Process robustness and impacts of hazardous events on membrane bioreactor performance

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PROCESS ROBUSTNESS AND IMPACTS OF HAZARDOUS EVENTS ON MEMBRANE BIOREACTOR PERFORMANCE

By

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Implementation of water treatment processes for recycling requires validation to demonstrate that such processes are capable of achieving required water quality objectives. In order to fully validate the performance of treatment systems, it is necessary to consider both normal (expected) operational conditions, as well as a range of potential "hazardous event" scenarios. The performance of membrane bioreactors (MBRs), including the removals of 48 trace organic chemical contaminants and key bulk water quality and operational parameters, was investigated under normal and hazardous event conditions. Full mass balance was achieved by monitoring both aqueous dissolved chemicals and those adsorbed to biomass. Hazardous events investigated included organic, salinity, 2,4-dinitrophenol (DNP) and, ammonia shock loads, feed starvation, loss of power and physical membrane damage.

Under organic, salinity, DNP, and ammonia shock conditions, removals of moderately and very hydrophobic chemicals were not affected. Since these chemicals are largely adsorbed to biomass, these results imply that biotransformation within the biomass structure itself was maintained. However, removals of hydrophilic chemicals were commonly observed to be impeded under hazardous event conditions, indicating loss of the bioactivity in the aqueous phase. This was observed primarily for chemicals of low or moderate ready biotransformability, while easily biotransformable chemicals were still largely removed. The removals of all chemicals were unaffected by the feed starvation (absence of new assimilable substances for 6 days) while the removals of some less readily biotransformable hydrophilic chemicals were measurably affected by the loss of power conditions (2 hours).

Impacts from physical membrane damage were investigated by sequentially cutting two hollow-fibre membranes within the MBR. Turbidity and chemical oxygen demand (COD) analyses revealed that these impacts were 'self-repaired' by blocking of the breach within approximately 15 minutes. Accordingly, these hazardous events were shown to have an insignificant impact to overall trace chemical removals.

The variability of trace chemical removals during hazardous event scenarios has enabled the identification of sensitive chemical indicators for the validation of MBR system performance. The application of these indicators for future risk assessment and management is described.

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Abstract

Implementation of water treatment processes for recycling requires validation to demonstrate that such processes are capable of achieving required water quality objectives. In order to fully validate the performance of treatment systems, it is necessary to consider both normal (expected) operational conditions, as well as a range of potential “hazardous event” scenarios. The performance of membrane bioreactors (MBRs), including the removals of 48 trace organic chemical contaminants and key bulk water quality and operational parameters, was investigated under normal and hazardous event conditions. Full mass balance was achieved by monitoring both aqueous dissolved chemicals and those adsorbed to biomass. Hazardous events investigated included organic, salinity, 2,4-dinitrophenol (DNP) and, ammonia shock loads, feed starvation, loss of power and physical membrane damage.

Under organic, salinity, DNP, and ammonia shock conditions, removals of moderately and very hydrophobic chemicals were not affected. Since these chemicals are largely adsorbed to biomass, these results imply that biotransformation within the biomass structure itself was maintained. However, removals of hydrophilic chemicals were commonly observed to be impeded under hazardous event conditions, indicating loss of the bioactivity in the aqueous phase. This was observed primarily for chemicals of low or moderate ready biotransformability, while easily biotransformable chemicals were still largely removed. The removals of all chemicals were unaffected by the feed starvation (absence of new assimilable substances for 6 days) while the removals of some less readily biotransformable hydrophilic chemicals were measurably affected by the loss of power conditions (2 hours).

Impacts from physical membrane damage were investigated by sequentially cutting two hollow-fibre membranes within the MBR. Turbidity and chemical oxygen demand (COD) analyses revealed that these impacts were ‘self-repaired’ by blocking of the breach within approximately 15 minutes. Accordingly, these hazardous events were shown to have an insignificant impact to overall trace chemical removals.

The variability of trace chemical removals during hazardous event scenarios has enabled the identification of sensitive chemical indicators for the validation of MBR system performance. The application of these indicators for future risk assessment and management is described.

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List of abbreviations

APCI: atmospheric pressure chemical ionisation

AS: activated sludge

ATU: allylthiourea

BOD: biochemical oxygen demand

CDNB: 1-chloro-2,4-dinitrobenzene

COD: chemical oxygen demand

CST: capillary suction time

DEET: N,N-Diethyl-meta-toluamide

DNP: 2,4-dinitrophenol

DOC: dissolved organic carbon

EDCs: endocrine disrupting chemicals

EEM: excitation-emission matrix

EPS: extracellular polymeric substance

ESI: electrospray ionisation

GC: gas chromatography

GC-MS/MS: gas chromatography tandem mass spectrometry

GC-MS: gas chromatography mass spectrometry

HPLC: high performance liquid chromatography

HPLC-MS: high performance liquid chromatography mass spectrometry

HRT: hydraulic retention time

IC: inorganic carbon

LC: liquid chromatography

LC-MS/MS: liquid chromatography tandem mass spectrometry

LLD: lower level of detection

LOD: level of detection

LOQ: limit of quantification

MBRs: membrane bioreactors

MDL: method detection limit

MLSS: mixed liquor suspended solids

MLVSS: mixed liquor volatile suspended solids

MRM: multiple reaction monitoring

MS/MS: tandem mass spectrometry

MS: mass spectrometry

PMT: photomultiplier tube

PPCPs: pharmaceutical and personal care products

RNA: ribonucleic acid

S/N: signal to noise ratio

SBR: sequencing batch reactor

SMP: soluble microbial product

SOUR: specific oxygen uptake rate

SPