *Pirellulaceae (Pirellulales)*, *Methylosinus (Rhizobiales)*, and *Phyllobacteriaceae* (*Rhizobiales*) (**Figure 4.4** and **Appendix Table A-4**). The members of the orders *Rhizobiales, Methylophilales, Rhodocyclales* are known to have denitrifying capacity (Morgan-Sagastume et al., 2008; Nielsen and Seviour, 2010). Therefore, their appearance in reduced proportions was probably the result of stopping recirculation that controlled the supply of nitrate from the aerobic zone to the anoxic zone.

Some bacterial phylotypes were enriched only in the anoxic reactor in absence of IR (Condition 4). The most abundant bacterial phylotype in anoxic sludge in Condition 4 was affiliated with *Cytophagaceae* (37.63%), which, however, comprised only 7 – 9 % of the anoxic/aerobic community in Condition 3 or aerobic community in Condition 4 (**Figure 4.4**). Their abundance can be explained by their capacity of consuming low- and high- molecular weight dissolved organic matter (Cottrell and Kirchman, 2000), which possibly gained them an advantage in competition with other organisms in the anoxic zone (where influent enters first) under Condition 4. The next dominant group of phylotypes in anoxic sludge of Condition 4 (i.e., without IR) were associated with *Enterobacteriaceae* (*Enterobacteriales*) (12.5%), and *Acinetobacter* (*Pseudomonadales*) (12%), but they were detected with a relative abundance of only 0.4 and 2%, respectively in aerobic/anoxic samples of Condition 3 or the aerobic sample of Condition 4 (**Figure 4.4**). Some bacterial phylotypes including *Chryseobacterium* (*Flavobacteriales*), *Fusibacter* (*Clostridiales*), and *Azospira* (*Rhodocyclales*) were present only in anoxic sludge of Condition 4 (1.2 - 3.1%) (**Figure 4.4**), which can be attributed to their growth characteristics as anaerobic/fermentative or facultative anaerobic bacteria.

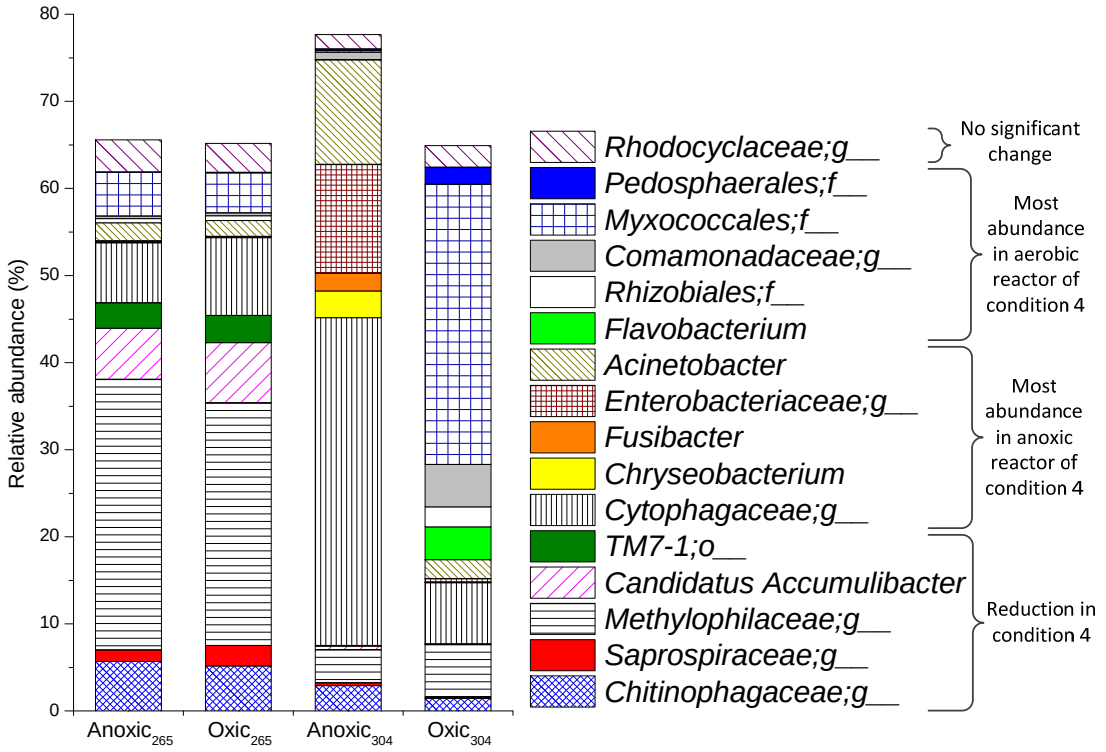

Figure 4.4: Relative abundance of the bacterial phylotypes at the deepest level of classification (order, family or genus) revealed by the data analysed. Anoxic and oxic samples were collected on day 265 (Condition 3: 25 d SRT, with TrOC, IR = 3) and 304 (Condition 4: 25 d SRT, with TrOC, IR = 0). The bacterial phylotypes showing at least an average relative abundance of 2% were arranged from the bottom to top in the following sequence: Significant reduction in Condition 4, most abundance in anoxic sample in Condition 4, most abundance in aerobic sample in Condition 4, and finally no significant change by cessation of IR. The protocol used in this study could provide information up to the genus level, but in some cases analysed data fell short to reveal the genus ("g__"), family ("f__") or even the order ("o__").

The removal performance of the aerobic reactor was similar in all four conditions. However, the aerobic sludge in Condition 4 (without IR) supported the growth of a unique bacterial community. The most dominant bacterial phylotype at this stage was affiliated with *Myxococcales*, which accounted for more than 32% of the community. This bacterial group comprised only around 5% of the community detected in aerobic/anoxic samples in Condition 3 (with IR), and a very low proportion (0.2%) of that in the anoxic sludge of Condition 4 (**Figure 4.4**), clearly demonstrating the impact of internal recirculation between anoxic/aerobic reactors on the composition of the

bacterial community. Notably, *Myxobacteria* are predominantly found in aerobic habitats and are capable of excreting hydrolytic enzymes and decomposing various complex biopolymers (Garrity et al., 2006). *Flavobacterium* showed a relative abundance of 3.75% in the aerobic sludge when IR was ceased, but this genus was not found during operation with IR, suggesting that this group may prefer strictly aerobic environment. Pentachlorophenol degradation by an aerobic, chlorophenol-utilizing *Flavobacterium* sp. has been reported (Steiert and Crawford, 1986). Phylotypes affiliated with *Rhizobiales*, *Comamonadaceae* (*Burkholderiales*) and *Pedosphaerales* showed significantly higher relative abundance (2 – 5% *vs*. <0.8%) in aerobic sludge in absence of IR. As discussed in Section **4.3.3.2**, members of *Rhizobiales* and *Burkhoderiales* are capable of utilizing TrOCs.

The differences in the bacterial communities developed under conditions with/without IR not only explain the variations in the nutrient removal performance by the system, but also provide insights into the variation in TrOC biodegradation and sorption under different redox conditions as described in the following sections.

4.3.4 Functional correlation

4.3.4.1 Bulk organic and nutrient removal

Although changes in the structure of bacterial communities in anoxic and aerobic samples between Conditions 1, 2 and 3 were noticed in response to changes in SRT or addition of TrOC in influent, the bulk organic and nutrient removal efficiency remained virtually unchanged. A high and stable (up to 90 %) overall TOC removal was achieved throughout the operation period (Error! Reference source not found.). Complete nitrification and over 80% TN removal was consistently achieved in all three conditions with an IR of 3. During operation with IR, more than 90% phosphate removal was maintained although the system was not designed specifically for phosphate removal (e.g., there was no strictly anaerobic reactor) (Phan et al., 2014). A possible explanation for this stable removal of TOC and TN despite the changes in relative abundance of the bacterial phylotypes is the functional redundancy of microbial community in activated sludge *i.e.*, the presence of a pool of species able to perform the same ecological function (Briones and Raskin, 2003). Possibly, an SRT of 25 d was adequate to sustain the development of functional bacteria for bulk organic and nutrient removal. For examples, the *Cytophaga-Flavobacter* cluster is thought to contain major bacterial

groups capable of dissolved organic matter consumption (Cottrell and Kirchman, 2000). Despite the variation of bacterial population, this cluster was consistently abundant (> 15%) for all conditions, explaining the consistently high removal of bulk organics.

The anoxic-aerobic MBR system was designed for nitrogen removal via nitrification/denitrification pathways by facilitating exposure of sludge to alternate anoxic/aerobic conditions. Autotrophic nitrification, the stepwise oxidation of ammonia to nitrate, is catalyzed by two different functional groups of microorganisms. First, ammonia is oxidized via hydroxylamine to nitrite by ammonia-oxidizing bacteria (AOB). Nitrite then is released and serves as a substrate for nitrite-oxidizing bacteria (NOB), which further oxidize it to nitrate, the end product of aerobic nitrification. Despite the efficient ammonia oxidization (ammonia in permeate was below the detection limit of 0.7 μg N/L as NH_3), only the AOB belonging to *Nitrosomonadaceae* were detected and that too at very low abundance (< 0.2%) (**Figure 4.5**). The observation made in the current study is consistent with previous studies (Ju and Zhang, 2015; Zhang et al., 2011). Possibly *Nitrosomonas* has high transcription activity despite the low abundance in activated sludge. It is also possible that there were unassigned or unidentified AOBs in activated sludge. Indeed the role of ammonia oxidizing archaea (AOA) in removing ammonia (Helbling et al., 2012; Park et al., 2006; Zhang et al., 2011) has been reported. Unfortunately, the primers designed for this study targeted only bacteria, not archaea.

Complete nitrification was achieved throughout the study. Notably only the *Nitrospira*-like NOBs were detected in this study irrespective of the SRT, although lower relative abundance of *Nitrospira*-like NOBs was noted under the shorter SRT (2.76 ± 0.45%, $n = 2$ in Condition 1, infinite SRT *vs*. 0.62 ± 0.22%, $n = 6$, 25 d SRT) (**Figure 4.5**). The observed key role of the *Nitrospira*-like NOB is in line with previous studies (Chiellini et al., 2013; Kim et al., 2013; Ma et al., 2013; Ye et al., 2011). The competitive abundance of the *Nitrospira*-like NOBs over the other NOBs may be attributed to their survival strategy. While *Nitrobacter* are regarded as *r*-strategists that can outgrow other NOBs quickly when substrate is not limited, *Nitrospira* have been shown to be *K*-strategists that display high substrate affinity and more resilience in case of substrate limitation (Schramm *et al.*, 1999; Nogueira and Melo, 2006). The usually low transient nitrite concentration in MBR because of high SRT (leading to low F/M ratio) favour the

growth of the *K*-strategists *Nitrospira* (Chiellini et al., 2013). This is consistent the with low nitrite concentration (under detection limit) in the MBR mixed liquor in this study. The *K*-strategy also explains the significant reduction of this NOB group under the shorter SRT (25 d) as mentioned above.

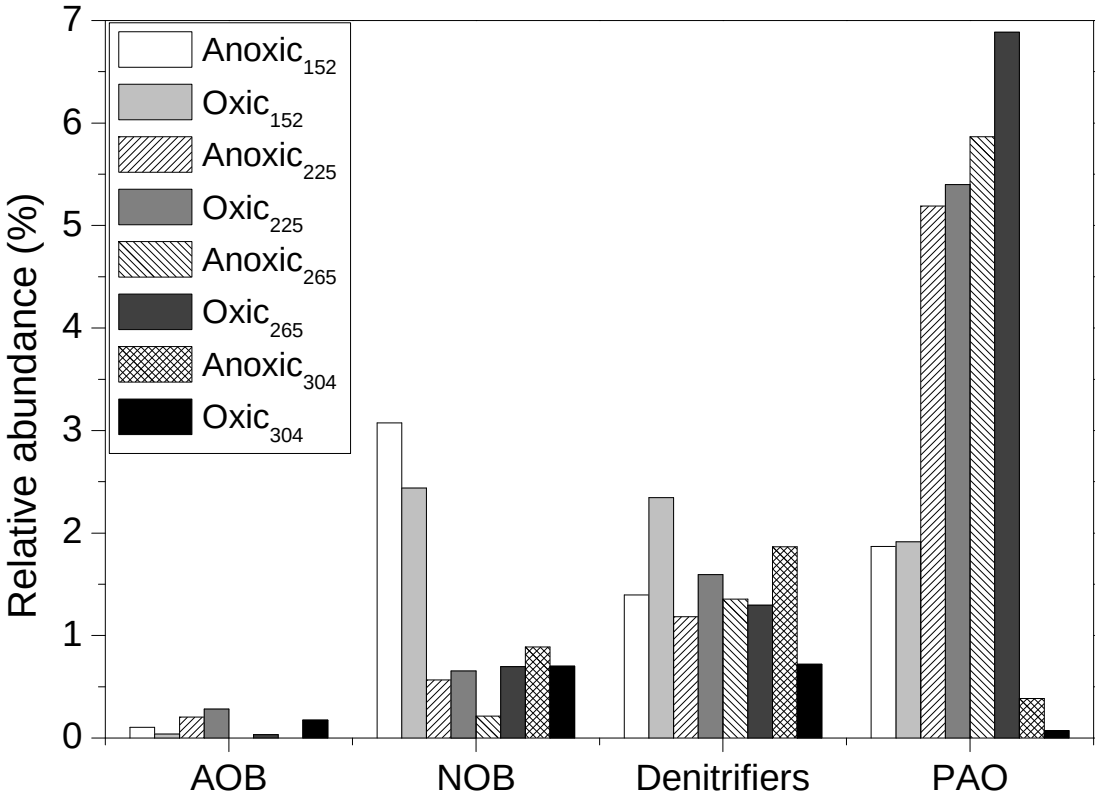

Figure 4.5: Relative abundance of potential functional bacterial groups including ammonia oxidizing bacteria (AOB: *Nitrosomonadaceae*), nitrite oxidizing bacteria (NOB: *Nitrospira*), polyphosphate accumulating organisms (PAO: "*Candidatus* Accumulibacter"), and denitrifers (*Hyphomicrobium, Rhodobacter, Acidovorax, Comamonas, Dechloromonas, Thauera* and *Zoogloea*).

Since ammonia removal via nitrification proceeds in the aerobic zone, stopping IR did not induce any impact on this process. Microbial community data verifies that AOB and NOB communities remained virtually unchanged when IR was ceased (**Figure 4.5**). By contrast, overall TN removal reduced by around 50% within 10 d of stopping recirculation because this ceased the supply of nitrate to the anoxic zone which is required for the denitrification process. Many bacterial groups have denitrifying capacity; however, *in situ* observation of their role in activated sludge would be rather complicated. Therefore, only the bacteria that are well known for their denitrifying role

in activated sludge have been closely analysed here. These include *Hyphomicrobium, Rhodobacter, Acidovorax, Comamonas, Dechloromonas, Thauera,* and *Zoogloea* (Guo et al., 2013; Nielsen and Seviour, 2010). Under an IR of 3 (conditions 1, 2 and 3), these bacteria showed stable abundance (1.2 – 1.4%) in the anoxic bioreactor. IR cessation did not change the abundance in the anoxic bioreactor, but significantly disrupted overall denitrification due to lack of supply of nitrate (**Figure 4.5**).

Under anaerobic conditions, PAOs assimilate fermentation products (i.e., volatile fatty acids) into storage products within the cells with the concomitant release of phosphorous from stored polyphosphates. Conversely, in the aerobic zone, energy is produced by the oxidation of storage products, and polyphosphate storage within the cell increases. When a portion of the biomass is wasted, the stored phosphorous is removed from the bioreactor for ultimate disposal with the wasted sludge. Therefore, integral to biological phosphorous removal are IR and sludge withdrawal. The role of PAOs in the current study is evident from the significant accumulation of phosphorus in the anoxic reactor in absence of either sludge withdrawal (Condition 1) or IR (Condition 4) (Phan et al., 2014). Consistent with the trend of phosphorus removal, *Candidatus* Accumulibacter, which is a PAO, showed higher abundance in presence of IR (5.84 ± 0.76%, *n = 4 vs*. 0.23 ± 0.22%, *n = 2*) (**Figure 4.5**). Furthermore, with IR, their relative abundance was significantly higher under an SRT of 25 d (5.84 ± 0.76%, *n =4*) than with infinite SRT i.e., no sludge withdrawal (1.89 ± 0.03%, *n =2*). The relative abundance correlated well with the phosphorus removal performance (Error! Reference source not found.). It is noteworthy that this PAO group is also regarded as an important denitrifying group (Nielsen and Seviour, 2010). Their higher relative abundance compared to other denitrifying groups (**Figure 4.5**) possibly demonstrates their important role in denitrification.

Plantomycetes was found to be the third dominant phylum (in presence of both IR and TrOC (5-10%), but they were detected only at a relative abundance of around 1.5% when either IR or TrOC was absent (**Figure 4.2**). It is notable that, anammox bacteria, which belong to the order of the *Brocadiales*, are affiliated to the *Plantomycetes* (Jetten et al., 2009). About 30 -50% of the global marine nitrogen loss is attributed to the activity of these anammox bacteria (Arrigo, 2005). A bacterial group affiliated with *Plantomycetes* was noted to induce significant nitrogen removal in a moving bed

biofilm reactor via anammox pathway (van Kessel et al., 2010). Coexistence of nitrifying, anammox and denitrifying bacteria was reported in a sequencing batch reactor (Langone et al., 2014). High relative abundance of *Plantomycetes* and their reduction due to cessation of IR in the current study suggest their highly likely role in TN removal. Their reduction in the absence of TrOC (Condition 2) also indicates their relationship with TrOC transformation as discussed in the next section.

4.3.4.2 Correlation between TrOC biodegradation and bacterial community

In this study TOC, TN and TP removal was unaffected by the operation SRT. However, better removal of two recalcitrant compounds namely carbamazepine and gemfibrozil was observed in absence of sludge withdrawal (i.e., theoretical infinite SRT). Analysis of bacterial community in the present study suggests that this difference in TrOC removal may be related to the diversity of microbial communities. Longer SRT resulted in higher bacterial diversity as demonstrated by alpha diversity indices (**Table 4.2**) and taxonomic profile (**Figure 4.2** and **Figure 4.3**). Higher microbial diversity may lead to a more diverse enzymatic profile (Ittisupornrat et al., 2014), which may achieve better removal of recalcitrant compounds. For example, *Burkholderiales* was found to be more abundant under infinite SRT (Condition 1). This bacterial order was previously identified to be predominant in sludge treating sulfamethoxazone (Esplugas et al., 2013) and also in chlorinated aliphatics and aromatic hydrocarbon-contaminated groundwater (Abbai and Pillay, 2013). Members of this order are characterized as methyl tert-butyl ether and tert-butyl alcohol degraders (Key et al., 2013).

Nitrification is biological oxidation of ammonium to nitrite and nitrate catalyzed by AOB and NOB. Because it is an aerobic process, stopping recirculation in between anoxic/aerobic reactor did not induce any impact on this process. Indeed, microbial community analysis verifies that AOB and NOB communities were unaffected by stopping IR (**Figure 4.5**). Consistent with that, similarly high biodegradation of TrOCs in the aerobic bioreactor continued in absence of IR. This indicates a pivotal role of nitrifiers in TrOC removal, which is consistent with recent literature (Fernandez-Fontaina et al., 2014; Tran et al., 2013).

In this study, the TrOC removal data demonstrates that anoxic condition may enhance the removal of nine of the 30 TrOCs added (**Figure 3.7**),. However, when IR was

absent, the anoxic removal of some compounds namely, triclosan, amitriptyline, 4-tert-octylphenol, benzophenol and octocrylene occurred mainly via adsorption to sludge (**Figure 3.7**), suggesting a low TrOC transformation capacity. Stopping recirculation led to the deterioration of denitrification and hence TN removal. The microbial community data clearly illustrates significant reduction of denitrifying bacteria in the anoxic reactor (**Figure 4.5**). This impact of IR on the bacterial structure explains the low TrOC biodegradation by the anoxic sludge under no IR. Some isolated/enriched representatives of denitrifiers have been shown to possess TrOC degradation capacity (Isamil and Chiang, 2011). Most prevalent among these in this study are the members affiliated with *Rhodocyclaceae* that showed significant relative abundance (around 10%) in the presence of IR than in absence (around 5%).

The change in abundance of *Plantomycetes* due to TrOC addition in this study is interesting. This change was driven by the bacterial phylotypes affiliated with *Gemmataceae*, *Pirellulaceae* and *Planctomycetaceae*. Under an IR of 3, their relative abundance was higher in presence of TrOCs (4.37 ± 0.52%, $n = 4$ *vs*. 0.52 ± 0.07%, $n = 2$) (**Figure 4.3**), which suggests their involvement in TrOC transformation. Indeed genomic analysis of 11 representatives of *Plantomycetales* revealed their capacity to degrade diverse toxic compounds including ethylbenzene, aminobenzoate, naphthalene, bisphenol A, chloroxyclohexane and polycyclic aromatic hydrocarbon (Guo et al., 2014). Further notable is that the relative abundance of the *Plantomycetes* decreased significantly (0.35 ± 0.17%, $n = 2$) when IR was stopped (**Appendix Table A-3**), which coincides with the period when nitrogen removal was significantly affected (Error! Reference source not found.). Thus the members of *Plantomycetes* were probably also involved in nitrogen removal. Nitrogen removal by this group may be attributed to anammox process; however, this speculation needs to be verified.

After stopping IR, aerobic TrOC biotransformation was not affected significantly, while a reduced TrOC biotransformation by the anoxic sludge was observed. Overall the TrOC removal by the MBR was stable. This indicates a key role of the aerobic reactor in TrOC degradation. In order to derive a clearer understanding of the bacteria that may have played a pivotal role in TrOC degradation, the bacteria that developed abundantly in aerobic sludge under no IR condition (but showed rare or no presence in the anoxic sludge) were closely examined. The most abundant bacteria in aerobic sludge under no

IR condition were the members of the order *Myxococcales*. This order accounted for more than 33% in aerobic sludge, but was only present at less than 0.3% in the anoxic sludge which showed low TrOC transformation in absence of IR (**Figure 4.4**). Their capacity of excreting hydrolytic enzymes and decomposing various complex biopolymers (Garrity et al., 2006) possibly contributed to biotransformation of TrOCs. The members of the order *Methylococcales* also may have played an important role in TrOC transformation. In absence of IR, this bacterial order was not detected in anoxic sludge. It was also absent in the sludge when the influent did not contain TrOCs (Condition 2: IR of 3, no TrOCs). By contrast, this bacterial group was present in all sludge treating TrOCs (0.28 – 0.35% and 0.03 – 0.08% in Condition 1 and 2, respectively), particularly in aerobic sludge under no IR (~0.2%) (**Figure 4.4**). It is noteworthy that the members of the orders *Bdellovibrionales* and *Sphingobacteriales* were detected at significant relative abundance in aerobic sludge (more than 0.5%), but did not appear (*Bdellobibrionales*) or rarely appeared (*Sphingobacteriales*) in anoxic sludge after stopping recirculation (**Figure 4.4**).

The bacteria that appeared in sludge only when the influent contained TrOCs may be relevant to TrOC biodegradation. Notably, the members of the order *Methylophilales* grew in sludge treating TrOCs in Condition 1 (~3.7 ± 0.8%), but disappeared when TrOC addition was stopped in Condition 2. However, they strongly reappeared to become the most abundant group (32 ± 2%) when TrOC addition was resumed in Condition 3 (**Figure 4.3**). When IR was ceased, their relative abundance again reduced to 4.94% and 6.64% in anoxic and aerobic sludge, respectively. Members belonging to *Methylophilales* are obligate methylotrohphs that only grow on reduced carbon compounds containing no C-C bonds such as methane, methanol, and methylated amines (Chistoserdova et al., 2007; Ginige et al., 2004). In the current study, the possible source of carbon in the synthetic wastewater was glucose, peptone, acetate and urea. Thus there is a possibility that the members of *Methylophilales* employed some TrOCs as the carbon source. TrOCs such as formononetin, ametryn, ibuprofen, ketoprofen, primidone, and naproxen (all without C-C bonds) potentially provided carbon source for the growth of *Methylophilales*. Their ability to degrade microcystin, a cyanobacterial toxin bearing nitrogen in its cyclic structure, (Mou et al., 2013) may indicate their capacity of degrading TrOCs.

4.4 CONCLUSIONS

The bacterial communities developed in the anoxic and aerobic bioreactor of the integrated anoxic-aerobic MBR were highly similar in structure and phylogenetic relationship due to internal recirculation (IR), which confirms IR as a key driving force shaping bacterial communities in the anoxic-aerobic MBR system. In absence of IR, significantly different microbial communities developed in anoxic and anaerobic bioreactors, consequently leading to significantly low nutrient and TrOC removal capacity of the anoxic bioreactor. Higher bacterial diversity under the longer SRT was evident; however, except for a few TrOCs the removal of TOC, TN and TrOCs were the same irrespective of the SRT. TrOC addition also induced shifts in bacterial communities, and UniFrac analysis (based on relative abundance of bacterial phylotypes) as well as taxonomic profile analysis indicated a relationship between microbial communities and TrOC biotransformation. Potential bacterial groups for TrOC biotransformation such as taxa within the order *Methylophilales* and *Myxococcales* were identified.

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CHAPTER 5. Comparison between Pilot- and Full-Scale Anoxic-Aerobic Membrane Bioreactors Regarding Nutrient and Trace Organic Contaminant Removal

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5.1 INTRODUCTION

Small to medium WWTPs are being progressively implemented in small townships and tourism hot spots around Australia to phase out unreliable septic tank systems. Several studies have assessed the occurrence of TrOCs in wastewater originating from various catchment areas including agricultural, rural, urban and industrial wastewater catchments (Lai et al., 2013; Leusch et al., 2014; Scott et al., 2014) and the treated effluent produced by various types and scales of WWTPs (Coleman et al., 2008; Leusch et al., 2014; Ying et al., 2009). However, only a few studies reported the TrOC profile of raw sewage generated from small Australian towns (Braga et al., 2005; Coleman et al., 2008; Leusch et al., 2014), particularly those which are tourist destinations (Le-Minh et al., 2010; Trinh et al., 2012b). Wastewater from small resort towns can have distinct characteristics in terms of volume of wastewater produced and also the frequency and concentration in which TrOCs may occur. A majority of the Australian studies investigated the removal efficiency of WWTPs for endocrine disrupting chemicals (EDCs), particularly the steroid hormones, with fewer studies also focusing on pharmaceuticals, industrial chemicals and pesticides (Le-Minh et al., 2010; Trinh et al., 2012a). Notably most available reports on TrOC removal from real sewage have documented the performance of conventional treatment technologies (e.g., activated sludge process, biofilters, and lagoons) (Ying et al., 2009).

Membrane bioreactors (MBRs) are an attractive option for decentralised wastewater treatment and reuse due to their ability to produce high quality effluent with a small footprint (Hai et al., 2014). MBRs account for the majority of new sewage treatment infrastructure in Australia. Most of these are small to medium MBR plants for water recycling applications in coastal towns and small cities, and are mostly driven by stringent environmental regulations, particularly targeting nutrient and TrOC removal, and to a lesser extent by freshwater scarcity. To date only a few Australian studies have investigated TrOC removal from real sewage, and these studies have been conducted mostly via pilot-scale MBRs (Coleman et al., 2008; Le-Minh et al., 2010; Trinh et al., 2012b). Available studies provide useful preliminary understanding; however the performance of current MBR technology as a barrier for a range of TrOCs and specific removal mechanisms involved remains unclear.

Sequential exposure to different redox conditions is a pre-requisite to nutrient (i.e., nitrogen and phosphorus) removal from wastewater. A simple two-stage pre-anoxic/aerobic reactor configuration can typically meet the total nitrogen (TN) disposal guideline of 10 mg L^{-1} (Hai et al., 2014). A series of aerobic/anoxic zones with supplemental organic carbon dosing to the anoxic zone may be required to achieve further improved TN removal to comply with a more stringent effluent TN guideline (sensitive areas). This may also facilitate stable removal when TN loading in wastewater fluctuates significantly. Notable in this context is that recent studies demonstrate close relationships between stable NH_4^+-N removal and the removal of TrOCs (Helbling et al., 2012). Most of the previous reports have shown the correlation of TrOC removal with the stability of NH_4^+-N removal via batch tests conducted with synthetic wastewater. Other than the only study by Vader et al. (2000) who showed a noticeable connection between NH_4^+-N and 17α- ethinyl estradiol removal, this correlation has not been validated at full scale level. Furthermore, compared with aerobic (nitrifying) conditions, fewer studies have investigated TrOC removal performance of combined anoxic/aerobic reactors (Phan et al., 2014; Xue et al., 2010). It is not clear whether, like TN, a combination of a number of aerobic and anoxic zones with different levels of dissolved oxygen concentration (DO) may be conducive to removal of different TrOC categories.

Given the research gaps discussed above, the aim of this study was to assess the occurrence and removal of a broad spectrum of TrOCs by a full-scale MBR plant serving a small resort town. Performance comparison with a pilot-scale MBR fed with the same sewage was used to clarify important aspects regarding bulk organics, TrOCs and nutrient removal. The potential impact of the application of multiple sequences of anoxic/aerobic regimes on nutrient and TrOC removal is also discussed.

5.2 MATERIALS AND METHOD

This study was conducted at the site of a decentralised full-scale MBR plant (designed for a maximum capacity of 743 m^3/d) located in Kangaroo Valley (New South Wales, Australia), which is a tourist destination known for caravan parks. The village has a permanent population of about 340 people; however this increases during peak holiday periods to approximately 1400. In addition to influent wastewater sampling over 15

events (from November 2012 to October 2014), a pilot-scale MBR was operated at the site. It was first operated for 11 weeks for acclimatization and performance stabilization. Then, a 10-week sampling campaign was carried out to compare the treatment performance of the pilot- and full-scale MBRs receiving the same sewage.

5.2.1 Description of the full-scale MBR

The MBR received wastewater via a pressurised sewerage network from the Kangaroo Valley township. A schematic diagram of the plant is presented in **Figure 5.1**. The treatment process comprised i) primary treatment, ii) parallel trains of activated sludge reactors integrated with membrane filtration cells, and iii) an UV disinfection unit (UV dose of 40 mJ/cm^2). One of the duplicate process trains was operated in stand-by mode. The activated sludge system consisted of a pre-anoxic zone (DO = 0-0.5 mg/L), aerobic zone-1 (DO = 0.5-1.0 mg/L), aerobic zone-2 (DO = 2-2.5 mg/L), and a post-anoxic zone (DO = 0-0.5 mg/L) receiving supplemental organic carbon (acetic acid, approximately 40 L/d) to enhance denitrification. The mixed liquor from the aerobic zone-2 was recycled to the pre-anoxic zone with an internal recirculation ratio of 4. The return activated sludge from the membrane cell was recycled to aerobic zone-2 and the pre-anoxic zone, also with a recirculation ratio of 4. The solids retention time (SRT) of the MBR was 25 d and the mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentration in the aerobic reactors were respectively 8.5 ± 0.7 and 6.2 ± 0.5 g/L (n=10) during the period of performance-comparison with the pilot MBR. The total hydraulic retention time (HRT) was 1.5 - 1.7 d with approximate HRTs in pre- anoxic zone, aerobic zone-1, aerobic zone-2, post-anoxic zone and the membrane cell of 0.45, 0.45, 0.45, 0.22 and 0.14 d, respectively. PVDF microfiltration membranes (Memcor, Evoqua Water Technologies, Australia) were submerged into the membrane cells to provide a surface area of 2400 m^2/cell.

During the course of this study, the total sewage flowrate was 146 ± 76 m^3/d (n = 66), and the membrane flux was 2.1 ± 1.1 (n = 66) and 1.1 ± 1.1 L/m^2 h (n = 31) for the primary and stand-by membrane cells, respectively. The final effluent was directed to a storage dam and then used for irrigation to farms and recreational facilities around the region.

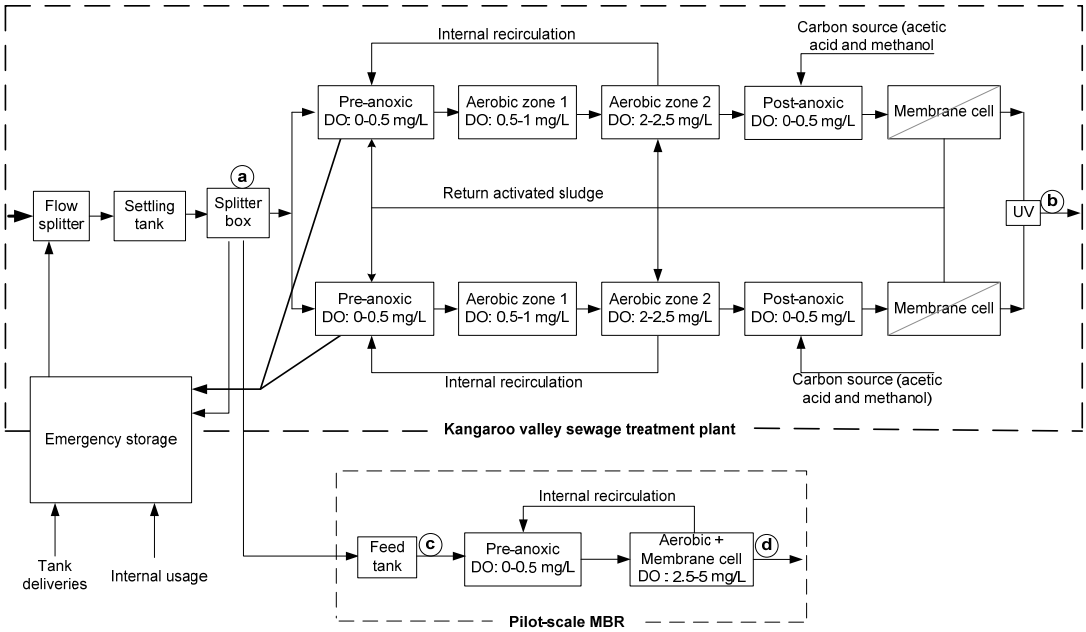

Figure 5.1: Layout of the full- and pilot-scale membrane bioreactors (MBRs), summarizing the key components. 'a' and 'b' indicate influent and effluent sampling points for the full-scale MBR. 'c' and 'd' indicate influent and effluent sampling points for the pilot MBR.

5.2.2 Pilot-scale MBR setup and operation

A pilot-scale anoxic-aerobic (**Figure 5.1**) MBR was operated parallel to the full-scale MBR. The pilot MBR was operated under the same SRT (25 d), total HRT (1.5 d) and with the same internal recirculation ratio (4) between anoxic-aerobic reactors. However, compared to the four reactors (2 x anoxic and 2 x aerobic) in the full-scale MBR, it contained only one pre-anoxic zone (working volume= 13.8 L, HRT= 0.8 d) and an aerobic zone (working volume= 11.7 L, HRT= 0.7 d). A hollow fibre ultrafiltration membrane (Zeweed-10) supplied by Zenon Environmental (Ontario, Canada) was submerged in the aerobic reactor. This membrane had a nominal pore size of 0.04 μm and an effective membrane surface area of 0.93 m^2, and was operated at a flux of 1.2 L/m^2 h. The transmembrane pressure (TMP) was continuously recorded via a high resolution (±0.1 kPa) pressure sensor connected to a data logging computer. All pumps were controlled via the same computer, and this computer was remotely controlled over the internet using the software TeamViewer. Throughout the whole experimental period, *in-situ* air scrubbing was found adequate to keep the TMP stable below 5 kPa, and no chemical cleaning was required. The mixed liquor pH was stable at

7.14 ± 0.35 (n = 14) and 7.43 ± 0.45 (n = 14) for the anoxic and aerobic bioreactors, respectively. DO was maintained in the range of 2.5 – 5 mg/L for the aerobic zone and 0 - 0.25 mg/L for the anoxic zone. The temperature inside the bioreactors varied according to the ambient temperature at 18 ± 3 ^{o}C. MLSS and MLVSS concentrations of the anoxic reactor were 4.13 ± 0.5 and 2.68 ± 0.3 g/L (n=18), respectively, with the corresponding values (n=18) of 2.35 ± 0.76 (MLSS) and 1.47 ± 0.57 (MLVSS) g/L for the aerobic bioreactor.

5.2.3 Sample collection and analysis

5.2.3.1 Sample collection

Amber glass bottles (500 mL) pre-rinsed with Milli-Q water were used for sample collection. Grab sewage samples (35) after primary settling (**Figure 5.1**) were collected over 15 sampling events to characterize the sewage originated from Kangaroo Valley. These influent samples were collected in duplicate (first 10 sampling events) or triplicate (last five sampling events) from November 2012 to October 2014 and analysed for both bulk organics and TrOCs. On the other hand, following the 11-week acclimatization period of the pilot MBR, effluent samples from the pilot- and full-scale MBRs along with the influent samples were collected to compare their performance over a period of 10 weeks. TrOC removal by the pilot- and full-scale MBRs was monitored during the last six week of sampling.

5.2.3.2 Analysis of basic parameters

Total organic carbon (TOC) and total nitrogen (TN) were analysed using a TOC/TN-V_{CSH} analyser (Shimadzu, Japan). Chemical oxygen demand (COD) was analysed using COD vials (0-1500 ppm, WatertestSystems, Australia) with a Hach DR 5000 spectrophotometer according to the Standard Method 5220 D (Eaton et al., 2005). NH_4^+-N and ortho- PO_4^{3-}-P concentrations were measured using flow injection analysis (Lachat instruments, Milwaukee, USA) following the Standard Methods 4500-NH_3 H and 4500-P G, respectively (Eaton et al., 2005). MLSS and MLVSS concentrations in bioreactors were measured according to the Standard Method 2540 (Eaton et al., 2005).

5.2.3.3 Trace organic contaminant analysis

In total, 45 TrOCs including 27 PPCPs, four industrial chemicals, eight steroid hormones and six pesticides were monitored in this study. Influent and MBR effluent samples (0.5 L) were collected and immediately transferred to the laboratory. The influent samples were filtered through 1 μm and then 0.45 μm glass fibre filters (Millipore, Australia), but the membrane-permeate samples were not further filtered. Concentrations of TrOCs were determined by solid phase extraction (Oasis HLB, Waters, Millford, MA, USA) followed by analysis using high performance liquid chromatography (HPLC) (Agilent 1200 series, Palo Alto, CA, USA) coupled with tandem triple quadrupole mass spectrometer (API 4000, Applied Biosystems, Foster City, CA, USA) employed in both positive and negative electro-spray modes and atmospheric pressure chemical ionization (APCI) in positive mode. Isotope dilution was used to quantify all analytes unless otherwise stated. The detailed method is available in **Appendix Table A-5**.

5.2.3.4 Statistical analysis of data

Average ± standard deviation values were used to compare the concentrations and removal/reduction efficiency of different parameters namely, TOC, COD, TN, NH_4^+-N and PO_4^{3-}-P. Distributions of TrOC concentrations were analysed in terms of maxima, minima, 95th and 5th percentiles and the median. Paired *t*-test of the TrOC removal data (pilot- *vs.* full-scale MBR) was conducted using the *t*-test function in Microsoft Excel. Values of $p < 0.05$ were considered to indicate statistical significance.

5.3 RESULTS AND DISCUSSION

5.3.1 Bulk organics removal

To assess the bulk organics removal by the pilot- and full- scale MBRs, both TOC and COD were analysed. During the period of comparison (Day 77 – 146), the influent TOC was 68 ± 25 mg/L (n = 10). The effluent TOC concentration for the pilot- and the full-scale MBR varied in the range of 21 ± 14 and 30 ± 15 mg/L, respectively (**Figure 5.2**). The removal efficiency was 68 ± 15 % for the pilot-scale MBR and 52 ± 22 % for the full-scale MBR. On the other hand, the influent COD varied in the range (n = 10) of 156 ± 91 mg/L (**Figure 5.3**). The range of effluent COD concentration was 25 ± 15 (pilot-

scale MBR) and 19±6 (full-scale MBR) mg/L. Accordingly, the removal efficiencies were 78 ± 17 % and 82 ± 14 % for the pilot- and full- scale MBRs, respectively.

External carbon source (acetic acid) was added to the post-anoxic reactor of the full-scale MBR in order to boost denitrification. Over-addition of carbon may, however, leave excess carbon in the effluent (Chou et al., 2003). This may explain the somewhat lower TOC removal by the full-scale MBR (**Figure 5.2**). Conversely, the COD removal efficiency of the MBRs was rather similar (**Figure 5.3**), indicating that TOC is a more sensitive parameter to capture variations in bulk organics removal performance.

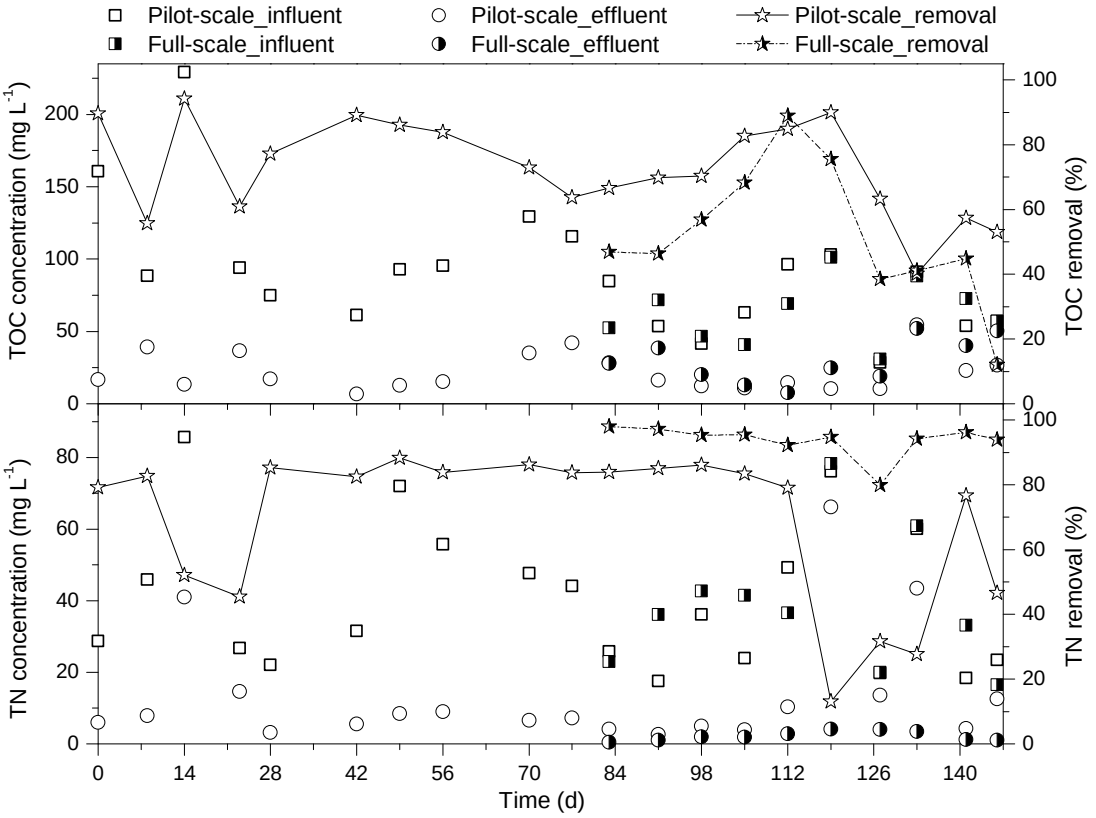

Figure 5.2: Total organic carbon (TOC) and total nitrogen (TN) concentrations and removals by the pilot-scale and the full- scale MBRs.

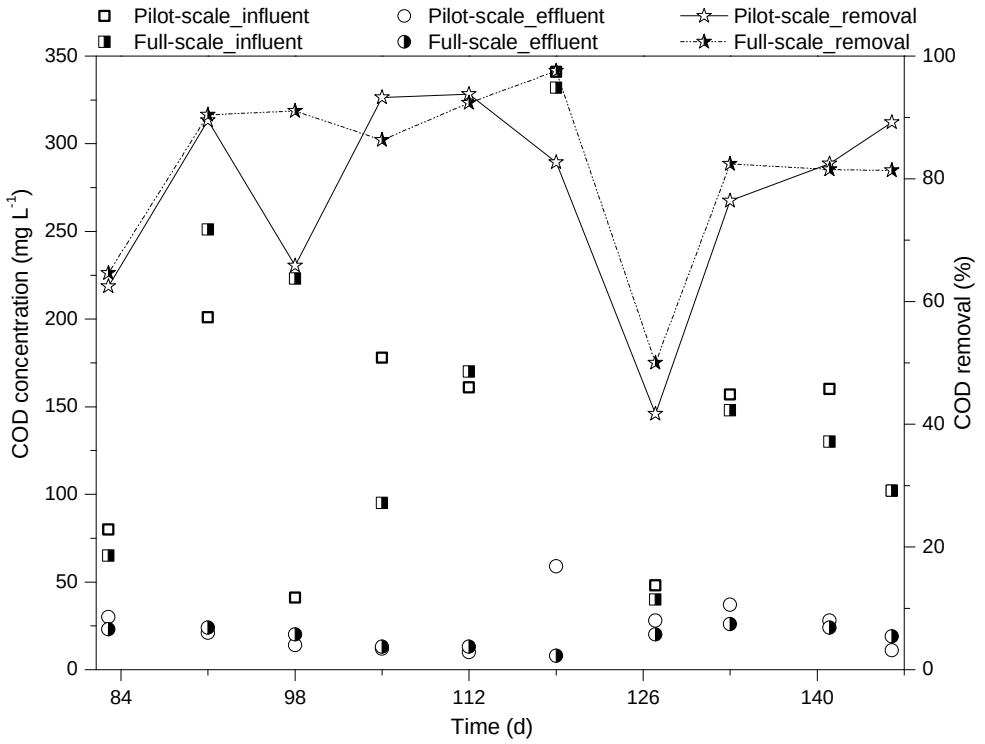

Figure 5.3: Chemical oxygen demand (COD) concentration and removal by the pilot- and full- scale MBRs (Pilot MBR operation scheme: Day 1-76, acclimatization; Day 77-146, period of pilot-and full-scale performance comparison).

5.3.2 Nutrients removal

During the period of comparison (Day 77 – 146), the influent TN varied significantly in the range of 39 ± 19 mg/L, while the effluent TN was 17 ± 21 and 2 ± 1 mg/L (n = 10) for the pilot- and full-scale MBRs, respectively (**Figure 5.2**). Thus the TN removal efficiency varied in the range of 62 ± 28 % (pilot-scale MBR) and 94 ± 5 % (full-scale MBR). The data demonstrate a high and stable TN removal by the full-scale plant that is significantly better than that of the pilot-scale MBR. The pilot-scale MBR comprised a pre-anoxic zone and an aerobic zone (**Figure 5.1**). It is noted that a complete denitrification may not be achieved by this configuration since part of the aerobic effluent is not recycled through the anoxic zone (Phan et al., 2014). The full-scale MBR utilized a four-stage nitrogen removal configuration (two aerobic zones plus pre-and post-anoxic zones) where the second anoxic zone provides for additional denitrification using remaining nitrate produced from aerobic stages as electron acceptor and external carbon source as the electron donor (Hai et al., 2014). Thus, despite significant

variations in influent TN, the full-scale plant achieved an effluent TN concentration of 2 ± 1 mg/L, which is considered the level of refractory dissolved organic nitrogen in wastewater treatment plant effluent (Hai et al., 2014).

Both MBRs were observed to achieve complete nitrification. That is the NH_4^+-N concentration in the effluent being below the detection limit of the method of analysis (**Figure 5.4**). This is consistent with the excellent NH_4^+-N removal achieved in another study involving a decentralised full-scale MBR plant (Trinh et al., 2012b). However, consistent with the case of TN removal, the full-scale MBR showed a more stable NH_4^+-N removal performance. This could again be attributed to the four-reactor configuration, particularly the existence of two aerobic zones in the full-scale MBR. The higher MLVSS concentration (approximately four-fold, see Materials and Methods in Section **5.2**) in the full-scale MBR may be another reason for such stable performance. The pilot-scale MBR was not designed for PO_4^{3-}-P removal; hence, as shown in **Figure 5.4**, the system achieved only marginal PO_4^{3-}-P removal performance (31 ± 15 %, n =10). By contrast, the full-scale MBR exhibited high and stable PO_4^{3-}-P removal (98 ± 4 %, n = 10). This excellent PO_4^{3-}-P removal can be explained by the higher MLVSS concentration and the combination of additional anoxic and aerobic bioreactors in the full-scale MBR.

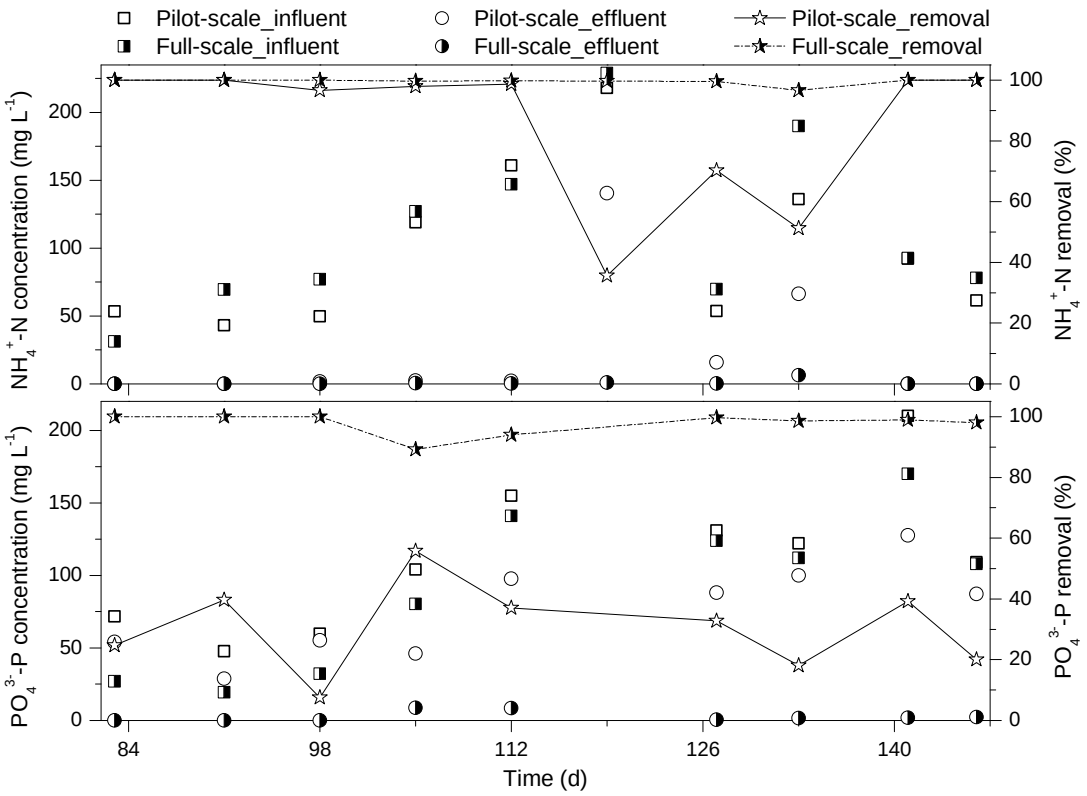

Figure 5.4: NH_4^+-N and PO_4^{3-}-P concentrations and removals by the pilot-scale and the full-scale MBRs (Pilot MBR operation scheme: Day 1-76, acclimatization; Day 77-146, period of pilot-and full-scale performance comparison).

5.3.3 Occurrence of TrOCs in influent wastewater

There are only a few studies reporting the TrOC profile of raw sewage generated from small towns in Australia (Le-Minh et al., 2010; Leusch et al., 2014; Scott et al., 2014; Trinh et al., 2012b). Thus a critical discussion regarding the frequency and concentration of the TrOCs detected in the influent wastewater is necessary to facilitate assessment of the TrOC removal capacity of the MBRs. Of the 45 monitored TrOCs (27 PPCPs, eight steroid hormones, four industrial chemicals and six pesticides), all except three pharmaceuticals (dilantin, risperidone and hydroxyzine) and one pesticide (linuron) were detected in the raw sewage samples at a wide range of concentrations above the detection limit (5 – 20 ng/L, **Appendix Table A-6**). High variability in the concentration of some TrOCs (**Figure 5.5**) may be explained by the fact that Kangaroo Valley has a permanent population of only 340, which, however, may get tripled in peak holiday periods.

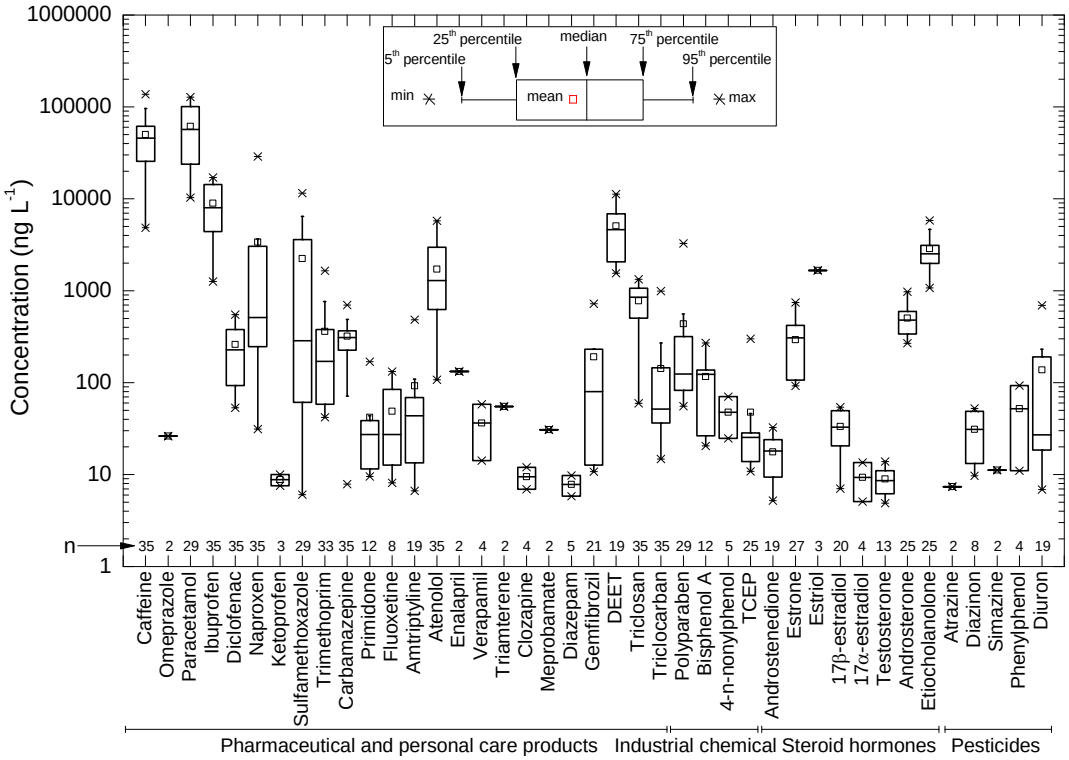

Figure 5.5: TrOC concentrations in raw sewage. 'n' indicates the number of samples in which the corresponding TrOC was detected. In total, 35 samples were collected from 15 sampling events in duplicate (first ten sampling events) or triplicate (last 5 sampling events) from November 2012 to October 2014 (Due to technical difficulties DEET and all steroid hormones could not be measured in 16 and 6 samples, respectively, thus for these TrOCs, 'n' shown are conservative estimates).

Among the PPCPs, caffeine was detected in all samples and with the greatest maximum TrOC concentration (140 µg/L) observed in raw sewage in this study (**Figure 5.5**). The common sources of caffeine are coffee, tea, soft and energy drinks, and caffeine supplements (stimulants), which explain why it is usually detected at high concentration in raw sewage (Luo et al., 2014). However, it is noteworthy that the maximum caffeine concentration detected in the current study is about 3.5 times higher than the value reported for raw sewage from a similar wastewater catchment in Australia (Trinh et al., 2012b) and significantly higher than the values reported overseas (Luo et al., 2014). Given its extensive consumption in Australia (PBS/DH, 2014), the high concentration of paracetamol (maximum detected concentration of 130 µg/L) observed in this study was not a surprise. Notably, non-prescription drugs were detected much more frequently

and at greater concentrations. For example, anti-inflammatory drugs ibuprofen, diclofenac and naproxen were detected in all 35 samples and at concentrations up to three orders of magnitude higher than the prescription anti-inflammatory drug ketoprofen (**Figure 5.5**). The maximum concentration (5.8 μg/L) of the antihypertensive drug atenolol was similar to that reported previously (Trinh et al., 2012b). Notable, however, is that unlike the rest of the antihypertensive drugs (i.e., enalapril, verapamil, triamterene), atenolol was detected in all samples and showed concentrations up to two orders of magnitude higher than the rest. This can be attributed to extensive use of atenolol in Australia for cardiovascular diseases (PBS/DH, 2014). The impact of usage-mode on the detected concentration was also noted in case of the two antibiotics sulfamethoxazole and trimethoprim – these antibiotics are often used in combination, for example, in 5:1 ratio, which may explain the significantly higher concentration of sulfamethoxazole detected in this study. Other prescription drugs detected frequently and at significant concentrations included the antilipidemic drug gemfibrozil (11 - 730 ng/L), the antidepressants fluoxetine (8 – 130 ng/L) and amitriptyline (7 – 480 ng/L), and the antiepileptic drugs carbamazepine (8 – 700 ng/L) and primidone (12 – 170 ng/L). The median age of the Kangaroo Valley population is 48 years, which is 11 years above the Australian average (ABS, 2011). This feature may have contributed to high consumption of prescription drugs in this area.

Ingredients of personal care products (i.e, triclosan, triclocarban, polyparaben and DEET) were frequently detected in the Kangaroo Valley raw sewage. For example, triclosan and triclocarban, which are antimicrobial agents used in toiletries, were detected in all samples and at maximum concentrations consistent with a previous study (Trinh et al., 2012b), although with significant week to week variation (concentration ranges of 60 -1300 ng/L and 15 – 1000 ng/L for triclosan and triclocarban, respectively). A similar behaviour was noted in case of polyparaben (a preservative used in cosmetics), which was detected at a wide concentration range of 56 – 3300 ng/L (**Figure 5.5**). However, DEET (an active ingredient of most commercial insect repellents) was detected at a relatively narrow concentration range of 1.5 – 11.3 μg/L – the maximum value surpassing the previously reported ones (Trinh et al., 2012b). Notable in this connection that two samples analysed with a different method probing some additional TrOCs confirmed few tens of microgram per litre of octocrylene and

benzophenone (ingredients of UV filters) and salicylic acid –an ingredients in medicinal/cosmetic products (data not shown).

Consistent with the rural nature of the area, the industrial xenoestrogens bisphenol A (21 – 270 ng/L, n = 12) and 4-n-nonylphenol (25 - 70 ng/L, n = 5) were detected with concentrations at the lower end of the previously reported values in Australia (Scott et al., 2014; Tan et al., 2007; Trinh et al., 2012b). TCEP, a flame retardant commonly found in products such as foams and plastics, was detected more frequently but at a concentration range of 23 ± 11 ng/L (n = 25), except for one sample with a high concentration of 300 ng/L. No Australian reports could be retrieved for comparison, but this TCEP concentration range is significantly lower than the few wastewater TCEP data available from Europe (Luo et al., 2014; van der Veen and de Boer, 2012).

All eight monitored steroid hormones were detected in the raw sewage (**Figure 5.5**). Among the androgenic hormones (i.e., testosterone, etiocholanolone and androsterone), the primary male sex hormone testosterone was detected at low concentrations (<5 - 14 ng/L), while its metabolites (i.e., etiocholanolone and androsterone) occurred at much higher concentrations (1.1 – 5.8 µg/L and 0.27 – 0.98 µg/L, respectively) and with greater frequency (**Figure 5.5**). This is in accordance with previous studies (Tan et al., 2007; Trinh et al., 2012b). The estrogen 17β-estradiol and its natural epimer 17α-estradiol were detected in low concentrations (7 – 54 ng/L and 5 – 14 ng/L, respectively). 17β-estradiol is the predominant estrogen during reproductive years, however, in wastewater this can swiftly degrade to estrone (Coleman et al., 2009), which can explain the high concentration of estrone (up to 0.75 µg/L) detected in this study (**Figure 5.5**). However, the maximum estrone concentration observed in this study was about seven times higher than the values reported for Australian sewage previously (Coleman et al., 2009; Trinh et al., 2012b). The extremely small sewage catchment area is the most likely source of such high variability and unusual results. However, it is interesting to note that estrone is the predominant estrogen in postmenopausal women, which matches the demography of the study area. Notable also in this connection is the fact that estriol, which is associated with pregnancy, was detected at a high concentration of 1.7 µg/L but only during one sampling event (n = 3) which coincides with a peak holiday period, indicating that this possibly came from the tourists.

Pesticides are hardly biodegradable TrOCs (Hai et al., 2012). Except linuron, all other pesticides monitored (i.e., atrazine, diazinon, simazine, phenylphenol and diuron) were detected in the raw sewage at different concentrations. Among these, diuron was detected frequently and at a concentration of up to 0.7 µg/L (**Figure 5.5**), which is higher than the values reported in a recent Australian study covering a few selected urban and rural wastewater treatment plants (Leusch et al., 2014). Diuron is used extensively in Australia for the control of weeds in certain crops (e.g., wheat, barley and sugarcane) and thus frequently detected in surface water. Its application to control a wide variety of broadleaf and grassy weeds along the roads and garden paths could be the source for their occurrence in Kangaroo Valley raw sewage.

5.3.4 Overall TrOC removal by the MBRs

Significantly hydrophobic compounds (approximately possessing a log *D* over 3) are generally well removed from the aqueous phase via sorption to biosolids. Depending on their biodegradability, the biosorbed TrOCs may be further degraded. In this study, the steroid hormones (log $D_{pH=8}$= 3.62 – 3.93) were efficiently removed by the MBRs (**Figure 5.6**). Similar removal from real wastewater has been reported for the steroid hormones in previous studies (Le-Minh et al., 2010; Trinh et al., 2012a). Furthermore, none of these TrOCs were detected in sludge (**Table 5.1**), evidencing their high biotransformation. Halogenated personal care products triclosan and triclocarban possess high hydrophobicity (log $D_{pH=8}$ of 4.93 and 6.07, respectively), and were significantly removed from the aqueous phase. However, their high resistance to biodegradation (Hai et al., 2011b) was evident as triclosan and triclocarban were detected in pilot-MBR sludge at a concentration of 190 - 230 and 790 - 1100 ng/g_{MLSS}, respectively.

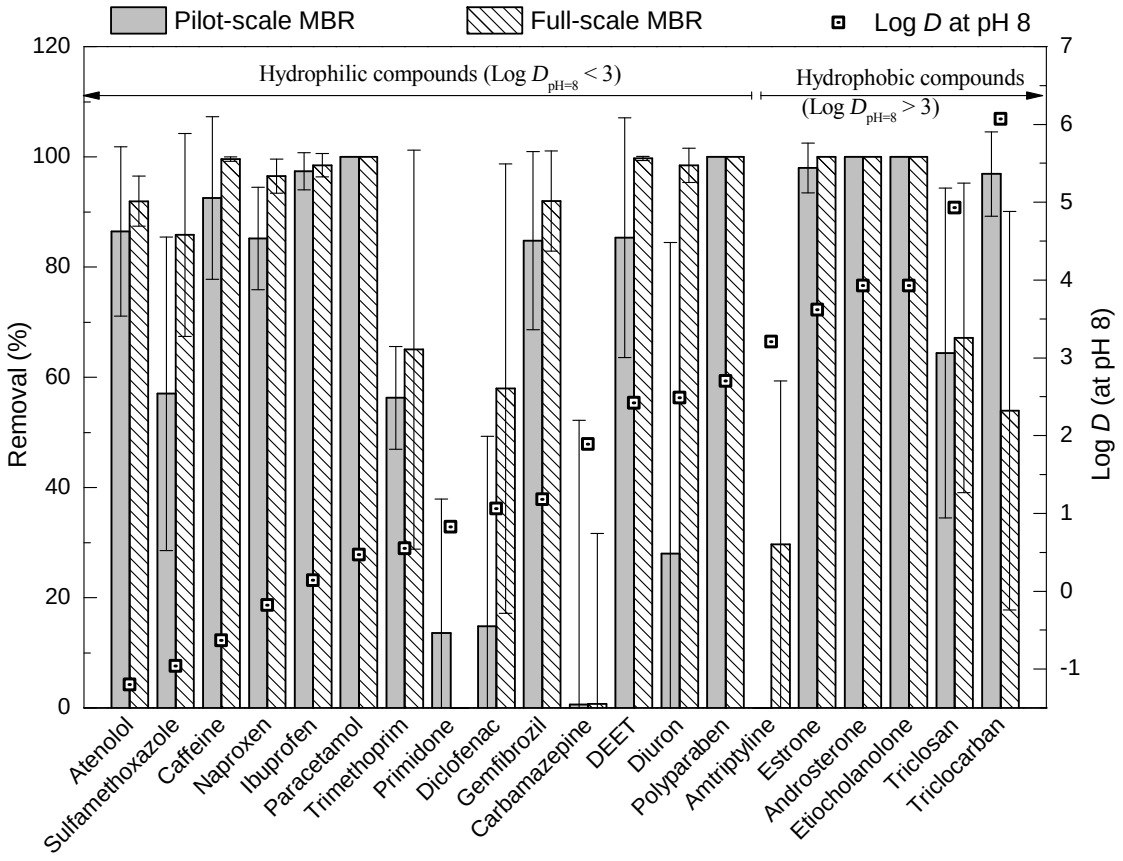

Figure 5.6: TrOC removal by the pilot- and full-scale MBRs. Error bars represent the standard deviation of duplicate samples taken once a week for six weeks.

The MBRs achieved high and stable removal (>90%) of eight PPCPs (atenolol, caffeine, naproxen, ibuprofen, paracetamol, gemfibrozil, DEET and propylparaben) (**Figure 5.6**). These compounds are hydrophilic (log $D_{pH=8} < 3$), and thus biodegradation is thought to be the major removal mechanism during biological treatment processes. These PPCPs are generally characterized as significantly biodegradable (Trinh et al., 2012b; Xue et al., 2010), although the removal of some compounds such as naproxen and DEET has been observed to be variable (Tadkaew et al., 2011). However, the MBRs showed little removal of the anticonvulsant drugs carbamazepine and primidone. Both compounds contain strong electron withdrawing amide groups, while primidone additionally contains a weak electron donating group (methyl). Occurrence of strong electron withdrawing groups and/or absence of electron donating groups impart resistance to biodegradation (Tadkaew et al., 2011). Indeed these TrOCs, particularly carbamazemine, have been widely reported to be resistant to biodegradation (Le-Minh et al., 2010; Tadkaew et al., 2011; Trinh et al., 2012b). A few resistant compounds (i.e.,

sulfamethoxazole, trimethoprim, diclofenac and diuron), which were detected at high concentrations in the raw sewage, were better removed by the full-scale plant (p<0.05, **Table 5.2**). This aspect is discussed in further detail in section **5.3.6**.

Table 5.1: Concentration (ng $_{TrOC}$/g $_{MLSS}$) of TrOCs in sludge (BDL = Below detection limit; NQ= Not quantifiable).

Compounds	Sample 1	Sample 2
Atenolol	BDL	36
Sulfamethoxazole	BDL	11
Caffeine	BDL	BDL
Naproxen	BDL	BDL
Ibuprofen	BDL	BDL
Paracetamol	17	BDL
Trimethoprim	42	45
Primidone	BDL	BDL
Diclofenac	13	12
Gemfibrozil	BDL	BDL
Carbamazepine	9	9
DEET	NQ	NQ
Diuron	BDL	BDL
Polyparaben	BDL	BDL
Amtriptyline	BDL	BDL
Estrone	BDL	BDL
Androsterone	NQ	NQ
Etiocholanolone	NQ	NQ
Triclosan	230	191
Triclocarban	786	1128

[Note:

1. TrOC concentration in sludge was not measured during the period of aqueous phase TrOC removal comparison between pilot- and full-scale MBRs (Day 105- 146). The pilot-scale MBR was continued to be operated beyond that, and TrOC concentration in sludge was measured on Day 174 when the TOC, TN and TrOC concentrations were at levels similar to that during Day 105 – 146.

*2. Regarding 'BDL': TrOCs from 0.5 g sludge (dry weight) was extracted (see Materials and Methods in Section **5.2.3.3**) into liquid samples on which TrOC analysis was conducted. Liquid samples which returned concentrations below the detection limits (see **Appendix Table A-6**) has been marked with 'BDL' here.*]

Table 5.2: Statistical analysis of pilot- *vs.* full-scale MBR TrOC removal data depicted in **Figure 5.6** (paired *t*-test was conducted using Microsoft Excel. Values of $p < 0.05$ were considered to indicate statistical significance).

Compounds	*p* value
Atenolol	0.210
Sulfamethoxazole	0.047
Caffeine	0.134
Naproxen	0.009
Ibuprofen	0.257
Paracetamol	-
Trimethoprim	0.289
Primidone	0.174
Diclofenac	0.038
Gemfibrozil	0.206
Carbamazepine	0.455
DEET	0.068
Diuron	0.023
Polyparaben	-
Amtriptyline	0.020
Estrone	0.173
Androsterone	-
Etiocholanolone	-
Triclosan	0.437
Triclocarban	0.009

It is important to note here that of the 35 raw sewage samples collected over 15 sampling events (**Figure 5.6**), TrOC removal estimation has been based on 12 samples (duplicate samples once a week over six weeks) for which the corresponding treated effluent samples were available. However, except for paracetamol, the median influent TrOC concentrations were the same for both sets of data (**Table 5.3**), indicating that the TrOC removal efficiencies reported in this study can be considered a reasonable representation of the full-scale plant capacity.

Table 5.3: Comparison of influent TrOC concentrations (ng/L) – all available samples (**Appendix Table A-6**) *vs*. samples used for performance comparison between pilot-scale and full-scale MBRs (n = number of samples).

TrOC	Detection limit	All available samples				Samples during period of comparison: pilot-scale *vs.* full-scale MBR			
		n	Max	Min	Median	N	Max	Min	Median
Atenolol	5	35	6140	79	1388	12	4040	548	785
Sulfamethoxazole	5	35	12340	5	185	12	2360	5	124
Caffeine	10	35	138200	3810	49000	12	138200	12840	30300
Naproxen	5	35	30600	23	508	12	9660	440	1382
Ibuprofen	5	35	17820	1040	8560	12	16620	1736	7550
Paracetamol	5	32	162400	5	58500	9	61200	5	23600
Trimethoprim	5	35	1830	5	114	12	1118	41	90
Primidone	5	35	176	5	5	12	176	5	27
Diclofenac	5	35	624	43	232	12	624	92	196
Gemfibrozil	5	35	974	5	11	12	234	5	81
Carbamazepine	5	35	740	6	312	12	500	222	318
DEET	5	19	12180	1540	4560	12	10740	1784	4640
Diuron	10	35	712	7	10	12	232	10	18
Polyparaben	10	35	3500	10	112	12	180	10	44
Amtriptyline	5	35	676	5	7	12	114	5	44
Estrone	5	29	834	5	258	12	834	5	233
Androsterone	5	29	1044	5	450	12	676	5	393
Etiocholanolone	5	29	6320	5	2220	12	6320	5	2570
Triclosan	5	35	1358	52	866	12	1358	384	960
Triclocarban	10	35	1110	12	54	12	164	19	44

5.3.5 Correlation between TN and TrOC removal

Of the six weeks of TrOC removal comparison (Day 105-146), on the 2nd to 5th week, the wastewater NH_4^+-N and TN concentrations fluctuated significantly (TN concentration of 50, 75, 20, and 60 mg/L in samples measured on day 112, 119, 127 and 133, respectively), leading to low NH_4^+-N (**Figure 5.4**) and TN (**Figure 5.7**) removal. Notably, as the influent TN leaped from 49 (on Day 112) to 76 mg/L on Day 119, an immediate drop in removal of eight TrOCs, namely, atenolol, caffeine, naproxen, ibuprofen, gemfibrozil, DEET, estrone and diuron by the pilot MBR was observed (**Figure 5.7**). Furthermore, the removal-profile of some of these TrOCs continued to closely follow the rise and fall in the (NH_4^+-N and) TN removal profile. By contrast, the full-scale MBR TN removal was little impacted by the fluctuation in TN concentration (**Figure 5.8**). TrOC removal (except that of atenolol on Day 119) by the full-scale MBR also remained stable (**Figure 5.8**) during the period of TN fluctuation in influent. Previous studies have shown a close relationship between stable NH_4^+-N removal and the removal of many TrOCs including atenolol (Helbling et al., 2012), ibuprofen and naproxen (Fernandez-Fontaina et al., 2014), and gemfibrozil (Maeng et al., 2013). Also DEET was shown to be metabolized only in the presence of nitrogen (Rivera-Cancel et al., 2007). Of particular relevance to the observed drop in TrOC removal due to rise in influent NH_4^+-N concentration is the study of De Gusseme et al. (2009) who showed that nitrifying cultures may preferentially oxidize ammonia rather than the synthetic estrogen 17α-ethinyl estradiol under elevated NH_4^+-N concentration. Most of the previous reports showing an association of TrOC removal with the stability of NH_4^+-N and TN removal were conducted with synthetic wastewater via batch tests. By contrast, this study shows the link between stable TN and TrOC removal via unique results from the pilot- and full-scale MBRs fed with the same raw sewage.

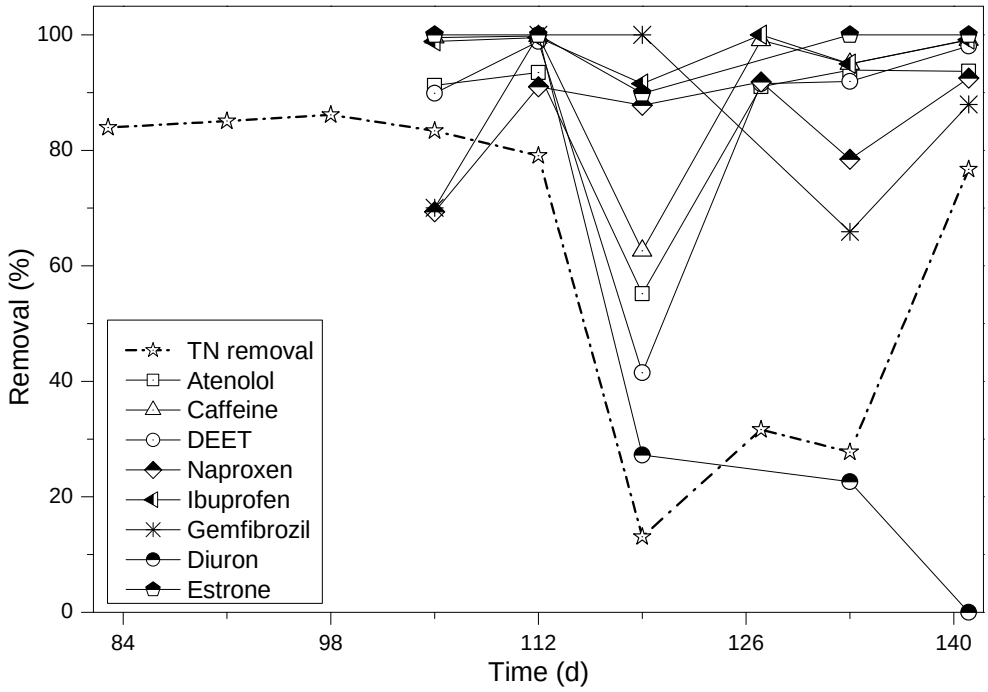

Figure 5.7: Variation in TN and TrOC removal by the pilot-scale MBR (Operation scheme: Day 1-76, acclimatization; Day 77-146, period of pilot- and full-scale performance comparison (TOC and TN); Day 105- 146, period when TrOC removal was monitored).

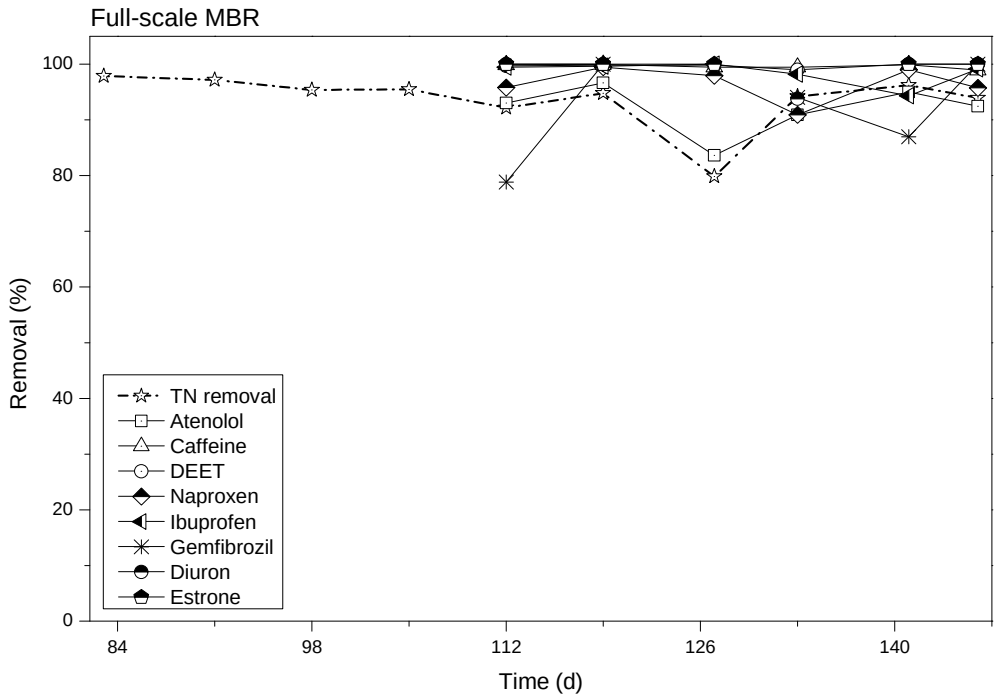

Figure 5.8: Variation in TN and TrOC removal by the full-scale MBR (TOC and TN removal by the Full- and pilot-scale MBR was compared from Day 77 to 146; however TrOC removal was monitored from Day 105 to 146).

5.3.6 Better TrOC removal by full-scale MBR: possible reasons

The full-scale MBR showed significantly better removal of four hydrophilic TrOCs namely, the pharmaceuticals sulfamethoxazole, trimethoprim and diclofenac, and the pesticide diuron. Additionally, in contrast to no removal by the pilot-scale MBR, a moderate removal of the antidepressant drug amitriptyline was achieved by the full-scale MBR (**Figure 5.6**). Amitriptyline is a significantly hydrophobic compound (log $D_{pH=8}$= 3.21), and due to its persistence in sludge, its removal by MBR has been attributed mainly to biosorption (Tadkaew et al., 2011; Trinh et al., 2011). Significant variability in amitriptyline removal, as observed in the current study as well as in previous work (Tadkaew et al., 2011; Trinh et al., 2011; Trinh et al., 2012b), may be attributed to the biosorption capacity, which may be site- and MBR-design (e.g., anoxic/aerobic sequences applied)-specific. Among the hydrophilic TrOCs, sulfamethoxazole has been shown to undergo biodegradation under a range of redox conditions, particularly at low DO (Hai et al., 2011a; Stadler et al., 2015), which were the conditions in the first aerobic reactor in the full-scale MBR. Conversely, to date the

biodegradation of the resistant TrOC diclofenac has been shown to occur only under stable nitrifying conditions (Vieno and Sillanpää, 2014), as was also achieved by the full-scale plant. A few reports additionally indicate that a delicate combination of aerobic and anoxic conditions such as that in attached growth systems may favour diclofenac degradation (Vieno and Sillanpää, 2014; Zwiener and Frimmel, 2003) – it is possible that in the current study the full-scale MBR had facilitated such redox conditions. Similarly, the excellent removal of diuron (98 ± 3 %) by the full-scale MBR was possibly facilitated by the combination of different redox zones as also suggested by Stasinakis et al. (2009).

Higher removal of the hydrophilic TrOCs by the full-scale plant (i.e., sulfamethoxazole, trimethoprim, diclofenac, and diuron) or more stable removal of other TrOCs (as discussed in the previous section) may be attributed to the existence of pre- and post-anoxic tanks, and combination of aerobic zones with different levels of DO as compared to a pre-anoxic and a single aerobic tank in the pilot MBR. For a clearer understanding, further studies specifically on different combinations of anoxic and aerobic reactors for TrOC removal by MBR are recommended.

Direct UV photolysis of TrOCs can occur at elevated dosages (Nguyen et al., 2013), but a significant body of literature has shown that at disinfection dosages direct UV photolysis is ineffective in removing most TrOCs (Yang et al., 2013). Thus, it is unlikely that better removal by the full-scale MBR compared to the pilot MBR observed here was due to full-scale effluent sample being collected after the UV disinfection unit.

Because TrOC concentrations in the raw sewage varied significantly (**Figure 5.5** and **Table 5.3**), in addition to monitoring the removal efficiency, the effluent TrOC concentrations were compared with the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies (NRMMC/EPHC/NHMRC, 2008b). The full-scale plant effluent was intended only to be reused in irrigation. However, comparing the effluent quality against these guidelines further facilitate the performance-comparison of the pilot- and full-scale MBRs. For example, caffeine usually registered a removal of 95 – 99% by the MBRs (**Figure 5.6**); however, when it was detected in the influent at the maximum concentration (138 µg/L), the pilot MBR effluent concentration (51.5 µg/L), but not that of the full-scale MBR effluent, exceeded

the guideline value of 3.5 μg/L (**Table 5.4**). Compared to caffeine, estrone was detected at much lower influent concentrations (0.005 – 0.80 μg/L) and estrone removal was consistently over 95%. Thus, with the exception of only one instance, the pilot MBR effluent could comply with the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies (NRMMC/EPHC/NHMRC, 2008b) despite the fact that a much stricter guideline value has been imposed for estrone (0.03 μg/L). By contrast, because triclosan removal varied from 35-95% (**Figure 5.6**), a third of the effluent samples (both the MBRs) could not comply with the moderate guideline value of 0.35 μg/L (**Table 5.4**). Interestingly, despite low removals of carbamazepine, diuron and amitriptyline by the pilot MBR (**Figure 5.6**), their effluent concentrations were within the limit of guideline values (**Table 5.4**). MBR-effluent TrOC concentrations observed in this study are consistent with that from the literature (Coleman et al., 2009; Trinh et al., 2012b). However, via the performance-comparison between the pilot- and full-scale MBRs, this study offers unique insight into the impact of application of multiple anoxic/aerobic treatment sequences on TrOC removal and compliance to water reuse guidelines.

Table 5.4: Concentrations of TrOCs detected in the permeate from the pilot- and full-scale MBRs and the Australian guideline values for augmentation of drinking water supplies (NRMMC/EPHC/NHMRC, 2008a). Data presented as 'concentration range (median value)'.

Compounds	Concentration of TrOCs (ng/L)		
	Pilot-scale MBR permeate	Full-scale MBR permeate	Australian guideline values
Atenolol	34 - 1700 (58)	42 - 210 (88)	Not available[a]
Sulfamethoxazole	<5 - 1700 (48)	<5 - 310 (49)	35×10^3
Caffeine	60 - 55800 (220)	31 - 230 (68)	35×10^2
Naproxen	35 - 1100 (250)	<5 - 290 (33)	22×10^4
Ibuprofen	<5 - 1400 (38)	<5 - 400 (45)	40×10^4
Paracetamol	<5	<5	17.5×10^4
Trimethoprim	13 - 490 (40)	20 - 210 (71)	70×10^3
Primidone	<5 - 180 (25)	<5 - 840 (27)	Not available
Diclofenac	87 - 270 (193)	<5 - 180 (87)	18×10^2

Gemfibrozil	<5 - 80 (<5)	<5 - 20 (11)	60 x 10^4
Carbamazepine	270 - 660 (330)	330 - 600 (454)	10 x 10^4
DEET	50 - 4200 (165)	<5 - 31 (12)	25 x 10^5
Diuron	<10 - 180 (17.6)	<10 - 190 (<10)	30 x 10^3
Polyparaben	<10	<10	Not available
Amtriptyline	53 - 260 (89)	40 - 99 (60)	70 x 10^3
Triclosan	47 - 1200 (180)	14 - 730 (160)	350
Triclocarban	<10 - 38 (<10)	<10 -46 (34)	Not available
Estriol	<5	<5	50
Androstenedione	<5	<5	Not available
Testosterone	<5	<5	7 x 10^3
Estrone	<5 - 82 (<5)	<5	30
17β-estradiol	<5	<5	175
17α-estradiol	<5	<5	175
Androsterone	<5	<5	14 x 10^3
Etiocholanolone	<5	<5	Not available

Note: [a]Values for other β-blockers are 350-40,000

5.4 CONCLUSIONS

To address a notable omission in the literature, this study analysed nutrient and TrOC removal performance by a full- and a pilot-scale MBR from wastewater originating from a resort town and showing significant fluctuations in concentrations of the target pollutants over time. The pilot-scale MBR demonstrated a very similar COD reduction as the full-scale MBR. Given the significantly higher MLVSS concentration and presence of additional anoxic and aerobic bioreactors in the full-scale plant, the removal of nutrients, particularly that of phosphorous by the full-scale MBR was significantly high (98 ± 4 % *vs.* 31 ± 15 % PO_4^{3-}-P removal by the pilot-scale MBR). Notably, any drop in TN or NH_4^+-N removal by the full-scale MBR was accompanied by a drop in the removal by the pilot-scale MBR, although the full-scale plant appeared to be more stable under influent load fluctuations. The full-scale MBR demonstrated higher and more stable removal of a few resistant and hydrophilic (log D < 3) TrOCs including sulfamethoxazole, trimethoprim, diclofenac and diuron. Performance comparison between the pilot- and full-scale MBRs reveals a link between stable TN and TrOC removals which were facilitated by a delicate combination of redox zones in the bioreactors.

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CHAPTER 6. Impact of Hazardous Events on the Removal of Nutrients and Trace Organic Contaminants by a Pilot-Scale Anoxic-Aerobic Membrane Bioreactor

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6.1 INTRODUCTION

In the field of risk assessment and water quality management, 'hazardous events' refer to departure from normal operational conditions including occurrence of changes in source water composition, extreme weather events, human error and mechanical malfunctions (Trinh et al., 2014). Hazardous events have been adopted by the World Health Organization (WHO) as a key parameter of risk management for the application of Water Safety Plans (Bartram et al., 2009) and the Guidelines for Drinking Water Quality (World Health Organization, 2011). Depending on both the likelihood and the consequences of these events, they may ultimately define the treatment reliability and level of risk regarding meeting final water quality objectives (Trinh, 2013). Advantages of MBRs over conventional activated sludge (CAS) processes have been assessed for removal of nutrient, bulk organics and trace organic contaminants (TrOCs) (Hai et al., 2014b). However, the validation of the robustness of the process to the risk of deviations in operating conditions during so called hazardous events has been scarce to date (Trinh et al., 2014).

Previous studies involving CAS processes indicate significant impact of hazardous events on wastewater treatment performance. Notwithstanding the aforementioned reports about the detrimental impact of hazardous events on CAS processes, important research gaps exist. For example, power failure ceases feeding, aeration and mixing of sludge as well as recirculation between different reactors in biological nutrient removal processes that combine aerobic, anoxic and/or anaerobic conditions. Besides the possible consequences caused by aeration failure, interrupted sludge recirculation between reactors can impair nitrogen and phosphorous removal via nitrification/denitrification pathways and the polyphosphate accumulation process, respectively. Such impacts have not been systematically studied to date. CAS performance under hazardous event circumstances may provide useful insights but may not completely represent impacts to MBR performance. MBRs combine membrane separation with biodegradation, and as such the potential impact of hazardous events on membrane hydraulic performance must be additionally considered. However, only one study (Trinh et al., 2015) to date has reported the impact of selected hazardous events on MBR performance. A further notable omission is that except for a limited coverage

in the study of Trinh (2013), the impact of hazardous events on TrOC removal remains largely unexplored.

Taking into consideration the above research gaps, this study examined the impact of four hazardous events, namely, aeration failure, power failure, ammonia shock and bleach shock on the performance of a pilot-scale anoxic-aerobic MBR receiving real wastewater. Impacts on the characteristics of mixed liquor and the removal efficiency of bulk organics, nutrient as well TrOC were systematically studied. The results of this study also provide unique insights to the impact of simulated hazardous events on effluent toxicity and estrogenicity.

6.2 MATERIALS AND METHODS

A pilot-scale MBR was set up on the site of a full-scale MBR plant located in Kangaroo Valley, New South Wales, Australia. The pilot-scale MBR was operated for total 270 d. It was first operated for 145 d for acclimatization, performance stabilization and performance comparison with the full-scale MBR. Following this, four simulated hazardous events (aeration failure, power failure, ammonia shock and bleach shock) were conducted over a period of 125 d (Day 146 - 270) to assess the impacts on basic effluent quality parameters and on attenuation of TrOC concentration and toxicity/estrogenicity from effluent.

6.2.1 Pilot-scale MBR setup and operation

The pilot-scale MBR comprised an anoxic reactor and an aerobic reactor with working volumes of 14 and 12 L, respectively. A hollow fibre ultrafiltration membrane (Zeweed-10 module, Zenon Environmental, Canada) was submerged in the aerobic reactor. With a nominal pore size of 0.04 μm and an effective membrane surface area of 0.93 m^2, the membrane was operated at an average flux of 1.2 $L/m^2.h$. To facilitate detachment of loosely attached particles on the membrane, effluent was extracted intermittently *i.e.*, a relaxation period of 3 min every 10 min was applied. The MBR was operated at a solids retention time of 25 d, a total hydraulic retention time of 1.5 d (0.8 d and 0.7 d for anoxic and aerobic reactors, respectively) and an internal recirculation ratio of 4 between anoxic-aerobic reactors.

Transmembrane pressure (TMP) was continuously recorded via a high resolution (±0.1 kPa) vacuum gauge. *In situ* air scrubbing was found adequate to keep the TMP stable within 5 kPa before conducting hazardous events experiments, and no chemical cleaning was required for whole operation period. Feeding, recirculation and effluent extraction was performed with peristaltic pumps (Masterflex L/S, USA). The mixed liquor in the upper quarter of the pre-anoxic tank was intermittently (1 min on and 15 min off) mixed by a mixer (200 rpm) to ensure that the sludge transferred from the aerobic tank did not get trapped within the anoxic tank. Mixed liquor temperature was stable at 18 ± 3 ^{o}C. During the steady state operation of MBR (before conducting hazardous events experiments), the mixed liquor pH was stable at 7.1 ± 0.4 (n = 14) and 7.4 ± 0.5 (n = 14) for the anoxic and aerobic bioreactors, respectively. Dissolved oxygen (DO) concentration in the bioreactors was maintained in the range of 2.5–5.0 mg/L for the aerobic zone and to below 0.25 mg/L for the anoxic zone.

6.2.2 Hazardous events experiment protocol

Four hazardous events, namely aeration failure, power failure, ammonia shock and bleach shock were selected based on a previously reported comprehensive literature review (Trinh et al., 2014). The MBR was subjected to the hazardous events in the following order: aeration failure (Day 146 -150); power failure (Day 174 – 178); ammonia shock (Day 231 – 234) and bleach shock (Day 258 – 261). The impact of the hazardous events on mixed liquor characteristics, membrane fouling and removal performance was assessed in terms of an array of selected parameters, namely, pH, DO concentration and oxidation reduction potential (ORP) of the mixed liquor, mixed liquor suspended solids (MLSS) concentration, mixed liquor volatile suspended solids (MLVSS) concentration, TMP, total organic carbon (TOC), chemical oxygen demand (COD), NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^{3-}-P as well as TrOCs and toxicity/estrogenicity.

To differentiate between the impacts of different hazardous events and to distinguish the changes due to hazardous events from that due to ambient variability in operational conditions, the following controls were in place:

(i) A monitoring period between the hazardous events was allowed to confirm retrieval of process stability;

(ii) Primary settled wastewater was collected weekly to a reservoir, and the same influent was fed to the pilot-scale MBR before, during and after the hazardous events over the week. It was also confirmed that the wastewater characteristics in terms of TOC, TN and TrOC did not change significantly during storage and use (data not shown).

(iii) Other key operational parameters were either controlled or remained stable as described in Section **6.2.1**.

6.2.2.1 Aeration and power failure

Aeration failure was simulated by ceasing aeration in the aerobic reactor for 18 h. The power failure was simulated by terminating the power supply to the MBR system for 18 h. Accordingly, influent feeding, aeration of the aerobic reactor, mixing of the anoxic reactor, and the sludge recirculation between aerobic and anoxic reactors were ceased for 18 h. Effluent withdrawal was discontinued during the periods without aeration or power supply, and samples were collected immediately before and 1, 3, 24 and 72h after the resumption of the aeration or power supply (i.e., the first sample was collected at 19^{th} hour).

6.2.2.2 Chemical shock

Chemical shocks (ammonia and bleach separately) were introduced as single dose directly to the bioreactors, and samples were collected immediately before and 1, 3, 24 and 72h after the shock application.

In line with the protocols used in previous CAS (Ding et al., 2014) and MBR (Trinh et al., 2015) studies, a NH_4^+-N concentration of 1000 mg/L (approximately 10 times the average concentration of NH_4^+-N in the raw sewage) was selected as a shock dose. This was applied in the form of ammonium bicarbonate (NH_4HCO_3).

Only two studies related to the impact of bleach on activated sludge processes were found. While Bodik et al. (2008) demonstrated a significant effect of disinfectants containing sodium hypochlorite (0.3 mL/L) on organics removal by activated sludge in batch tests, no discernible effect of a bleach dose of 0.4 mL/L on the performance of bench- or pilot-scale MBRs was observed by Knops (2010). Accordingly in the current study, bleach shock was conducted by a single dose of 0.8 mL/L of commercial bleach

(Domestos, Unilever) to the mixed liquor of both bioreactors, *i.e.*, 38 ppm of active chlorine, plus 9.6 ppm of sodium hydroxide and 0.4 ppm of alkaline salts (active ingredients of Domestos bleach: sodium hypochlorite 49.9 g/L, active chlorine 4.75% (m/v), sodium hydroxide 12.0 g/L and alkaline salts 0.5 g/L).

6.2.3 Sample analysis

6.2.3.1 Analysis of basic parameters

TOC and TN were analysed using a TOC/TN-V_{CSH} analyser (Shimadzu, Japan). COD was analysed using COD vials (0-1500 ppm, WatertestSystems, Australia) with a Hach DR 5000 spectrophotometer according to the Standard Method 5220 D (Eaton et al., 2005). NH_4^+-N and ortho- PO_4^{3-}-P concentrations were measured using flow injection analysis (Lachat instruments, Milwaukee, USA) following the Standard Methods 4500-NH_3 H and 4500-P G, respectively (Eaton et al., 2005). MLSS and MLVSS concentrations in bioreactors were measured according to the Standard Method 2540 (Eaton et al., 2005).

6.2.3.2 Trace organic contaminant analysis

In total, 45 TrOCs including 27 PPCPs, four industrial chemicals, eight steroid hormones and six pesticides were monitored in this study (**Appendix Table A-7**). Influent and MBR effluent samples (0.5 L) were collected and immediately transferred to the laboratory. The influent samples were filtered through 1 µm and then 0.45 µm glass-fibre filter papers (Millipore, Australia). The effluent (membrane-permeate) samples were not pre-filtered. Concentrations of TrOCs were determined using an analytical method previously described by Phan et al. (2015). This method involves solid phase extraction (SPE) using Oasis HLB cartridges (Waters, Millford, MA, USA) followed by analytical quantification by a high performance liquid chromatography (Agilent 1200 series, Palo Alto, CA, USA) coupled with tandem triple quadrupole mass spectrometer (API 4000, Applied Biosystems, Foster City, CA, USA) in positive and negative electro-spray modes as well as atmospheric pressure chemical ionization (APCI) in positive mode. Isotope dilution was used for SPE correction of all analytes. Sludge samples were extracted by a previously developed solvent extraction method (Wijekoon et al., 2013) prior to analysis as described above.

6.2.3.3 Estrogenicity and toxicity analysis

The filtered influent and MBR effluent samples (0.5 L) were extracted on Oasis HLB solid-phase cartridges preconditioned and eluted with equal volume (10 mL) of methanol and reconstituted in 500 μL methanol. Estrogenicity in solid-phase extracts was measured using GeneBLAzer ERα-UAS-*bla* GripTite cells (Life Technologies, Carlsbad, CA), following methods described by Escher et al. (2014) and the manufacturers protocols, with slight modifications. The GeneBLAzer ERα assay is a reporter gene assay that measures estrogen receptor-mediated gene activation, indicating the presence of estrogens or estrogen mimics in the sample. The assay was performed in 384-well plate format, run in both agonist and antagonist (in combination with an EC_{80} concentration of the agonist) modes, and a 3-5 point serial dilution curve of each sample was tested on at least two separate occasions. Fluorescence was measured in a Fluostar plate reader (BMG Labtech, Germany) at 460 and 520 nm after excitation at 410 nm, and the data expressed as the ratio of fluorescence at 460 divided by 520. The results were then compared to a concentration-effect curve with the reference standards and expressed as 17β-estadiol (agonist) and tamoxifen (antagonist) equivalent concentrations. The limits of detection were 0.01 ng/L and 6 μg/L for 17β-estadiol (EEQ) and tamoxifen (TMXEQ) equivalent concentrations, respectively.

The aquatic toxicity of the solid-phase extracts was assessed using the bacterial luminescence toxicity screen (BLT-Screen) described by van de Merwe and Leusch (2015). Briefly, solid-phase extracts were added to phosphate-buffered saline medium and serially diluted in a 96-well plate, which also contained a reference compound (pentachlorophenol), negative controls and inter-assay samples for quality control. Naturally luminescent bacteria, *Photobacterium leiognathi*, was then added to each well (from a cryopreserved stock) to mark the start of the exposure period. Exactly 30 minutes later the luminescence of each well was measured in a Fluostar plate reader (BMG Labtech, Germany), and the inhibition of luminescence was calculated relative to controls. The toxicity of each sample was expressed as relative Toxic Unit (rTU).

6.3 RESULTS AND DISCUSSION

6.3.1 Impact on mixed liquor characteristics

6.3.1.1 pH and ORP

Except for the ammonia shock event, mixed liquor pH was within the normal operation range during all simulated hazardous events (**Figure 6.1**). The mixed liquor pH increased from 7.1 to 8.3 and 7.2 to 8.4 for the anoxic and the aerobic bioreactors, respectively following ammonia shock. In agreement with Trinh (2013), the pH levels returned to the normal operation range within 72 h. Such temporary pH variation may not significantly affect bulk organics removal since the optimum pH for biological process is thought to lie between pH 6.5 and 8.0 (Baldwin and Campbell, 2001). However, this may have significant impact on the removal of some specific TrOCs as further discussed in Section **6.3.3**.

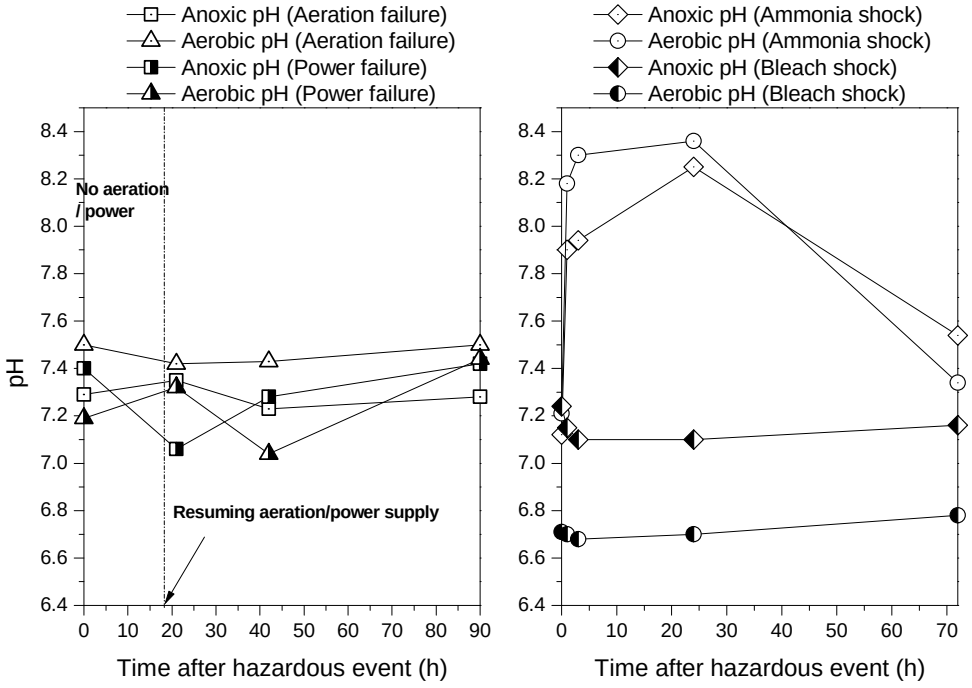

Figure 6.1: Impact of hazardous events on mixed liquor pH in anoxic and aerobic bioreactors of the pilot-scale MBR.

As expected, the mixed liquor ORP of both reactors instantly decreased due to aeration/power failure and ammonia shock, while the ORP of the anoxic reactor increased from -40 to 53 mV immediately (1 h) after applying the bleach shock (**Figure 6.2**), although in all cases the ORP levels returned to their original levels within 24 h.

Biological nutrient removal is extremely sensitive to ORP in bioreactors, and different metabolic processes, namely, nitrification, denitrification and phosphate accumulation/release dominate in different ORP ranges (Phan et al., 2014). Therefore, the observed ORP swing was likely a key reason for the disruption in nutrient removal as discussed in Section **6.3.2**.

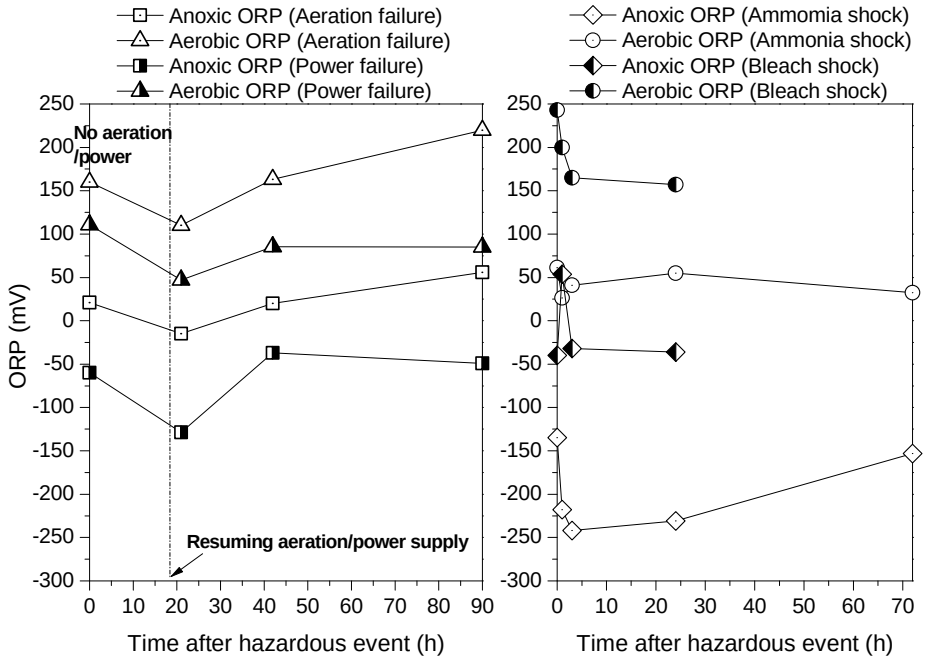

Figure 6.2: Impact of hazardous events on the oxidation-reduction potential (ORP) in anoxic and aerobic bioreactors of the pilot-scale MBR.

6.3.1.2 MLSS and MLVSS concentration

Of the entire period of the pilot plant operation (270 d), the aerobic MLSS concentration was at its highest immediately prior to the application of ammonia shock (Day 231). This may have been caused by the high suspended solid concentration in the influent that was fed to the pilot plant from Day 210 to 234. The influent suspended solid concentration in that period was 0.6 g/L (approximately 10 times the usual suspended solid concentration in influent, 0.06 ± 0.07 g/L, $n = 26$). Ammonia shock caused notable gradual reduction in MLVSS concentration (aerobic: from 2.6 to 2.0 g/L; anoxic from 2.5 to 1.6 g/L) (**Figure 6.3**) and MLVSS/MLSS ratio (aerobic: 0.59 to 0.55; anoxic: 0.73 to 0.51) (**Appendix Figure A-8**), and possibly contributed to the observed reduction in bulk organics, nutrient and TrOC removal (see Section **6.3.2** and **6.3.3**). The observed impact of ammonia shock on MLVSS concentration was expected as it

has been demonstrated to inhibit the growth of activated sludge (Ding et al., 2014). A similar impact of ammonia shock on sludge concentration was observed in a previous MBR study (Trinh, 2013). In the current study, 18 h power failure caused notable reduction in aerobic MLVSS concentration (2.5 to 1.5 g/L) which did not recover within 72 h, while the MLVSS concentration was only slightly affected due to 18 h aeration failure (**Figure 6.3**). Although sludge lysis was evident through the brownish colour in effluent which appeared within 1 h of adding bleach and continued for the next 72 h (data not shown), little variation in MLVSS or MLSS concentration was observed. This may be attributed to the fact that among all the hazardous events, the influent TOC (200 mg/L) and TN (75 mg/L) during bleach shock was the highest, which perhaps sustained the sludge concentration despite sludge lysis by bleach.

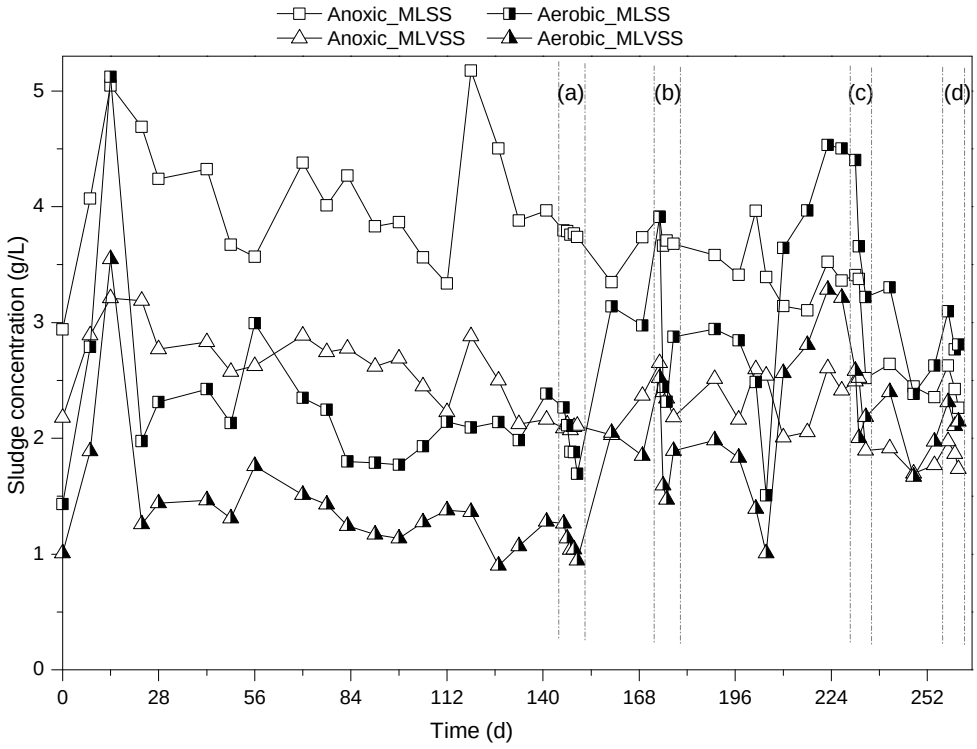

Figure 6.3: Sludge concentration profiles over the entire operation period of the pilot-scale MBR. The hazardous events tests were conducted in the following order: (a) aeration failure (Day 146 – 150), (b) power failure (Day 174 – 178), (c) ammonia shock (Day 231 – 234) and (d) bleach shock (Day 258 – 261). The variation in MLSS and MLVSS profiles during normal operation period can be attributed to the variation in the influent strength.

6.3.2 Impact on bulk organics and nutrient removal

The influent TOC, COD and TN concentrations to the MBR varied significantly (TOC = 110 ± 50 mg/L, *n = 41*; COD = 210 ± 120 mg/L, *n = 29*; and TN = 50 ± 20 mg/L, *n = 41*) over the observation period. Thus the bulk organics and nutrient removal efficiency also fluctuated considerably (**Figure 6.4**). Nevertheless, the influent was not changed during conducting hazardous events and the impacts of hazardous events were investigated at periods of relatively stable TOC, COD and TN removal.

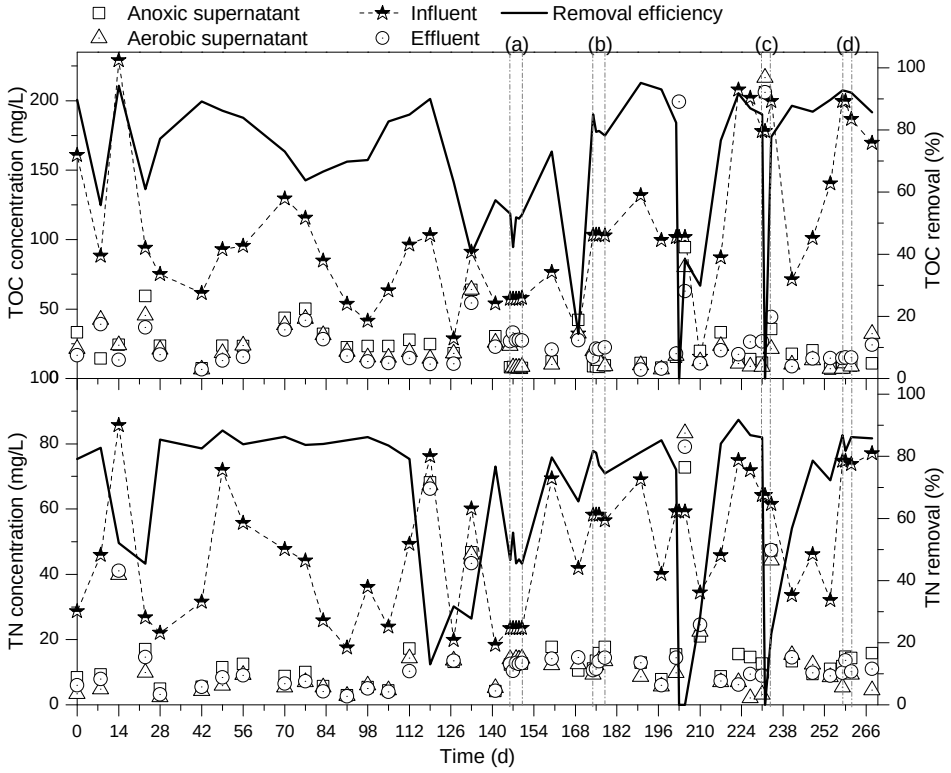

Figure 6.4: TOC and TN concentration and removal efficiency profiles over the entire operation period of the pilot-scale MBR. The hazardous events were conducted in the following order: (a) aeration failure (Day 146 -150); (b) power failure (Day 174 – 178); (c) ammonia shock (Day 231 – 234) and (d) bleach shock (Day 258 – 261). (Note: ammonia shock was initially attempted on Day 202, but later abandoned as it was found that prior to this attempt the mixing in anoxic reactor was operating inadequately for a day. The second attempt was made on Day 231 after stabilization of the reactor performance).

6.3.2.1 TOC and COD removal

In agreement with a recent study on the impact of oxygen cut off and starvation conditions on biological activity of activated sludge (Villain et al., 2013), in this study, aeration and power failure caused slight and temporary drops in TOC and COD removal (**Figure 6.5** and **Figure 6.6**). Conversely, ammonia shock led to immediate loss of TOC removal capacity by the MBR, and no removal was observed for 24 h, although the removal performance significantly recovered within 72 h (**Figure 6.7**). A significant but less prominent impact of ammonia shock on COD removal was also observed. While the impact of ammonia shock observed in the current study generally resonates with that from previous CAS and MBR studies (Ding et al., 2014; Trinh et al., 2015), the results from this study additionally indicate that TOC may be a more sensitive parameter than COD to assess the impact of these types of hazardous events.

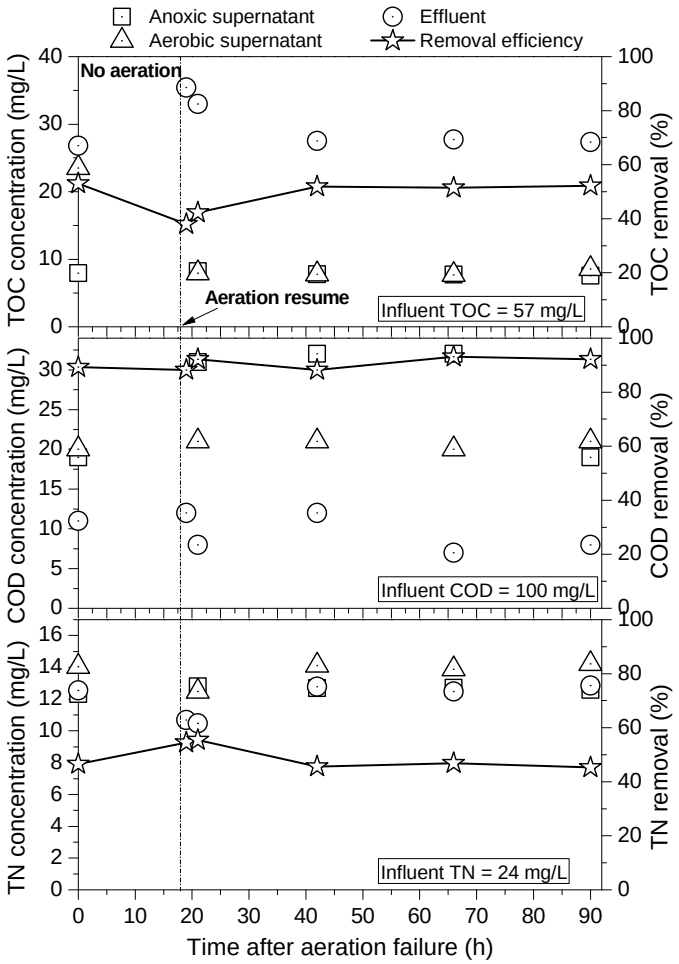

Figure 6.5: Impact of aeration failure on TOC/COD/TN removal performance by the pilot-scale MBR. Aeration was stopped for 18 h, and removal performance was monitored in samples collected from the 19th hour i.e., 1 h after resuming aeration.

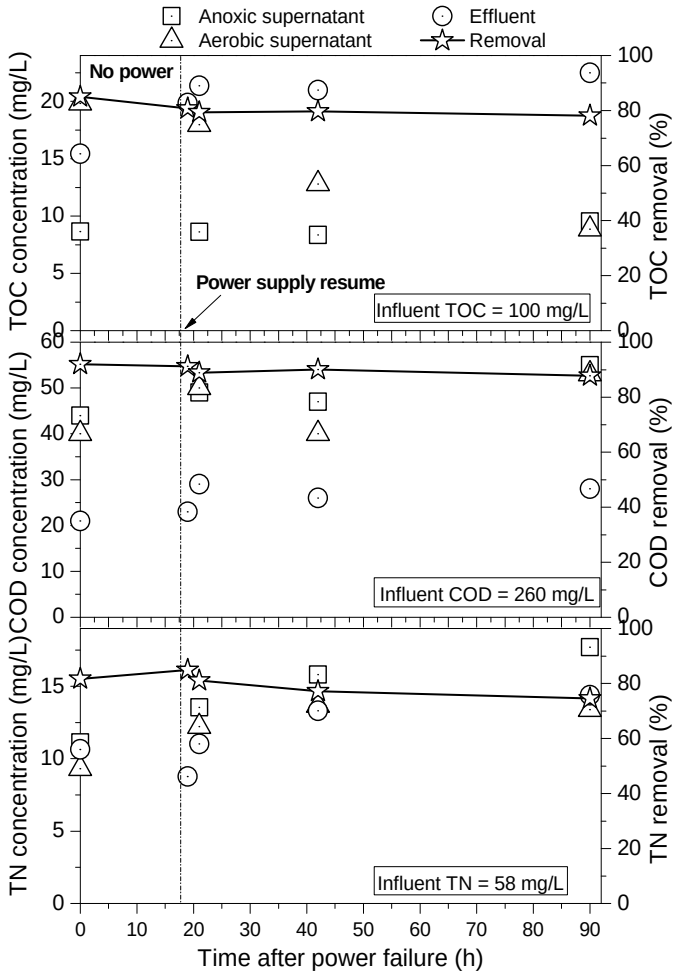

Figure 6.6: Impact of power failure on TOC/COD/TN removal performance by the pilot-scale MBR. Power supply was stopped for 18 h, and removal performance was monitored in samples collected from the 19th hour i.e., 1 h after resuming power supply.

Bodik et al. (2008) reported a 30-100% drop in COD removal due to bleach dosage of 4.75 – 47.5 mg Cl_2/g MLSS in batch tests. However, although an average bleach dosage of *ca.* 13 mg Cl_2/g MLSS was applied in the current study, in agreement with the MBR study of Knops (2010), a negligible impact of bleach shock on TOC and COD removal was observed (**Figure 6.8**). The discrepancy between the observations made in the current study and that in Bodik et al. (2008) highlights that relying on batch tests to predict the impact on continuous flow MBRs may be misleading.

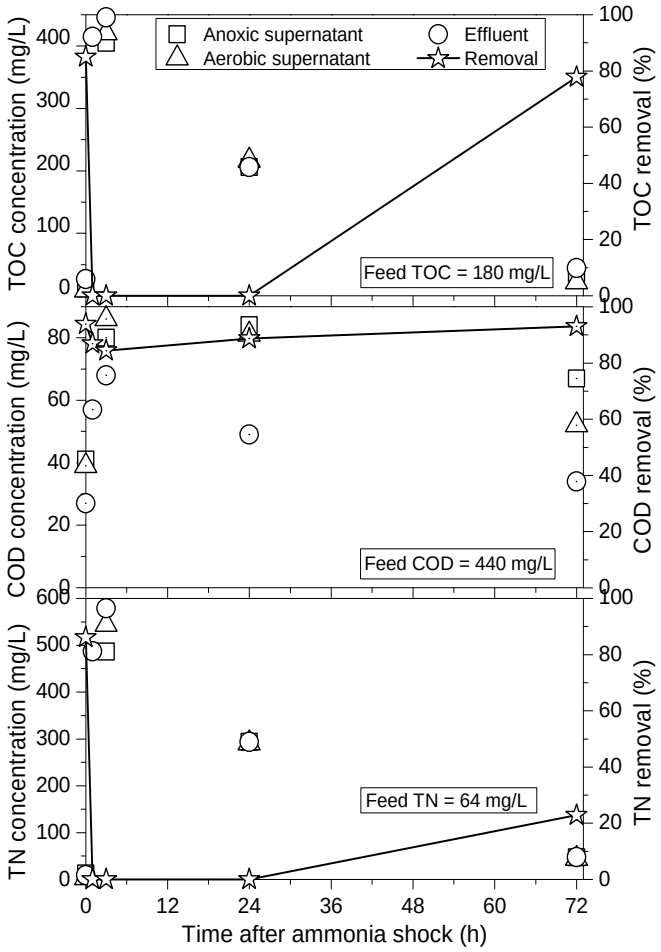

Figure 6.7: Impact of ammonia shock on TOC, COD and TN removal performance by the pilot-scale MBR. Addition of NH_4HCO_3 to achieve a NH_4^+-N concentration of 1000 mg/L caused an instantaneous additional TOC and TN load of 670 and 780 mg/L, respectively.

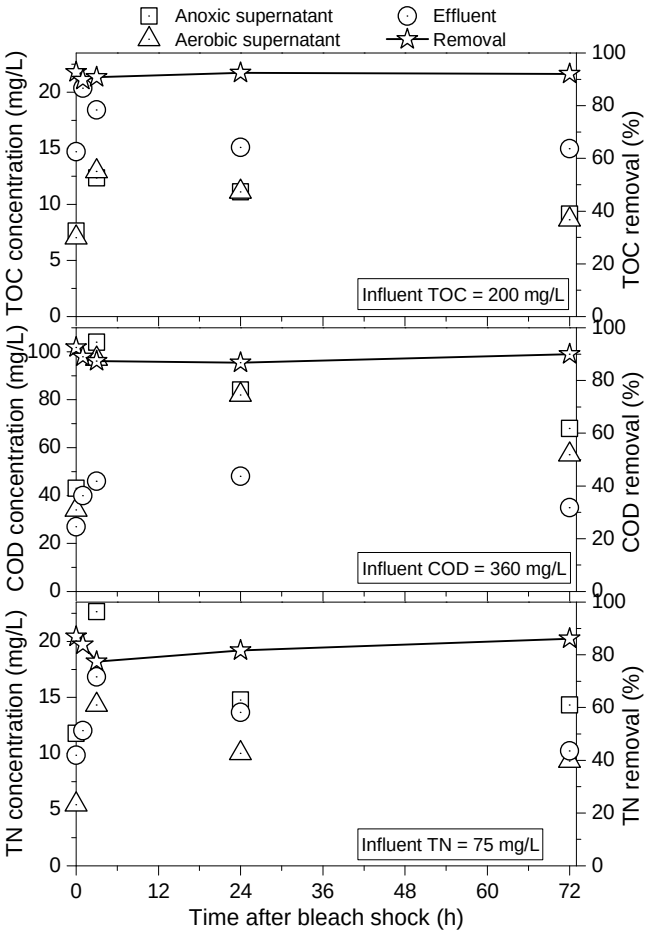

Figure 6.8: Impact of bleach shock on TOC/COD/TN removal performance by the pilot-scale MBR.

6.3.2.2 Nutrient removal

All hazardous events, most significantly ammonia shock, caused significant deterioration in TN and ammonia removal. Notably, addition of NH_4HCO_3 to achieve a NH_4^+-N concentration of 1000 mg/L caused an instantaneous additional TOC and TN load of 670 and 780 mg/L, respectively. However, in addition to this increased load of TOC and TN, ammonia is notorious for its cytotoxic effects. Furthermore, ammonia shock caused increase in mixed liquor pH (Section **6.3.1.1**). Consequently, ammonia shock led to an immediate and complete cessation of TN and ammonia removal for 24 h (**Figure 6.9**), and only a 25% recovery of TN and ammonia removal was observed within 72 h. By contrast, a 10-30% reduction in TN removal was observed during the other hazardous events, and a complete recovery was usually observed within 72 h (**Figure 6.10**, **Figure 6.11** and **Figure 6.12**).

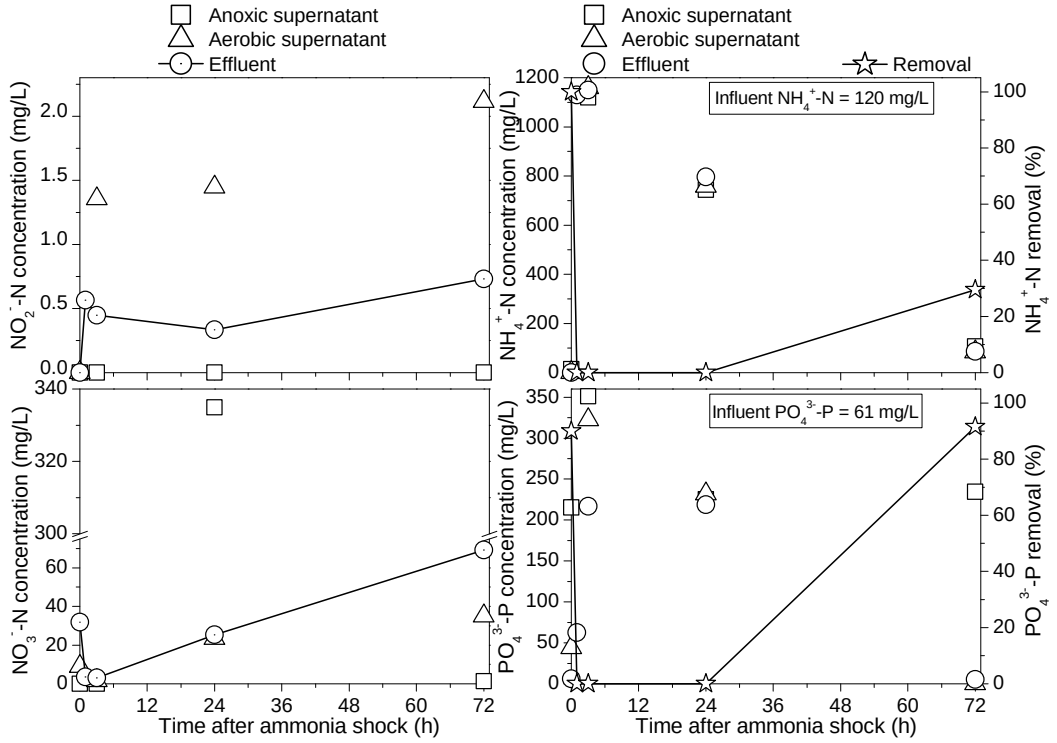

Figure 6.9: Impact of ammonia shock on the formation of NO_x^--N and the removal performance of NH_4^+-N and PO_4^{3-}-P by the pilot-scale MBR.

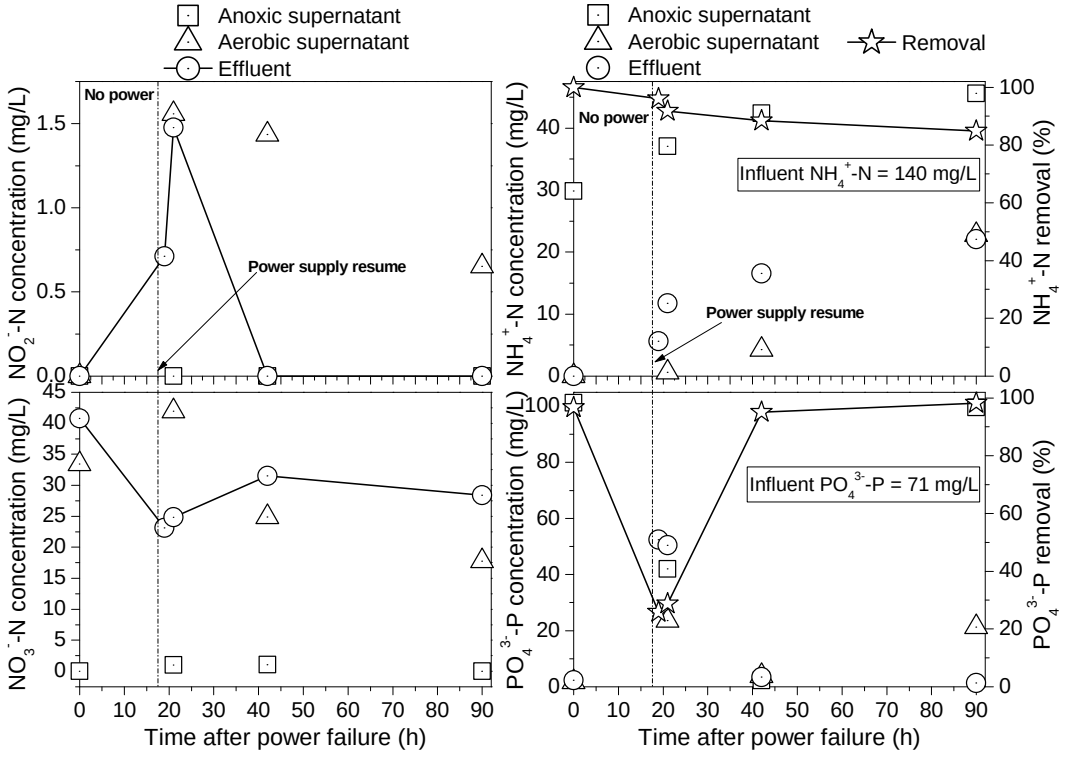

Figure 6.10: Impact of power failure on the formation of NO_x^--N and the removal performance of NH_4^+-N/ PO_4^{3-}-P by the pilot-scale MBR. Power supply was stopped for

18 h, and removal performance was monitored in samples collected from the 19th hour i.e., 1 h after resuming power supply.

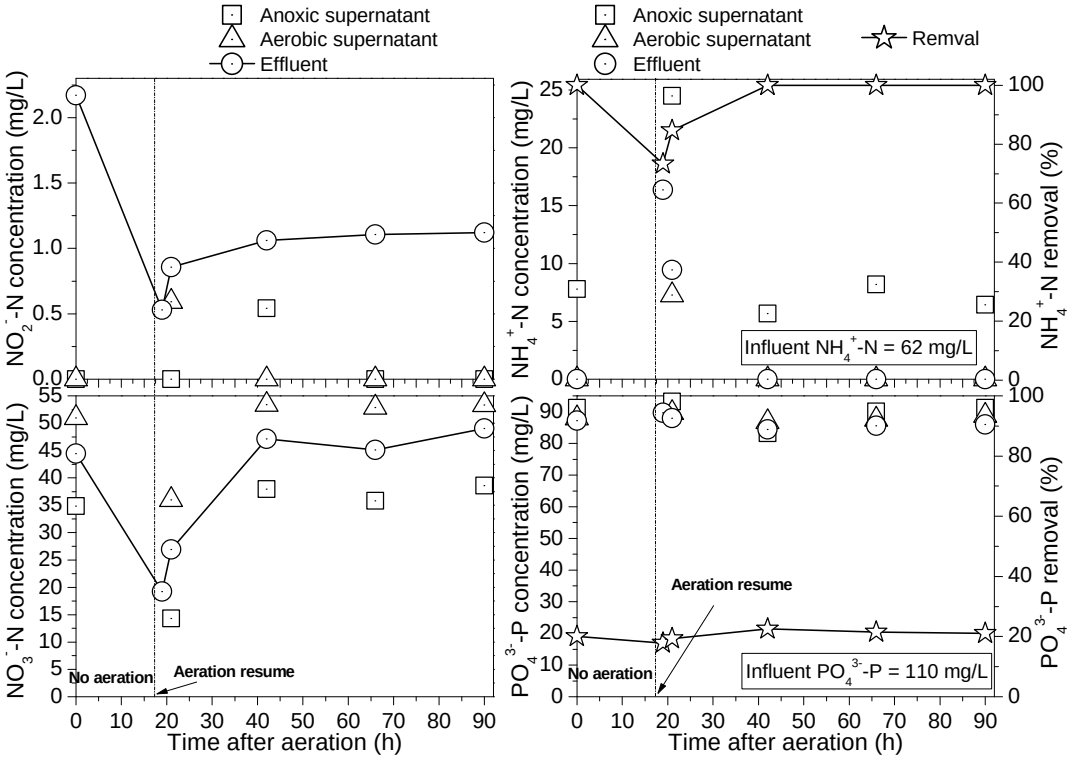

Figure 6.11: Impact of aeration failure on the formation of NO_x^--N and the removal performance of NH_4^+-N/PO_4^{3-}-P by the pilot-scale MBR. Aeration was stopped for 18 h, and removal performance was monitored in samples collected from the 19th hour i.e., 1 h after resuming aeration.

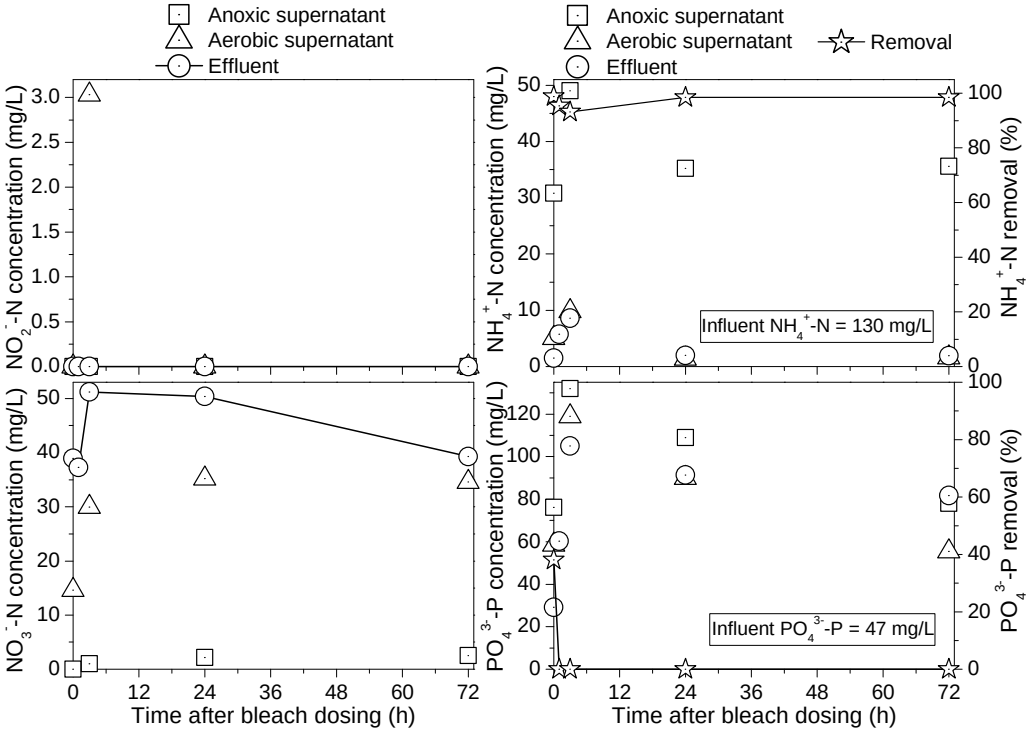

Figure 6.12: Impact of bleach shock on the formation of NO_x^--N and the removal performance of NH_4^+-N/ PO_3^{3-}-P by the pilot-scale MBR.

The drop in ammonia and TN removal was accompanied by the appearance of nitrite in effluent (**Figure 6.9**). The appearance of nitrite following ammonia shock indicates impaired nitrification, *i.e.*, incomplete conversion of ammonia, which may be attributed to the elevated ammonia load as well as the inhibition of the nitrifiers due to oxygen limitation caused by ammonia shock. Similar to the current study, ammonia shock to a pilot-scale aerobic CAS system led to a sharp increase of ammonia concentration with the concurrent occurrence of nitrite in effluent (Burgess et al., 2002). However, an additional aspect revealed in this study was the increase in nitrate concentration (following an initial drop), with simultaneous detection of nitrite in effluent (**Figure 6.9**). This suggests that not only nitrification (i.e., conversion of ammonia to nitrate) but also denitrification (i.e., conversion of nitrate to nitrogen gas) was affected. This can be explained by the upsets in ORP and MLVSS concentrations caused by the hazardous events (See Section **6.3.1**). Notably, ammonia removal recovery was confirmed 10 d following the ammonia shock, while that of TN was not achieved before an additional 7 d (data not shown), possibly indicating that the denitrifiers were more susceptible to ammonia shock.

The pilot-scale MBR was not specifically designed for phosphate removal. For example, it lacked a strictly anaerobic reactor. Accordingly, the PO_4^{3-}-P removal performance was poor throughout this study (32 ± 16%, n = 18), except for the period of Day 174 – 190 and Day 231 – 248, when the PO_4^{3-}-P removal was as high as 96 and 90%, respectively. The power failure and ammonia shock events coincided with the periods of high PO_4^{3-}-P removal, and a significant impact of these hazardous events on PO_4^{3-}-P removal performance was observed. For example, ammonia shock resulted in a complete cessation of PO_4^{3-}-P removal for 24 h, although the removal was recovered entirely within 72 h after shock load (**Figure 6.9**). Similarly, the samples taken after 1 h of resuming power supply showed a 73% drop in PO_4^{3-}-P removal (**Figure 6.10**), possibly due to release of stored PO_4^{3-}-P via biomass autolysis under starvation conditions (Yogalakshmi et al., 2007). The impact of aeration failure and bleach shock was significant but less pronounced, which may be attributed to the fact that PO_4^{3-}-P removal was already low even before the hazardous events were conducted (**Figure 6.11** and **Figure 6.12**).

6.3.3 Impact on trace organic contaminant removal

Among the 45 TrOCs monitored (**Supplementary Data Table A-4**), 13 compounds were consistently detected in influent samples during the experimental period of four simulated hazardous events, while 14 TrOCs were detected only occasionally (**Figure 6.13**).

6.3.3.1 Aqueous phase removal

The MBR achieved high removal (>90%) of significantly hydrophobic TrOCs (Log D > 3), namely, polyparaben, estrone, etiocholanolone, androsterone and 17β-estradiol irrespective of the hazardous events (**Figure 6.13**). These compounds possess similar chemical backbone structures, and the fact that they were not detected in sludge (see Section **6.3.3.2**) confirmed their removal by biotransformation. High biodegradability of these TrOCs can be attributed to the presence of strong electron withdrawing group hydroxyl in their structures (Tadkaew et al., 2011). On the other hand, negligible to up to 25% removals of the well-known resistant compounds diclofenac, carbamazepine, primidone and amitriptyline were observed throughout the experimental period. Occurrence of strong electron withdrawing groups and/or absence of electron donating

groups is thought to be related to their poor removal by CAS processes or MBRs (Luo et al., 2014).

A significant impact of one or more hazardous events was noted in the case of removal of eight pharmaceuticals, namely, atenolol, sulfamethoxazole, naproxen, trimethoprim, ibuprofen, paracetamol, caffeine, and triclosan. With the exception of triclosan, all these TrOCs are significantly hydrophilic and thus biodegradation can be considered the main mechanism of their removal by MBR. Similar to TOC or TN removal, ammonia and bleach shocks were generally observed to exert greater impact on TrOC removal than aeration and power failure events (**Figure 6.13**).

Atenolol, caffeine, ibuprofen and paracetamol are generally classified as easily biodegradable (Hai et al., 2014; Tadkaew et al., 2011), but in this study a significant reduction in removal efficacy was noted for these TrOCs after the hazardous events. Consistent with the available literature, in this study, over 90% removal of atenolol was observed (**Figure 6.13**). However, a 5-10% reduction in atenolol removal was noted during the hazardous events. The presence of the strong electron donating group amine in atenolol is thought to make it amenable to biodegradation (Tadkaew et al., 2011), but atenolol also contains a strong electron withdrawing group (amide), which perhaps renders its removal by MBR susceptible to process changes. Nevertheless, in all instances a complete recovery of atenolol removal was achieved within 72 h. Paracetamol was detected in raw sewage at concentrations up to 120 µg/L. Due to bleach shock, its concentration in effluent increased noticeably from below detection limit (<5 ng/L) to approximately 200 ng/L within 1 h of bleach addition. During the observation period, the raw sewage caffeine concentration varied over 13 – 98 µg/L. While caffeine removal efficiency was found to range between 96 – 99% regardless of the hazardous events, effluent caffeine concentration increased significantly (from around 100 up to 2500 ng/L) immediately after hazardous events occurred. Effluent concentration returned to baseline within 24 h in case of aeration failure, but a full recovery was not achieved even within 72 h for the other events.

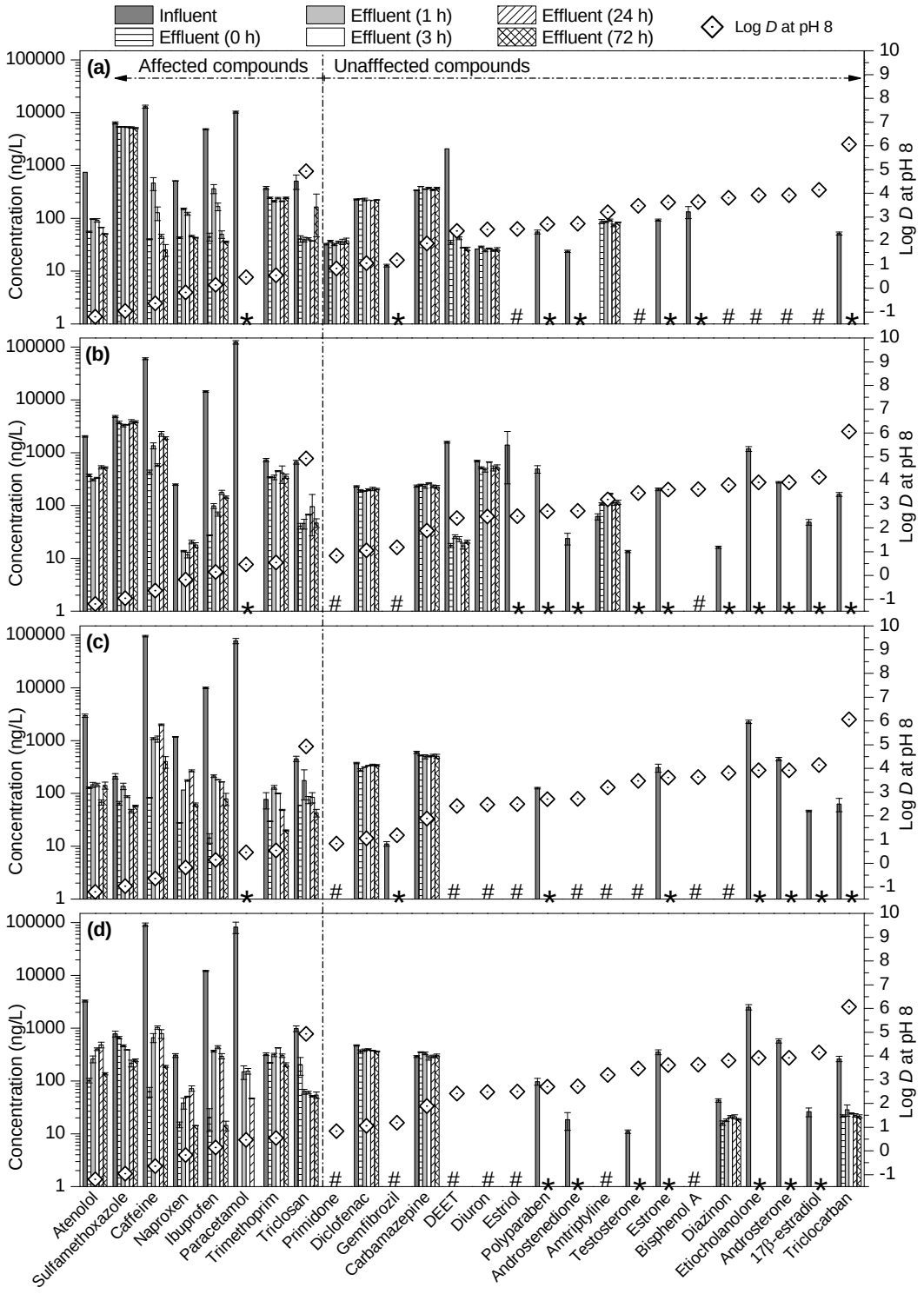

Figure 6.13: Impact of hazardous events (a. aeration failure, b. power failure, c. ammonia shock, and d. bleach shock) on the effluent concentrations of 26 TrOCs which were detected beyond detection limit in influent during at least one of the hazardous events (Note: "*" indicates that the concentration of this TrOC in effluent was below detection limit in that event; "#" indicates that this TrOC remained undetected in both

influent and effluent in that specific event). Error bars indicate the standard deviation from triplicate samples.

It was a similar outcome for ibuprofen which had a concentration of 5 to 15 µg/L in the influent during the observation period. While over 90% removal was consistently achieved, all hazardous events caused significant increase in effluent ibuprofen concentration. For example, in case of ammonia shock, effluent ibuprofen concentration increased from 14 ± 3 ng/L (before adding ammonia) to 215 ±13 ng/L within 1 h of ammonia addition. By contrast, caffeine and ibuprofen were only slightly affected by ammonia shock or power failure in a previous MBR study (Trinh, 2013), but this discrepancy may be explained by the fact that the influent TrOC concentrations in that study were lower.

Naproxen removal was observed to gradually reduce from 98 to 77% within 24 h of ammonia shock; however, the removal had mostly recovered within 72 h (**Figure 6.13**). A similar outcome for naproxen removal was observed with the other hazardous events. Compared to the study of Trinh (2013), a greater impact on naproxen removal was observed in the current study, possibly because a higher ammonia shock dose (260 *vs.* 140 mg NH_4^+-N/ g MLSS) and a longer power failure period (18 *vs.* 2 h) were applied here. Trimethoprim has been reported to be degraded only by slow-growing bacteria such as nitrifiers (Pérez et al., 2005) that are known to be more sensitive to environmental factors. Indeed, a wide variation (0-90%) in trimethoprim removal by activated sludge treatment has been reported in the literature (Tadkaew et al., 2011). In this study, trimethoprim removal dropped from around 60% to no removal within 1 h of ammonia shock, although a complete recovery of removal was observed within 72 h. A similar impact was observed after bleach shock, while a slight fluctuation in removal was found for aeration and power failure events. Gemfibrozil removal has been previously correlated to NH_4^+-N removal (Phan et al., 2015). Indeed Trinh (2013), reported significant impact of ammonia shock on gemfibrozil removal. In the current study, gemfibrozil was detected in influent only during aeration failure and ammonia shock experiments, and, unaffected by the hazardous events, it was completely removed. The discrepancy with the study of Trinh (2013) may be explained by the significantly lower influent gemfibrozil concentration in this study (<15 ng/L *vs.* 1 µg/L).

Influent sulfamethoxazole concentration during the current study varied from 0.02 (ammonia shock) to 6.7 µg/L (aeration failure), which led to significant variation in removal (20-69%) even without occurrence of the hazardous events (**Figure 6.13**), suggesting kinetic limitations. This is consistent with the high variability (50-90%) in sulfamethoxazole removal reported in the literature (Tadkaew et al., 2011). Furthermore, consistent with another study (Trinh, 2013), sulfamethoxazole removal decreased from 69 to 35% within 1 h of ammonia shock. However, interestingly, 24 h after applying the ammonia shock, the removal increased to around 80%. No other hazardous event studies (CAS or MBR) have reported such a phenomenon, but drawing on the report of Gulde et al. (2014) regarding increased biotransformation rate of cationic-neutral TrOCs such as sulfamethoxazole due to pH increase from 7 to 8, the observation made in this study may also be attributed to the increased pH of the bioreactors due to ammonia addition. Additionally, the improved removal of sulfamethoxazole (20 *vs.* 75%) after bleach shock may be attributed to the oxidative degradation of this TrOC by chlorine. Gao et al. (2014) reported effective removal over an initial sulfamethoxazole concentration range of 0.05 – 5 mg/L after addition of a chlorine dose of 2 mg/L, which is well below that applied in the current study. Similarly, the improved aqueous triclosan removal during bleach shock may be attributed to the formation of chlorinated triclosan derivatives (Buth et al., 2011), and not necessarily breakdown to smaller metabolites.

6.3.3.2 TrOC concentration in sludge

Of the 45 monitored TrOCs, nine pharmaceuticals were detected in sludge at concentrations over the detection limit during the monitoring period (**Figure 6.14**). All significantly hydrophobic TrOCs (i.e., compounds having log D>3), except triclosan and triclocarban, were consistently well removed from the aqueous phase by the MBR irrespective of the hazardous events (**Figure 6.13**). Furthermore, these compounds were not detected in sludge, which confirms their biodegradation. Conversely, triclosan (140 – 940 ng/g MLSS) and triclocarban (960 – 1500 ng/g MLSS) were detected in sludge at concentrations much higher than all other TrOCs (5 – 450 ng/g MLSS) (**Figure 6.14**). In fact the accumulated mass of triclosan and triclocarban on sludge accounted for up to 14 and over 95%, respectively, of their influent load. This suggests that triclocarban underwent insignificant biotransformation. Similar observations were reported by Trinh

(2013). Sludge-adsorption data for the aeration failure event was unavailable, however, no significant impact of the toxic shocks or the power failure event on triclocarban adsorption was observed. On the other hand, triclosan concentration in both aqueous and sludge phases increased following the ammonia shock (**Figure 6.14**), indicating that triclosan biotransformation was significantly affected by ammonia shock.

Several hydrophilic TrOCs, whose aqueous phase removal was susceptible to one or more hazardous events, were also detected in sludge (**Figure 6.14**). This is consistent with previous reports that TrOC accumulation in sludge may depend on their inherent biodegradability and concentration in wastewater in addition to adsorption capacity (Wijekoon et al., 2013), and thus hydrophilic TrOCs which are resistant and/or occur at high concentrations in wastewater can also accumulate in sludge. Hazardous events appeared to influence the sludge concentration of these hydrophilic compounds to some extent but a clear relationship between concentration in sludge and their aqueous phase removal could not be established, indicating that their removal was controlled more by biodegradation. For example, within 3 h of ammonia shock, the concentration of paracetamol increased from 110 to 450 ng/g MLSS, but was then reduced to 15 ng/g MLSS within 24 h. However, the effluent concentration of paracetamol was always below the detection limit following the ammonia shock. Consistent with the current study, Trinh (2013) observed no clear impact of hazardous events including ammonia shock and power failure on TrOC adsorption to MLSS.

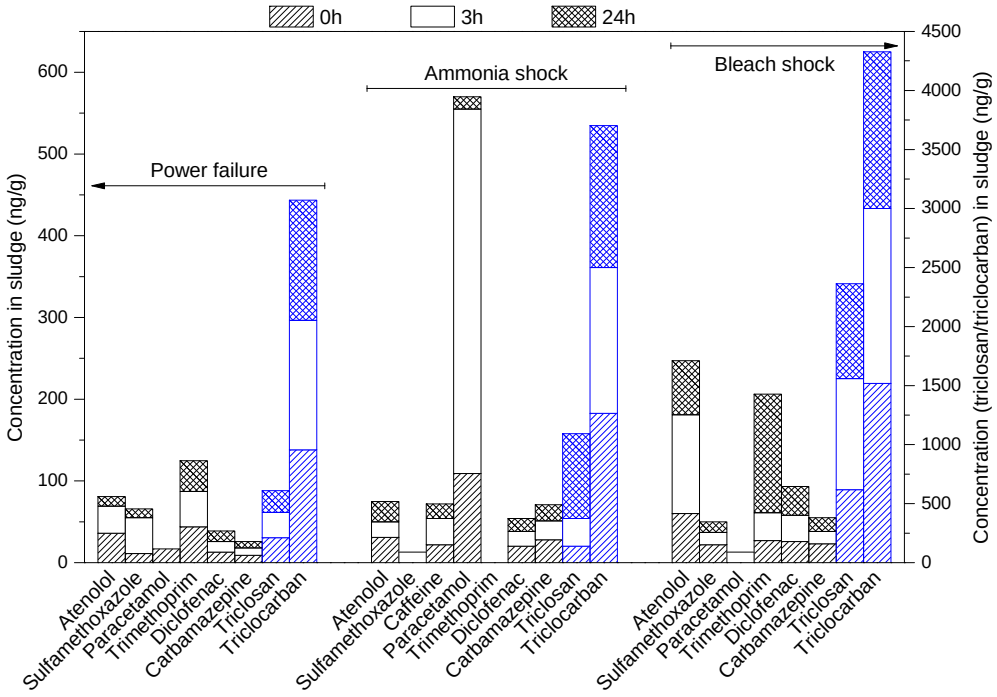

Figure 6.14: Concentration of nine TrOCs detected over detection limit in sludge before (0 h) and at 3 and 24 h after the hazardous events (power failure, ammonia shock and bleach shock). Data plotted are average of duplicate samples.

6.3.4 Impact on effluent estrogenicity and toxicity

There was a significant variation in estrogenicity (**Figure 6.15**) and toxicity (**Table 6.1**) of the raw sewage (feed water) between the various simulated hazardous events. For example, higher estrogenicity was measured in influent during the power failure simulation compared to the aeration failure experiment. This variation was also reflected in chemical analysis results, with greatly higher influent concentration of estrogen hormones (17β-estradiol, estrone and estriol) during the power failure simulation compared to those detected in influent during the aeration failure experiment. Similarly, the extremely low influent toxicity (0.52 rTU compared to 44 - 79 rTU in other events) during the aeration failure test is reflected in the low influent COD (about 70 – 75% less than in the other events) during that period. However, significant reduction of both estrogenicity (**Figure 6.15**) and toxicity (**Table 6.1**) was achieved following MBR treatment irrespective of the occurrence of any simulated hazardous events. This was again in line with the stable removal of estrogenic TrOCs during the hazardous events established by chemical analysis (**Figure 6.13**). An estrogenic activity of 1 ng/L EEQ is commonly accepted as unlikely to cause significant endocrine effects

in exposed aquatic biota (Leusch et al., 2014; Scott et al., 2014), and the MBR effluent estrogenicity in this study was consistently below this threshold value even during hazardous events.

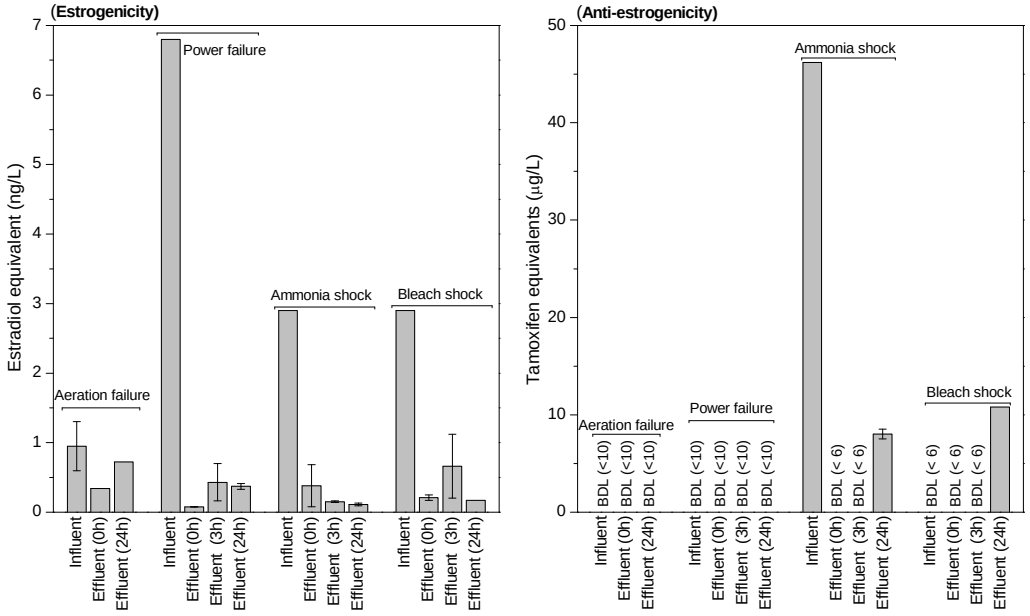

Figure 6.15: Estrogenic (17β-estradiol equivalent, EEQ) and anti-estrogenic (Tamoxifen equivalent, TMXEQ) activities measured in influent and effluent of the pilot-scale MBR during hazardous event experiments. Effluent samples were collected immediately before hazardous events (0 h) and at 3 and 24 h after resuming normal operation (following aeration or power failure) or application of shock loading. Where applicable, error bars represent the standard deviation from duplicate samples.

Anti-estrogenic activity was detected in raw sewage (feed water) during the ammonia shock simulation, and in MBR effluent 24 h after the ammonia and bleach shock (**Figure 6.15**). Although much less is known about the significance of anti-estrogenic activity in wastewater, both estrogenic and anti-estrogenic activity can lead to endocrine disruption in aquatic organisms (Pike et al., 2001). The appearance of anti-estrogenic activity during bleach shock appears to indicate the formation of anti-estrogenic by-products, possibly the result of chlorination of aromatic amino acid and humic/fulvic acid components of wastewater matrix (Tang et al., 2014). Ammonia shock has been reported to cause abiotic nitration of estrogens in wastewater treatment in case of high

influent NH_4^+-N concentration (>200 mg/L) or in process conditions that prevent nitrification of NO_2^--N to NO_3^--N (Gaulke et al., 2008). The nitrated forms of these chemicals show lower estrogenic activity than the parent compounds; however, their anti-estrogenic activity has not been established (Sun et al., 2012). Nevertheless, this is the first report on effluent estrogenicity and toxicity following simulated hazardous events, and results from this study confirm that MBRs can efficiently reduce estrogenicity and toxicity from wastewater, but toxic shocks such as that from ammonia or bleach could temporarily increase the endocrine activity of effluent after significant process disturbances.

Table 6.1: Toxicity in influent and effluent of the pilot-scale MBR, expressed as relative toxicy unit (rTU). Effluent samples were collected immediately before hazardous events (0 h) and at 3 and 24 h after resuming normal operation (following aeration or power failure) or application of shock loading. Values indicate average ± standard deviation (n = 2).

Sample	Toxicity (rTU)			
	Aeration failure	Power failure	Ammonia shock	Bleach shock
Feed	0.52[a]	44[a]	79[a]	--
Effluent (0 h)	0.19 ± 0.01	0.27 ± 0.02	0.14 ± 0.02	0.15 ± 0.01
Effluent (3 h)	--	0.20 ± 0.04	0.22 ± 0.02	0.35 ± 0.03
Effluent (24 h)	0.22[a]	0.21 ± 0.01	0.23 ± 0.04	0.34[a]

[a]Single samples.

6.3.5 Impact on membrane fouling

Hazardous events such as toxic shock can alter sludge settling and dewatering properties and consequently affect filterability of the mixed liquor. For example, exchange of monovalent ammonium with divalent cations in sludge can result in weaker and fragile flocs (Novak, 2001), or elevated release of soluble microbial product and extracellular polymeric substance into the mixed liquor can occur upon exposure to toxic shocks (Kimura et al., 2014) – all of which can result in aggravated membrane fouling. In this study, slight but discernible increase in TMP (up to 5 kPa) was observed during the simulated hazardous events (**Figure 6.16**). This is in agreement with previous reports regarding the impact of power failure and ammonia shock (Trinh et al., 2015) and bleach shock (Knops, 2010) on membrane fouling. However, the extent of membrane fouling may be governed by the chemical shock-dose and particularly the applied

membrane flux. In this context, it is noteworthy that, compared to Trinh et al. (2015) and Knops (2010), a significantly higher ammonia (140 *vs.* 260 mg NH_4^+-N/ g MLSS) and bleach dose (3.3 *vs.* 13 mg Cl_2 /g MLSS), respectively was applied in the current study. By contrast, the applied membrane flux (1.2 L/m^2.h) in the current study was only 5-10% of that applied by Trinh et al. (2015) and Knops (2010). Thus the results reported here do not necessarily imply that hazardous events will always have minor impacts on membrane hydraulic performance. Indeed, despite seemingly moderate impact on TMP during MBR operation, heightened filtration resistance was recorded during processing (for analysis) of bleach-shock samples via high-flux filtration, indicating the role of flux on membrane fouling during hazardous events.

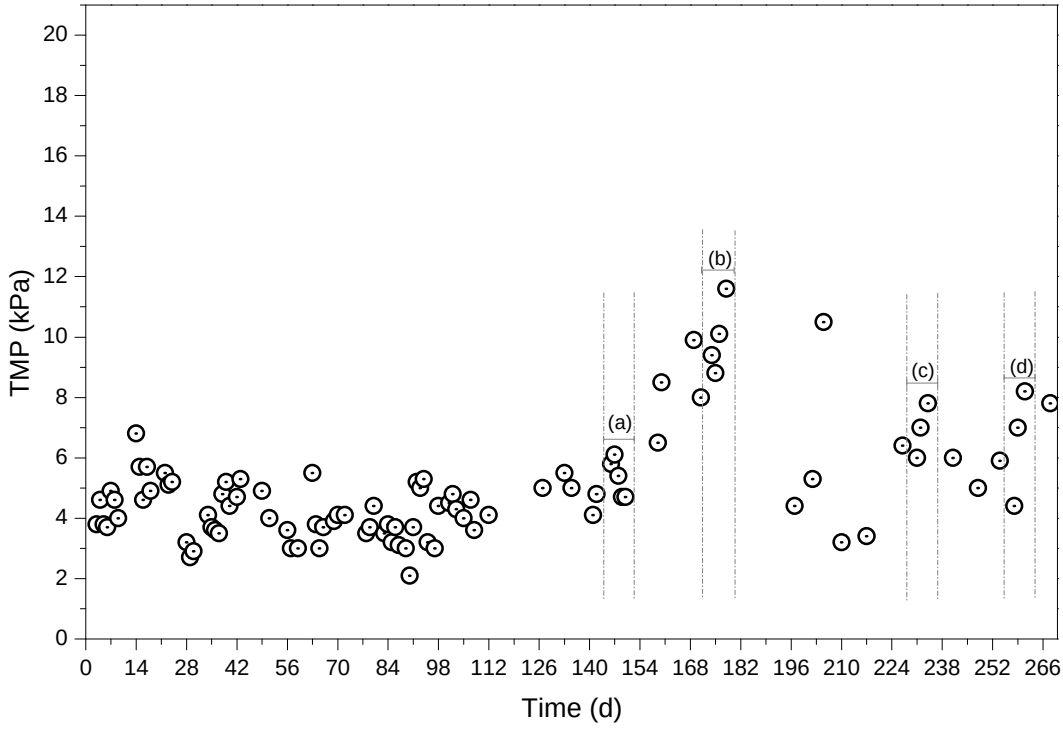

Figure 6.16: TMP profile over the whole operation period of the pilot-scale MBR. The periods of four hazardous events i.e., aeration failure, power failure, ammonia shock and bleach shock are indicated as (a), (b), (c) and (d), respectively.

6.4 CONCLUSIONS

The impacts of aeration and power failure and ammonia and bleach shock on the performance of a pilot-scale MBR fed with real sewage were investigated. Ammonia shock showed the greatest impact that led to an immediate loss of TOC, TN, NH_4^+-N,

and PO_4^{3-}-P removal capacity. The TOC removal recovery was swift (72 h), while TN removal recovery took over two weeks. Other hazardous events induced less severe and brief impact. Removal of a number of TrOCs which are resistant and/or occur at high concentrations in wastewater were affected. Of interest was the impact on effluent estrogenicity and membrane fouling.

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CHAPTER 7. Conclusions and Recommendations for Future Work

7.1 CONCLUSIONS

The performance of an integrated anoxic-aerobic MBR was evaluated at both laboratory and pilot-scales, and via performance-comparison with a full-scale MBR in terms of removal of bulk organics, nutrients, and traces organic contaminant (TrOCs). A series of experiments provided insight into the role of redox conditions on the removal performance, the impact of shift in bacterial community in response to operating conditions on removal performance, and the robustness of the system to hazardous events.

(i) Long-term operation of a lab-scale integrated anoxic-aerobic MBR revealed that low DO or ORP (*i.e.*, anoxic) regimes are conducive to biodegradation of some TrOCs. However, an important prerequisite to anoxic biodegradaton of TrOCs is internal recirculation (IR) between the anoxic and aerobic bioreactors, in absence of which anoxic/anaerobic regimes alone may only enhance biosorption. Dependence of TN removal on IR, that controls the supply of nitrate to the anoxic reactor, was also evident. Despite the significantly different removal of certain TrOCs by the preceding anoxic bioreactor (depending on the IR), TrOC concentration in effluent from the aerobic MBR was stable, which confirms a key role of aerobic biodegradation in TrOC removal.

(ii) Monitoring of the dynamics of bacterial communities in the lab-scale anoxic-aerobic MBR revealed IR as the primary driving force shaping bacterial communities in the system. With IR, the bacterial communities in anoxic and aerobic bioreactors of the integrated MBR were highly similar in community structure and phylogenetic relationships. A community with greater diversity developed under longer SRT. TrOC addition induced changes in bacterial communities. Relative abundance of bacterial phylotypes indicated a relationship between microbial communities and capacity of nutrient and TrOC biotransformation. Based on the shifts of bacterial communities and variation in removal performance in response to operating conditions, potential bacterial groups responsible for nutrient and TrOC removal were identified.

(iii) During comparison of bulk organics, nutrients and TrOC removal performance by a full- and a pilot-scale MBR from real wastewater originating from a resort town, the pilot-scale MBR demonstrated a very similar COD reduction as the full-scale MBR. Given the significantly higher MLVSS concentration and presence of additional anoxic

and aerobic bioreactors in the full-scale plant, the removal of nutrients, particularly that of phosphorous by the full-scale MBR was significantly high (98 ± 4 % *vs.* 31 ± 15 % PO_4^{3-}-P removal by the pilot-scale MBR). Notably, any drop in TN or NH_4^+-N removal by the full-scale MBR was accompanied by a drop in the removal by the pilot-scale MBR, although the full-scale plant appeared to be more stable under influent load fluctuations. The full-scale MBR demonstrated higher and more stable removal of a few resistant and hydrophilic (log D < 3) TrOCs including sulfamethoxazole, trimethoprim, diclofenac and diuron. Performance comparison between the pilot- and full-scale MBRs reveals a link between stable TN and TrOC removals which were facilitated by a delicate combination of multiple redox zones in the bioreactors of the full-scale plant.

(iv) Assessment of the impact of aeration/power failure and ammonia/bleach shocks on the performance of the pilot-scale MBR fed with real sewage revealed the greatest impact of ammonia shock that led to an immediate loss of TOC, TN, NH_4^+-N, and PO_4^{3-}-P removal capacity. The TOC removal recovery was swift (72 h), while TN removal recovery took over two weeks. Other hazardous events induced less severe and brief impact. Removal of a number of TrOCs which are resistant and/or occur at high concentrations in wastewater were affected. The MBR effectively reduced estrogenicity and toxicity from wastewater, but chemical shocks temporarily increased the effluent endocrine activity. Additionally, hazardous events may exacerbate membrane fouling depending on the dose of chemical shock and the membrane flux.

7.2 RECOMMENDATIONS FOR FUTURE WORK

The higher removal of some hydrophilic TrOCs by the full-scale plant or more stable removal of other TrOCs by the full-scale plant as observed in this study may be attributed to the existence of pre- and post-anoxic tanks, and combination of aerobic zones with different levels of DO as compared to a pre-anoxic and a single aerobic tank in the pilot MBR. For a clearer understanding, further studies specifically on different combinations of anoxic and aerobic reactors for TrOC removal by MBR are recommended. Incorporation of the monitoring of pathogenic indicators in addition to removal of bulk and trace organic contaminants would better validate the performance of MBR systems for application in water reuse.

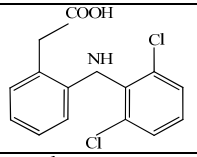
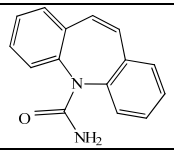
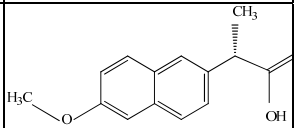
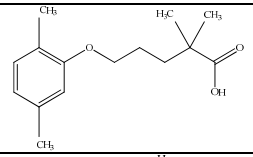
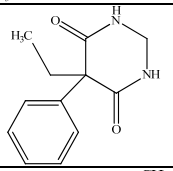
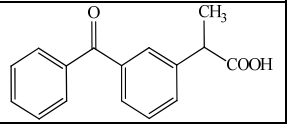
A more focused assessment of the bacterial communities responsible for biodegradation of specific TrOC groups in MBR systems is recommended. Advanced molecular technology such as next generation sequencing is a promising tool to identify these bacterial groups. An interesting approach may be to combine the stable isotope probing technique with pyrosequencing or other next generation sequencing methods, whereby activated sludge is inoculated with isotope-labelled TrOCs, and after total genomic DNA extraction, isotope-enriched DNA is separated by gradient centrifugation. DNA sequencing and then bacterial characterization will provide the profile of TrOC-degrading bacteria. Also the application of the FISH/FISH-MAR process will assist to clarify the role of bacterial structure on TrOC degradation.

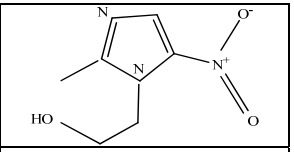
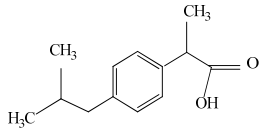
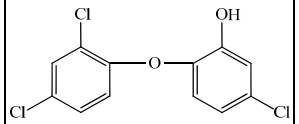
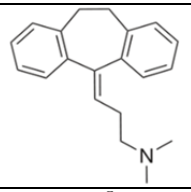
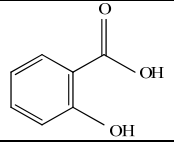
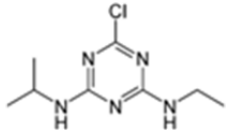
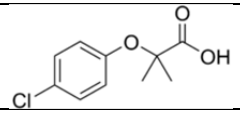
Depending on which microbial products will go through the membrane, attach to membrane or rebound back from the membrane surface, the extent of membrane fouling may vary. An assessment of the interactions between microbial products (*e.g.*, humic acids, polysaccharides, proteins, amino acids, antibiotics, and extracellular enzymes) and different types of membranes under different redox conditions is deemed necessary to optimize membrane performance in anoxic-aerobic MBRs.

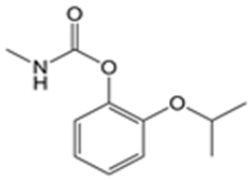
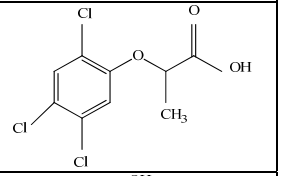
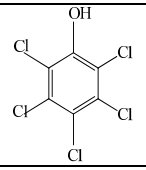
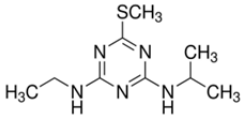
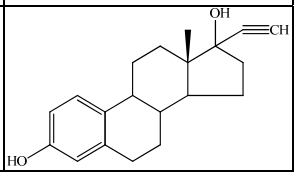
Finally, investigations on the impacts of other forms of hazardous events (than investigated in this study) under different operating conditions (*e.g.*, SRT, HRT and MLSS concentration) are recommended. A combination of chemical analysis, toxicity and estrogenicity bioasssays, and molecular biology is likely to provide a more comprehensive insight to the impacts of hazardous events on the performance of anoxic-aerobic MBRs.

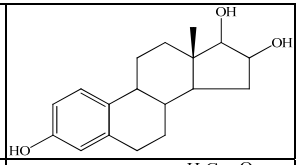
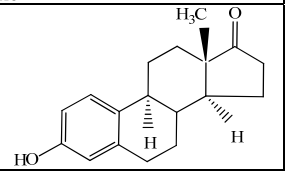
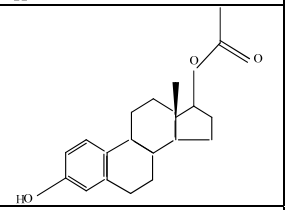
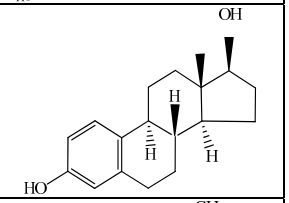
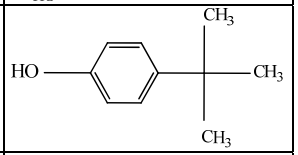
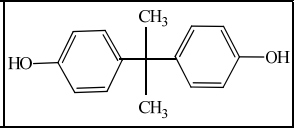
APPENDIX

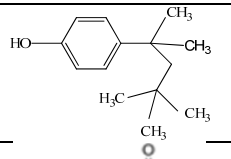
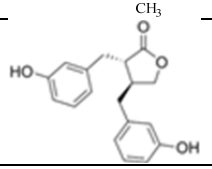
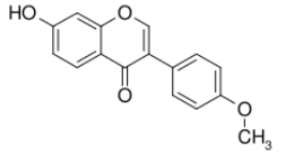
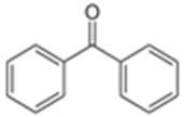
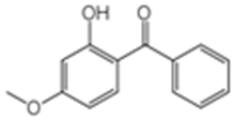
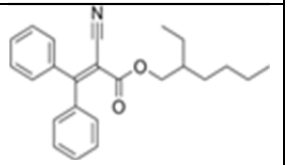
Table A-1: Physicochemical properties of the trace organic contaminants (TrOC) investigated in Chapter 3 and 4

Category	Chemical formula	Molecular weight (g/mol)	log*D* (pH 8)[a]	Henry's Law constant at 25°C (atm m^3/mol)[a]	Molecular structure
Pharmaceuticals and personal care products	Diclofenac ($C_{14}H_{11}Cl_2NO_2$)	296.15	1.06	2.69×10^{-11}	
	Carbamazepine ($C_{15}H_{12}N_2O$)	236.27	1.89	9.41×10^{-12}	
	Naproxen ($C_{14}H_{14}O_3$)	230.30	-0.18	6.08×10^{-12}	
	Gemfibrozil ($C_{15}H_{22}O_3$)	250.30	1.18	1.83×10^{-11}	
	Primidone ($C_{12}H_{14}N_2O_2$)	218.25	0.83	1.16×10^{-14}	
	Ketoprofen ($C_{16}H_{14}O_3$)	254.30	-0.55	1.92×10^{-13}	

	Metronidazole ($C_6H_9N_3O_3$)	171.15	-0.14	2.07×10^{-12}	
	Ibuprofen ($C_{13}H_{18}O_2$)	206.30	0.14	5.54×10^{-10}	
	Triclosan ($C_{12}H_7Cl_3O_2$)	287.50	4.92	9.49×10^{-6}	
	Amitriptyline ($C_{20}H_{23}N$)	277.40	3.21	1.24×10^{-10}	
	Salicylic acid ($C_7H_6O_3$)	138.12	-1.14	1.42×10^{-8}	
Pesticides	Atrazine ($C_8H_{14}ClN_5$)	215.68	2.64	5.22×10^{-8}	
	Clofibric acid ($C_{10}H_{11}ClO_3$)	214.64	-1.29	2.91×10^{-10}	

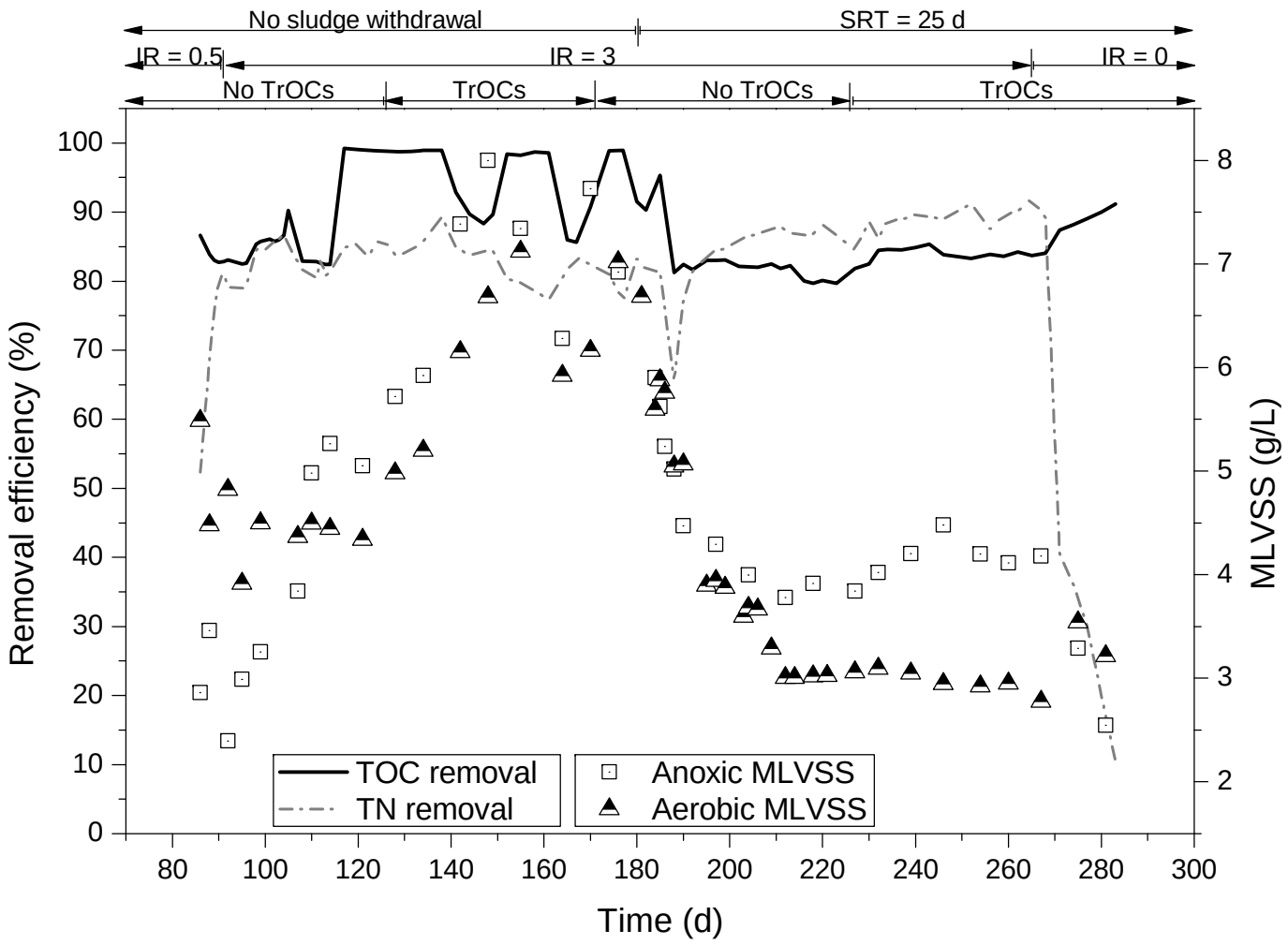
	Propoxur ($C_{11}H_{15}NO_3$)	209.24	1.54	5.26×10^{-7}	
	Fenoprop ($C_9H_7Cl_3O_3$)	269.51	-0.28	4.72×10^{-12}	
	Pentachlorophenol (C_6HCl_5O)	266.38	2.19	1.82×10^{-7}	
	Ametryn ($C_9H_{17}N_5S$)	227.33	2.97	3.67×10^{-9}	
Steroid hormones	17α-Ethinylestradiol (EE2) ($C_{20}H_{24}O_2$)	296.48	4.11	3.74×10^{-10}	

	Estriol (E3) ($C_{18}H_{24}O_3$)	288.40	2.53	1.75×10^{-11}	
	Estrone (E1) ($C_{18}H_{22}O_2$)	270.36	3.62	9.61×10^{-10}	
	17β-Estradiol-17-acetate (E2Ac) ($C_{20}H_{26}O_3$)	314.42	5.11	2.15×10^{-9}	
	17β-Estradiol (E2) ($C_{18}H_{24}O_2$)	272.38	4.14	1.17×10^{-9}	
Industrial chemicals	4-tert-Butylphenol ($(CH_3)_3CC_6H_4OH$)	150.22	3.39	7.51×10^{-6}	
	Bisphenol A ($C_{15}H_{16}O_2$)	228.29	3.64	9.16×10^{-12}	

	4-tert-Octylphenol ($C_{14}H_{22}O$)	206.33	5.18	8.67×10^{-6}	
Physoestrogens	Enterolactone ($C_{18}H_{18}O_4$)	298.33	1.88	8.07×10^{-13}	
	Formononetin ($C_{16}H_{12}O_4$)	268.26	1.81	2.91×10^{-10}	
UV filters	Benzophenone ($C_{13}H_{10}O$)	182.22	3.21	1.31×10^{-6}	
	Oxybenzone ($C_{14}H_{12}O_3$)	228.24	3.42	1.22×10^{-8}	
	Octocrylene ($C_{24}H_{27}N$)	361.48	6.89	3.38×10^{-9}	

[a] *Source: SciFinder database* https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf

Log D is logarithm of the distribution coefficient which is the ratio of the sum of concentrations of all forms of the compound (ionised and unionised) in octanol and waterat a given pH.



Appendix Figure A-2: Variation in TOC/TN removals and MLVSS concentration over the entire period of operation of the lab-scale anoxic-aerobic MBR

Appendix Table A-3: Relative abundance of bacterial phyla. Anoxic and oxic samples were collected on day 152 (Condition 1: Infinite SRT, with TrOC, IR =3), day 225 (Condition 2: 25 d SRT, no TrOC, IR = 3), day 265 (Condition 3: 25 d SRT, with TrOC, IR = 3), and day 304 (Condition 4: 25 d SRT, with TrOCs, IR =0). 'Unclassified' indicates the sequences that could not be classified up to phylum level.

Taxon	$Anoxic_{152}$	$Oxic_{152}$	$Anoxic_{225}$	$Oxic_{225}$	$Anoxic_{265}$	$Oxic_{265}$	$Anoxic_{304}$	$Oxic_{304}$
Unclassified	10.53	10.90	14.90	12.52	9.63	7.77	4.44	14.96
Acidobacteria	3.84	3.85	1.13	2.22	1.27	1.55	0.5	0.58
Actinobacteria	0.65	0.23	0.31	0.47	0.69	0.85	0.03	0.84
Armatimonadetes	1.61	3.13	0.67	0.53	0.28	0.13	0	0.02
BRC1	0	0	0.1	0	0	0	0	0
Bacteroidetes	17.55	22.38	47.69	43.99	14.87	17.6	46.1	14.67
Chlorobi	1.66	2.25	1.08	0.97	0.21	0.09	0.12	0.02
Chloroflexi	5.46	5.93	0.36	0.62	2.21	2.59	0	0.02
Cyanobacteria	0.23	0.21	0.1	0.19	0.11	0.13	0.03	0.23
Elusimicrobia	0	0.08	0	0	0	0	0.03	0
Firmicutes	0.38	0.41	0.41	0.37	0.86	1.39	3.61	0.39
GN02	0.02	0.02	0	0	0	0	0	0
Gemmatimonadetes	0.7	0.99	0.36	0.12	0.09	0.06	0.03	0.04
Lentisphaerae	0	0	0	0	0	0	0.77	0
NKB19	0.35	0.32	0	0.03	0.02	0.09	0	0
Nitrospirae	3.11	2.44	0.57	0.66	0.21	0.7	0.89	0.7
OD1	0.65	0.71	0.26	0.31	0.52	0.41	1.45	0.33
OP11	0.02	0	0	0	0	0	0	0
Planctomycetes	10.65	9.7	1.54	1.56	5.09	5.34	1.45	0.53
Proteobacteria	40.57	34.76	22.76	26.1	60.06	57.57	39.14	64.11
Spirochaetes	0.03	0	0.05	0.03	0	0	0.68	0

TM6	0	0	0	0	0.09	0.09	0.03	0.14
TM7	0.38	0.54	6.63	7.12	2.92	3.13	0.53	0
Thermotogae	0	0	0	0	0	0	0	0.02
Verrucomicrobia	1.45	0.86	0.82	1.65	0.54	0.28	0.15	2.33
WPS-2	0.16	0.28	0.26	0.53	0.34	0.22	0	0.11
[Thermi]	0.02	0.02	0	0	0	0	0	0

Appendix Table A-4: Relative abundance of the bacterial phylotypes at the deepest level of classification (order, family or genus) revealed by the data analysed. Anoxic and oxic samples were collected on day 152 (Condition 1: Infinite SRT, with TrOC, IR = 3), 225 (Condition 2: 25 d SRT, no TrOC, IR = 3), 265 (Condition 3: 25d SRT, with TrOC, IR = 3), and 304 (Condition 4: 25 d SRT, with TrOC, IR = 0). The bacterial phylotypes that were observed at less than 0.45% average abundance were grouped in 'Others'. The protocol used in this study could provide information up to the genus level, but in some cases analysed data fell short to reveal the genus ("g__"), family ("f__"), order ("o__") or even the class (c__). All values in percentage (%).

Consensus lineage (*Phylum*;*Class*;*Order*;*Family*;*Genus*)	$Anoxic_{152}$	$Oxic_{152}$	$Anoxic_{225}$	$Oxic_{225}$	$Anoxic_{265}$	$Oxic_{265}$	$Anoxic_{304}$	$Oxic_{304}$
Acidobacteria;Holophagae;Holophagales;Holophagaceae;g__	0.23	0.47	0.21	0.19	0.04	0.09	0.41	0.23
Acidobacteria;[Chloracidobacteria];PK29;f__;g__	0.59	0.53	0.05	0.06	0.15	0.00	0.00	0.02
Acidobacteria;[Chloracidobacteria];RB41;Ellin6075;g__	2.04	2.03	0.57	1.62	0.82	1.33	0.03	0.00
Actinobacteria;Actinobacteria;Actinomycetales;f__;g__	0.21	0.09	0.31	0.28	0.26	0.32	0.03	0.53
Armatimonadetes;[Fimbriimonadia];[Fimbriimonadales]; [Fimbriimonadaceae];g__	0.91	0.86	0.51	0.44	0.24	0.13	0.00	0.00
Armatimonadetes;[Fimbriimonadia];[Fimbriimonadales]; [Fimbriimonadaceae];Fimbriimonas	0.68	2.01	0.15	0.09	0.04	0.00	0.00	0.00
Bacteroidetes;Bacteroidia;Bacteroidales;f__;g__	0.02	0.04	0.00	0.12	0.00	0.00	0.68	0.00
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae; Bacteroides	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.02
Bacteroidetes;Cytophagia;Cytophagales;f__;g__	1.15	1.89	0.00	0.06	0.00	0.03	0.03	0.74
Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae;g__	8.24	12.40	35.20	29.44	6.92	8.94	37.63	7.04
Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae;Runella	0.79	1.13	0.67	0.19	0.17	0.22	0.00	0.49

Bacteroidetes;Flavobacteriia;Flavobacteriales; Cryomorphaceae;g__	2.60	2.36	5.86	5.78	0.41	0.35	0.24	0.16
Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae; Flavobacterium	0.00	0.00	0.00	0.06	0.00	0.00	0.00	3.75
Bacteroidetes;Flavobacteriia;Flavobacteriales;[Weeksellaceae]; Chryseobacterium	1.00	0.51	0.21	0.44	0.04	0.00	3.08	0.02
Bacteroidetes;Sphingobacteriia;Sphingobacteriales;f__;g__	0.31	0.47	0.31	0.09	0.30	0.41	0.09	0.68
Bacteroidetes;[Saprospirae];[Saprospirales];f__;g__	3.11	2.96	0.00	0.25	0.00	0.13	0.24	0.12
Bacteroidetes;[Saprospirae];[Saprospirales];Chitinophagaceae;g__	0.30	0.53	5.09	6.43	5.69	5.15	2.90	1.45
Bacteroidetes;[Saprospirae];[Saprospirales];Saprospiraceae;g__	0.02	0.04	0.26	1.12	1.33	2.37	0.36	0.14
Chlorobi;SJA-28;o__;f__;g__	1.20	1.82	0.87	0.75	0.21	0.09	0.06	0.00
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolinaceae; Longilinea	1.33	1.52	0.00	0.00	0.09	0.00	0.00	0.00
Chloroflexi;Anaerolineae;Caldilineales;Caldilineaceae;Caldilinea	0.09	0.02	0.10	0.19	1.31	1.39	0.00	0.00
Chloroflexi;Anaerolineae;SBR1031;f__A4b;g__	1.03	1.61	0.05	0.28	0.00	0.00	0.00	0.00
Chloroflexi;Anaerolineae;SHA-20;f__;g__	0.40	0.23	0.05	0.12	0.52	0.73	0.00	0.00
Chloroflexi;Anaerolineae;envOPS12;f__;g__	1.17	1.28	0.10	0.00	0.02	0.06	0.00	0.00
Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Lactococcus	0.14	0.23	0.10	0.16	0.56	1.20	0.03	0.02
Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Anaeromusa	0.00	0.00	0.00	0.00	0.11	0.00	0.68	0.02
Firmicutes;Clostridia;Clostridiales;[Acidaminobacteraceae]; Fusibacter	0.16	0.06	0.05	0.00	0.00	0.00	2.01	0.02
Gemmatimonadetes;Gemmatimonadetes;o__;f__;g__	0.42	0.56	0.05	0.06	0.02	0.03	0.03	0.04
Lentisphaerae;[Lentisphaeria];Victivallales;Victivallaceae;g__	0.00	0.00	0.00	0.00	0.00	0.00	0.77	0.00
Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira	3.07	2.44	0.57	0.66	0.21	0.70	0.89	0.70
OD1;SM2F11;o__;f__;g__	0.30	0.45	0.10	0.16	0.52	0.41	1.21	0.19
Planctomycetes;Phycisphaerae;Phycisphaerales;f__;g__	5.08	5.42	0.92	0.75	0.54	1.36	0.74	0.12
Planctomycetes;Planctomycetia;Gemmatales;Gemmataceae;g__	1.62	1.35	0.36	0.19	1.74	1.77	0.12	0.18
Planctomycetes;Planctomycetia;Gemmatales;Gemmataceae; Gemmata	0.63	0.38	0.10	0.03	0.32	0.28	0.00	0.04

Planctomycetes;Planctomycetia;Pirellulales;Pirellulaceae;g__	0.87	0.56	0.05	0.16	0.49	0.47	0.06	0.00
Planctomycetes;Planctomycetia;Planctomycetales; Planctomycetaceae;Planctomyces	2.08	1.74	0.05	0.09	1.85	1.33	0.30	0.02
Proteobacteria;Alphaproteobacteria;o__;f__;g__	0.26	0.60	0.00	0.22	0.11	0.03	0.92	0.68
Proteobacteria;Alphaproteobacteria;o__Rhizobiales;f__;g__	0.59	1.16	0.62	0.59	0.47	0.54	0.06	2.28
Proteobacteria;Alphaproteobacteria;Rhizobiales; Hyphomicrobiaceae;Hyphomicrobium	0.14	0.15	0.00	0.06	0.45	0.60	0.12	0.23
Proteobacteria;Alphaproteobacteria;Rhizobiales; Methylocystaceae;Methylosinus	0.00	0.00	0.00	0.00	0.37	0.85	0.00	0.04
Proteobacteria;Alphaproteobacteria;Rhizobiales; Phyllobacteriaceae;g__	0.09	0.13	0.10	0.16	0.54	0.28	0.00	0.04
Proteobacteria;Alphaproteobacteria;Rhodobacterales; Hyphomonadaceae;g__	0.86	0.54	0.05	0.34	0.11	0.13	0.00	1.68
Proteobacteria;Alphaproteobacteria;Rhodospirillales;f__;g__	0.07	0.06	0.05	0.06	0.45	0.09	0.09	0.28
Proteobacteria;Alphaproteobacteria;Rhodospirillales; Acetobacteraceae;g__	0.14	0.04	0.10	0.22	0.19	0.13	0.65	0.04
Proteobacteria;Alphaproteobacteria;Rhodospirillales; Rhodospirillaceae;g__	0.72	0.62	0.41	0.56	1.14	1.04	0.36	0.89
Proteobacteria;Betaproteobacteria;Burkholderiales; Other;Other	0.02	0.04	0.05	0.06	0.09	0.06	0.03	0.96
Proteobacteria;Betaproteobacteria;Burkholderiales; Alcaligenaceae;g__	0.02	0.00	0.46	0.06	0.13	0.06	0.39	0.47
Proteobacteria;Betaproteobacteria;Burkholderiales; Comamonadaceae;g__	1.47	1.05	0.72	0.81	0.30	0.35	0.83	4.90
Proteobacteria;Betaproteobacteria;Burkholderiales; Comamonadaceae;Comamonas	0.21	0.08	0.10	0.00	0.02	0.00	0.65	0.04
Proteobacteria;Betaproteobacteria;Burkholderiales; Comamonadaceae;Methylibium	0.09	0.06	0.00	0.12	0.09	0.03	0.06	0.47
Proteobacteria;Betaproteobacteria;Burkholderiales; Oxalobacteraceae;g__	2.30	2.34	0.92	0.62	0.77	1.04	0.21	0.75
Proteobacteria;Betaproteobacteria;Ellin6067;f__;g__	1.47	0.83	0.62	0.87	0.62	0.95	0.21	0.04

Proteobacteria;Betaproteobacteria;Methylophilales; Methylophilaceae;g__	3.91	2.78	0.00	0.00	31.05	27.87	3.79	6.04
Proteobacteria;Betaproteobacteria;Methylophilales; Methylophilaceae;Methylotenera	0.31	0.34	0.00	0.00	1.46	1.55	0.15	0.60
Proteobacteria;Betaproteobacteria;Neisseriales; Neisseriaceae;g__Aquitalea	0.75	0.23	0.00	0.00	0.00	0.00	0.12	0.00
Proteobacteria;Betaproteobacteria;Rhodocyclales; Rhodocyclaceae;Other	0.56	0.77	0.72	0.59	0.37	0.70	0.15	0.82
Proteobacteria;Betaproteobacteria;Rhodocyclales; Rhodocyclaceae;g__	8.36	6.27	1.59	2.44	3.67	3.32	1.66	2.49
Proteobacteria;Betaproteobacteria;Rhodocyclales; Rhodocyclaceae;Azospira	0.00	0.00	0.10	0.00	0.00	0.00	1.16	0.14
Proteobacteria;Betaproteobacteria;Rhodocyclales; Rhodocyclaceae;Candidatus Accumulibacter	1.87	1.91	5.19	5.40	5.87	6.89	0.39	0.07
Proteobacteria;Betaproteobacteria;Rhodocyclales; Rhodocyclaceae;Dechloromonas	0.89	2.01	0.92	1.47	0.71	0.57	1.04	0.14
Proteobacteria;Betaproteobacteria;Methylophilales; Methylophilaceae;Other	0.00	0.00	0.00	0.00	0.43	0.54	0.15	0.18
Proteobacteria;Betaproteobacteria;Rhodocyclales; Rhodocyclaceae;Methyloversatilis	0.79	0.53	0.00	0.00	0.09	0.06	0.06	0.04
Proteobacteria;Betaproteobacteria;SC-I-84;f__;g__	0.80	0.51	0.21	0.09	0.52	0.47	0.27	0.00
Proteobacteria;Deltaproteobacteria;Bdellovibrionales; Bdellovibrionaceae;Bdellovibrio	0.56	0.90	0.31	0.47	0.15	0.03	0.00	0.49
Proteobacteria;Deltaproteobacteria;Myxococcales;f__;g__	6.08	4.75	5.04	4.65	5.07	4.61	0.24	32.17
Proteobacteria;Deltaproteobacteria;Myxococcales; 0319-6G20;g__	0.02	0.04	0.05	0.03	0.00	0.03	0.00	0.82
Proteobacteria;Deltaproteobacteria;Myxococcales; Nannocystaceae;Nannocystis	0.94	0.69	0.21	0.12	0.19	0.06	0.00	0.32
Proteobacteria;Gammaproteobacteria;Chromatiales; Chromatiaceae;Allochromatium	0.09	0.34	0.05	0.00	0.11	0.47	0.00	0.00
Proteobacteria;Gammaproteobacteria;Enterobacteriales; Enterobacteriaceae;g__	0.26	0.19	0.41	0.09	0.17	0.16	12.50	0.42

Proteobacteria;Gammaproteobacteria;HOC36;f__;g__	0.47	0.09	0.00	0.06	0.21	0.38	0.00	0.00
Proteobacteria;Gammaproteobacteria;Pseudomonadales; Moraxellaceae;Acinetobacter	0.94	1.33	2.42	3.25	2.06	1.80	12.00	2.19
Proteobacteria;Gammaproteobacteria;Xanthomonadales; Sinobacteraceae;g__	0.89	0.41	0.15	0.72	0.28	0.16	0.09	0.51
Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae; Treponema	0.03	0.00	0.05	0.03	0.00	0.00	0.68	0.00
TM7;TM7-1;o__;f__;g__	0.26	0.47	6.63	7.12	2.92	3.13	0.09	0.00
TM7;TM7-3;Blgi18;f__;g__	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00
Verrucomicrobia;Opitutae;Opitutales;Opitutaceae;g__	0.59	0.30	0.00	0.06	0.00	0.00	0.00	0.00
Verrucomicrobia;[Pedosphaerae];[Pedosphaerales];f__;g__	0.30	0.15	0.36	0.37	0.00	0.03	0.12	1.94
Verrucomicrobia;[Pedosphaerae];[Pedosphaerales];Ellin515;g__	0.14	0.13	0.15	0.56	0.00	0.06	0.03	0.05
WPS-2;c__;o__;f__;g__	0.16	0.28	0.26	0.53	0.34	0.22	0.00	0.11
Others	9.29	8.05	3.13	4.37	3.93	3.63	2.76	5.06
Unclassified at phylum level	10.53	10.90	14.90	12.52	9.63	7.77	4.44	14.96

Table A-5. Analysis procedure for the trace organic contaminants investigated in Chapter 5 and 6.

Table A-5a. Method description

Analytical methods using electrospray ionization (ESI) are based on that of Vanderford et al. Environmental Science and Technology, **2006**, volume 40, pp 7312-7320. The method employing atmospheric pressure chemical ionization (APCI) was based on that reported by Vanderford et al. Analytical Chemistry, **2003**, volume 75, pp 6265-6274.

Solid-Phase Extraction. Analytes were extracted using 5 mL, 500 mg hydrophilic/lipophilic balance (HLB) cartridges (Waters, Millford, MA, USA). Cartridges were pre-conditioned with 5 mL of methanol and 5 mL of reagent water. Samples were spiked with a solution containing 50 ng of an isotopically labeled version of each analyte. The sample was then loaded onto the cartridges at 10 mL/min, after which the cartridges were rinsed with 5 mL of reagent water and dried with a stream of nitrogen for 30 min. Loaded cartridges were stored at 4 °C in sealed bags under nitrogen until elution and analysis. Analytes were eluted from the cartridges with 5 mL of methanol followed by 5 mL of 1/9 (v/v) methanol/MTBE into centrifuge tubes. The resulting extract was concentrated using vacuum assisted evaporation to approximately 100 µL. The extract was brought to a final volume of 1mL with methanol.

Liquid Chromatography. Analytes were separated using an Agilent (Palo Alto, CA, USA) 1200 series high performance liquid chromatography (HPLC) system equipped with a 150 x 4.6 mm, 5 µm particle size, Luna C18 (*2*) column (Phenomenex, Torrence CA, USA) . A binary gradient consisting of 5 mM ammonium acetate in water (A) and 100% methanol (B) at a flow rate of 800 µL/min was used. For ESI positive analyses, the gradient was as follows: 10% B held for 0.50 min, stepped to 50% B at 0.51 min and increased linearly to 100% B at 8 min, then held at 100% B for 2 min. For ESI negative analyses, the gradient was as follows: 10% B held for 0.50 min, stepped to 60% B at 0.51 min and increased linearly to 100% B at 8 min, then held at 100% B for 3 min. A 5 min equilibration step at 10% B was used at the beginning of each run. For APCI analysis the eluants consisted of milli-Q grade water (A) and 0.1% v/v formic acid in methanol with the following ramp at a flow rate of 700 µL/min. 60% B held for 5 min, increased linearly to 100% B at 20 min, then held at 100% B for 3 min. A 3 min equilibrium step preceded injection. An injection volume of 10 µL was used for all methods.

Mass Spectrometry. Mass spectrometry was performed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a turbo-V ion source employed in both positive and negative electro-spray modes. Steroids were analysed the source configured for (APCI) in positive mode. Using multiple reaction monitoring (MRM) two mass transitions for all but three of the analytes were monitored for unequivocal confirmation. One mass transition for the labeled internal standard was monitored. Only the first transition was used for quantitiation. Relative retention times of the analyte and isotopically labeled internal standard were also monitored to ensure correct identification.

Calibration and limits of Detection. Standard solutions of all analytes were prepared at 1, 5, 10, 50, 100, 500 and 1000 ng/mL. A relative response ratio of analyte/internal standard over a 1 – 1000 ng concentration range was generated enabling quantitation with correction for losses due to ion suppression and incomplete SPE recovery. All calibration curves had a correlation coefficient of 0.99 or better. Detection limits were defined as the concentration of an analyte giving a signal to noise (s/n) ratio greater than 3. The Limits of Reporting were determined using a s/n ratio of greater than 10.

Table A-5b. Transitions for compounds using ESI positive mode

Compound	Precursor Ion (m/z)	Product Ion (m/z)
Atenolol 1	267.2	145.1
Atenolol 2	267.2	190.2
Atenolol-D7	274.1	145.1

Paracetamol	152.1	110.1
Paracetamol-$^{15}N^{13}C$	155.0	111.0
Sulfamethoxazole 1	254.0	156.1
Sulfamethoxazole 2	254.0	92.0
Sulfamethoxazole-D4	258.1	160.1
Caffeine 1	195.0	138.1
Caffeine 2	195.0	110.1
Caffeine-D9	204.1	144.2
Trimethoprim 1	291.1	230.2
Trimethoprim 2	291.1	261.1
Trimethoprim-D9	300.3	234.2
TCEP 1	284.9	223.0
TCEP 2	284.9	62.9
Dilantin 1	253.1	182.1
Dilantin 2	253.1	104.1
Dilantin-D10	263.1	192.2
Carbamazepine 1	237.0	194.2
Carbamazepine 2	237.0	192.1
Carbamazepine-D10	247.1	204.3
Norfluoxetine 1	296.0	134.0
Norfluoxetine 2	296.0	30.2
Norfluoxetine-D5	301.0	139.0
Fluoxetine 1	310.0	44.1
Fluoxetine 2	310.0	148.2
Fluoxetine-D5	315.1	44.2
Enalapril 1	377.1	234.1
Enalapril 2	377.1	91.1
Enalapril-D5	382.2	239.2
Risperidone 1	411.1	191.2
Risperidone 2	411.3	110.0
Risperidone-D4	415.1	195.2
Atrazine 1	216.0	174.2
Atrazine 2	216.0	96.1
Atrazine-D5	221.3	179.1
Linuron 1	249.0	182.2
Linuron 2	249.0	160.1
Linuron-D6	255.0	160.1
Atorvastatin 1	559.1	440.1
Atorvastatin 2	559.1	250.3
Atorvastatin-D5	564.2	445.4
Omeprazole 1	346.2	198.2
Omeprazole 2	346.2	136.1
Omeprazole D3	349.2	198.0
Clozapine 1	327.1	270.2
Clozapine 2	327.1	192.1
Clozapine_D4	331.2	272.0
Amtriptyline 1	278.2	233.0
Amtriptyline 2	278.2	117.1
Amtriptyline-D6	284.4	233.1
DEET 1	192.2	119.0
DEET 2	192.2	108.9
DEET-D7	199.2	126.1
Primidone 1	219.2	162.2
Primidone 2	219.2	119.0

Primidone-D5	224.2	167.0
Verapamil 1	455.4	165.1
Verapamil 2	455.4	150.0
Verapamil-D6	461.4	165.2
Triamterene 1	254.2	237.0
Triamterene 2	254.2	104.0
Triamterene-D5	259.2	242.2
Polyparaben 1	181.2	139.1
Polyparaben 2	181.2	121.0
Metformin 1	130.1	113.1
Metformin 2	130.1	112.5
Metformin-D6	136.1	119.2
Meprobamate 1	218.9	158.2
Meprobamate 2	218.9	115.1
Meprobamate-D3	221.9	161.2
Hydroxyzine 1	375.3	201.1
Hydroxyzine 2	375.3	165.1
Hydroxyzine-D8	383.3	201.1
Diazepam 1	285.1	193.1
Diazepam 2	285.1	154.2
Diazepam-D5	290.1	198.1

Table A-5c. Transitions for compounds using ESI negative mode

Compound	Precursor Ion (m/z)	Product Ion (m/z)
Ketoprofen	252.8	208.8
Ketoprofen-D3	255.6	211.7
Naproxen 1	228.9	184.6
Naproxen 2	228.9	169.8
Naproxen-D3	231.9	187.8
Bisphenol A 1	226.9	211.8
Bisphenol A 2	226.9	132.9
Bisphenol A-D6	232.9	214.9
Ibuprofen 1	204.9	160.8
Ibuprofen 2	204.9	158.8
Ibuprofen-D3	208.0	163.9
Gemfibrozil 1	248.9	120.8
Gemfibrozil 2	248.9	126.8
Gemfibrozil-D6	254.9	120.9
Triclosan	286.6	35.0
Triclosan-D3	289.7	34.9
Simvastatin-hydroxyacid 1	435.1	318.9
Simvastatin-hydroxyacid 2	435.1	114.9
Simvastatin-hydroxyacid-D6	441.1	319.0
Simvastatin 1	399.0	114.9
Simvastatin 2	399.0	282.8
Simvastatin-D6	405.4	121.1
Diclofenac 1	293.9	249.7
Diclofenac 2	293.9	213.7
Diclofenac-D4	297.9	253.8
Triclocarban 1	312.9	159.8
Triclocarban 2	312.9	125.7

Triclocarban-D4	317.0	159.8
t-Octylphenol 1	205.2	132.9
t-Octylphenol 2	205.2	134.0
n-Octylphenol-D17	222.1	108.0
Polyparaben 1	179.0	135.7
Polyparaben 2	179.0	136.9
Phenylphenol 1	168.9	114.8
Phenylphenol 2	168.9	140.8
Nonylphenol 1	219.0	106.0
Nonylphenol 2	219.0	119.0
Nonylphenol-D4	223.1	110.0

Table A-5d. Transitions for compounds using APCI positive mode

Compound	Precursor Ion (m/z)	Product Ion (m/z)
Estriol 1	271.1	253.1
Estriol 2	271.1	133.0
Estriol-D2	273.2	255.2
Androstendione 1	287.2	97.1
Androstendione 2	287.2	109.2
Androstendione-D3	290.2	100.1
Etiocholanolone 1	273.2	255.3
Etiocholanolone 2	273.2	91.1
Etiocholanolone-D2	275.2	257.1
Androsterone 1	273.2	255.2
Androsterone 2	273.2	91.0
Estrone 1	271.2	159.2
Estrone 2	271.2	133.0
Estrone-D4	275.1	161.0
17β-Estradiol 1	255.2	159.3
17β-Estradiol 2	255.2	133.2
17β-Estradiol-D4	259.1	161.1
17α-Estradiol 1	255.2	159.3
17α-Estradiol 2	255.2	133.2
17α-Ethynylestradiol 1	279.2	133.1
17α-Ethynylestradiol 2	279.2	159.2
17α-Ethynylestradiol-D4	283.1	135.1
Testosterone 1	289.2	97.2
Testosterone 2	289.2	109.1
Testosterone-D2	291.2	99.1

Table A-6: Raw sewage concentration (ng/L) of 45 monitored trace organic contaminants (TrOCs) including 27 pharmaceutical and personal care products (PPCPs), four industrial xenoestrogens, eight steroid hormones and six pesticides. In total, 35 samples were collected from 15 sampling events in duplicate (the first ten sampling events) or triplicate (the last five sampling events) from November 2012 to October 2014.

Compounds	Detection limit	13 November 2012		27 June 2013		29 July 2013		22 April 2014		29 April 2014		6 May 2014	
		Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
Phamarceutical and personal care products (PPCPs)													
Caffeine	10	52000	49000	3810	5840	43000	49800	29200	30200	41800	40800	137400	138200
Omeprazole	5	26	26	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Paracetamol	5	<5	<5	8660	13400	55700	55800	53800	61200	24400	N.Q.	N.Q.	N.Q.
Ibuprofen	5	16520	17560	1040	1470	4530	4260	7300	6440	7800	7880	15520	16620
Diclofenac	5	556	546	43	64	86	88	106	114	476	624	356	380
Naproxen	5	27000	30600	23	39	224	226	1296	1286	440	484	8000	9660
Ketoprofen	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Sulfamethoxazole	5	<5	<5	<5	<5	7	6	161	177	<5	21	<5	<5
Trimethoprim	5	40	56	<5	<5	57	59	41	43	764	1118	114	468
Carbamazepine	5	660	740	6	10	73	71	230	222	302	306	356	374
Primidone	5	<5	<5	<5	<5	<5	<5	11	12	<5	<5	21	23
Fluoxetine	5	118	146	<5	<5	<5	<5	46	28	8	9	<5	<5
Amtriptyline	5	290	676	9	15	7	<5	48	38	52	54	41	47
Atenolol	5	5400	6140	79	136	196	191	648	598	900	880	4040	3180
Enalapril	5	129	135	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Verapamil	5	52	65	<5	<5	<5	<5	19	9	<5	<5	<5	<5
Triamterene	5	52	58	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Clozapine	5	<5	<5	5	9	<5	<5	15	9	<5	<5	<5	<5
Meprobamate	5	<5	<5	<5	<5	<5	<5	32	29	<5	<5	<5	<5
Diazepam	5	5	7	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5

Gemfibrozil	5	42	40	474	974	613	606	67	61	96	97	<5	11
DEET	5	10380	12180	Not measured				2040	1978	4660	4560	7140	6640
Triclosan	5	866	900	52	67	149	178	1308	1298	1304	1358	1020	1120
Triclocarban	10	880	1110	12	18	17	18	164	127	138	102	24	19
Dilantin	5	Not measured						<5	<5	<5	<5	<5	<5
Risperidone	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Hydroxyzine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Industrial chemicals													
Polyparaben	10	3500	3080	89	44	457	461	148	97	78	77	180	174
TCEP	10	304	296	<10	<10	<10	<10	57	35	26	28	24	27
Bisphenol A	20	20	21	27	<20	129	147	143	396	N.Q.	N.Q.	N.Q.	N.Q.
4-n-nonylphenol	5	<5	<5	<5	<5	<5	<5	93	48	<5	<5	<5	<5
Steroid hormones													
Androstendione	5	Not measured						<5	<5	11	11	5	6
Estrone	5	Not measured						298	326	458	432	834	656
Estriol	5	Not measured						NQ	NQ	NQ	NQ	NQ	NQ
17β-estradiol	5	Not measured						16	15	24	28	40	39
17α-estradiol	5	Not measured						<5	5	<5	14	<5	<5
Testosterone	5	Not measured						<5	<5	6	7	8	9
Androsterone	5	Not measured						286	260	316	362	556	566
Etiocholanolone	5	Not measured						1578	1624	1928	2040	3100	3140
Pesticides													
Atrazine	5	<5	<5	<5	<5	6	9	<5	<5	<5	<5	<5	<5
Diazinon	10 (5)*	53	52	<10	<10	<10	<10	<5	<5	<5	10	<5	<5
Simazine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Phenylphenol	10 (20)*	94	92	<10	<10	<10	11	<20	<20	<20	<20	<20	<20
Diuron	5 (10)*	178	202	23	38	7	7	<10	<10	16	20	29	25

Linuron	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5

Table A-6 (continued)

Compounds	Detection limit	14 May 2014		20 May 2014		28 May 2014		2 June 2014		30 June 2014		
		Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 3
Phamarceutical and personal care products (PPCPs)												
Caffeine	10	13440	12840	26000	25400	32000	30400	13680	12480	57600	64600	60000
Omeprazole	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Paracetamol	5	<5	<5	23000	23600	28800	22200	10660	10040	123200	110400	124600
Ibuprofen	5	1736	1738	7840	8160	3440	3140	4780	4980	14600	13900	14460
Diclofenac	5	258	270	131	134	95	92	224	232	228	224	222
Naproxen	5	3040	3080	1468	1506	494	448	508	516	248	242	246
Ketoprofen	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Sulfamethoxazole	5	87	77	2340	2360	378	344	6680	6200	5140	4640	4820
Trimethoprim	5	108	94	79	86	55	61	396	362	734	778	780
Carbamazepine	5	476	500	306	314	346	322	338	348	232	228	226
Primidone	5	163	176	32	36	47	40	33	32	<5	<5	<5
Fluoxetine	5	18	16	<5	<5	<5	<5	<5	<5	<5	<5	<5
Amtriptyline	5	114	105	17	10	<5	<5	<5	<5	70	66	70
Atenolol	5	1302	1388	652	690	548	594	740	740	2080	2080	1984
Enalapril	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Verapamil	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Triamterene	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Clozapine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Meprobamate	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Diazepam	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Gemfibrozil	5	<5	<5	226	234	113	110	12	13	<5	<5	<5
DEET	5	10240	10740	1784	2340	4620	4660	2080	2080	1540	1566	1540

Triclosan	5	624	384	900	810	712	680	616	400	640	596	748
Triclocarban	10	46	43	39	45	40	38	50	54	148	167	155
Dilantin	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Risperidone	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Hydroxyzine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Industrial chemicals												
Polyparaben	10	<10	<10	<10	<10	<10	<10	52	59	600	542	536
TCEP	10	13	15	<10	19	28	28	27	28	<10	<10	<10
Bisphenol A	20	NQ	NQ	NQ	NQ	107	118	109	157	NQ	NQ	NQ
4-n-nonylphenol	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	25	<5
Steroid hormones												
Androstendione	5	<5	<5	33	32	8	8	23	25	19	18	18
Estrone	5	<5	<5	162	167	93	96	95	90	206	200	197
Estriol	5	<5	<5	NQ	NQ	NQ	NQ	NQ	NQ	1066	3520	394
17β-estradiol	5	<5	<5	<5	<5	<5	<5	<5	7	55	47	56
17α-estradiol	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Testosterone	5	<5	<5	<5	<5	<5	<5	<5	<5	15	13	14
Androsterone	5	<5	<5	676	520	450	424	<5	<5	264	266	274
Etiocholanolone	5	<5	<5	6320	5360	4600	4680	<5	<5	1068	1056	1094
Pesticides												
Atrazine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Diazinon	5	<5	<5	<5	<5	<5	<5	<5	<5	17	17	17
Simazine	5	11	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Phenylphenol	20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Diuron	5	<10	<10	230	232	19	18	26	26	712	680	680
Linuron	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5

Table A-6 (continued)

Compounds	Detection limit	26 August 2014			5 September 2014			22 September 2014			2 October 2014		
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Phamarceutical and personal care products (PPCPs)													
Caffeine	10	91800	98600	97800	64200	60200	59400	82800	93200	99800	49800	43800	43400
Omeprazole	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Paracetamol	5	83000	67800	84200	111200	108800	128800	111600	80800	65400	103200	118200	162400
Ibuprofen	5	9820	10480	9920	9680	8560	8360	12440	12520	12000	17820	15760	17000
Diclofenac	5	366	382	386	60	53	52	492	484	464	334	338	318
Naproxen	5	1176	1196	1158	41	33	32	330	296	328	3660	3800	3520
Ketoprofen	5	<5	11	4	<5	<5	<5	<5	<5	10	<5	<5	<5
Sulfamethoxazole	5	185	234	220	12340	10620	11540	826	814	908	39	48	33
Trimethoprim	5	61	63	108	1830	1532	1580	336	286	340	246	230	244
Carbamazepine	5	564	624	618	376	348	332	312	288	290	158	150	162
Primidone	5	<5	<5	<5	<5	<5	<5	10	<5	<5	<5	12	<5
Fluoxetine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Amtriptyline	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Atenolol	5	2740	3040	3180	1418	1240	1210	3480	3200	3400	1600	1540	1656
Enalapril	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Verapamil	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Triamterene	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Clozapine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Meprobamate	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Diazepam	5	10	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Gemfibrozil	5	10	11	12	<5	<5	<5	<5	<5	<5	<5	<5	<5
DEET	5	Not measured											
Triclosan	5	404	452	512	1178	1188	1076	1154	874	1110	968	960	910

Triclocarban	10	42	75	72	103	100	110	218	284	308	51	27	32
Dilantin	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Risperidone	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Hydroxyzine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Industrial chemicals													
Polyparaben	10	127	123	130	96	84	83	116	98	113	134	128	112
TCEP	10	16	14	13	<10	<10	<10	14	11	12	11	11	<10
Bisphenol A	20	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
4-n-nonylphenol	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Steroid hormones													
Androstendione	5	<5	<5	<5	19	17	17	30	21	22	<5	<5	<5
Estrone	5	364	258	300	103	112	105	306	332	376	402	428	430
Estriol	5	NQ	NQ	NQ	NQ	NQ	<10	NQ	NQ	NQ	NQ	NQ	NQ
17β-estradiol	5	48	45	47	<5	<5	<5	29	21	29	54	57	51
17α-estradiol	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Testosterone	5	<5	<5	<5	6	5	4	12	11	10	<5	<5	<5
Androsterone	5	490	426	442	1044	1028	864	622	638	590	532	500	482
Etiocholanolone	5	2480	2140	2220	3340	3120	2700	2800	2740	2780	2340	2140	2200
Pesticides													
Atrazine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Diazinon	5	<5	<5	<5	<5	<5	<5	47	43	45	<5	<5	<5
Simazine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Phenylphenol	20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Diuron	5	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Linuron	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5

***The detection limit of diazinon, phenylphenol and diuron were 10, 10, and 5 ng/L, respectively for first six samples collected in 2012 and 2013, but then changed to 5, 20, and 10 ng/L, respectively, for the rest of the samples. **NQ**: not quantifiable.*

Table A-7: List of 45 monitored trace organic contaminants (TrOCs) and their detection limits

Compounds	Detection limit (ng/L)	Compounds	Detection limit (ng/L)
Pharmaceutical and personal care products (PPCPs)		**Industrial chemicals**	
Atenolol	5	Polyparaben	10
Paracetamol	5	TCEP	10
Sulfamethoxazole	5	Bisphenol A	20
Caffeine	10	4-n-nonylphenol	5
Trimethoprim	5	**Steroid hormones**	
Dilantin	5	Estriol	10
Carbamazepine	5	Androstendione	5
Fluoxetine	5	Testosterone	5
Enalapril	5	Estrone	5
Risperidone	5	17β-estradiol	5
Omeprazole	5	17α-estradiol	5
Clozapine	5	Etiocholanolone	5
Amtriptyline	5	Androsterone	5
DEET	5	**Pesticides**	
Primidone	5	Diazinon	5
Verapamil	5	Simazine	5
Triamterene	5	Atrazine	5
Meprobamate	5	Linuron	5
Hydroxyzine	5	Phenylphenol	20
Diazepam	5	Diuron	10
Ketoprofen	5		
Naproxen	5		
Ibuprofen	5		
Gemfibrozil	5		
Triclosan	5		
Diclofenac	5		
Triclocarban	10		

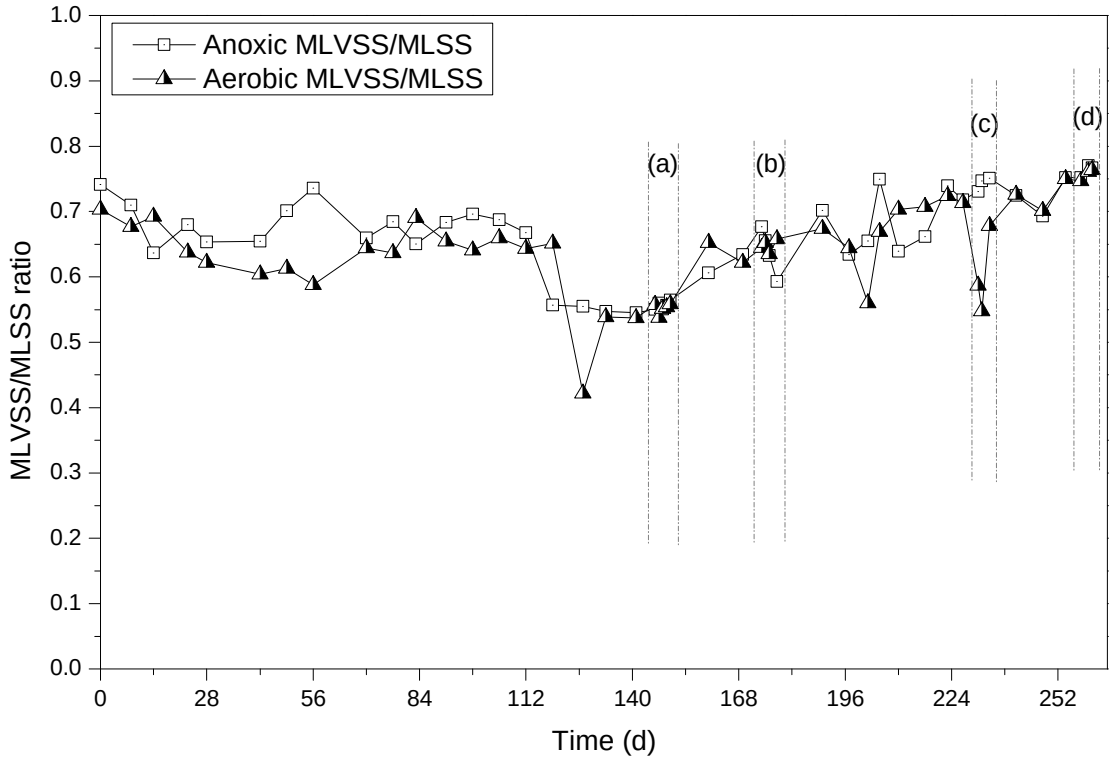


Figure A-8: The MLVSS/MLSS ratio over the entire operation period of the pilot-scale MBR. The hazardous events tests were conducted in the following order: (a) aeration failure (Day 146 – 150), (b) power failure (Day 174 – 178), (c) ammonia shock (Day 231 – 234) and (d) bleach shock (Day 258 – 261). The variation in MLVSS/MLSS ratio during normal operation period can be attributed to the variation in the influent strength.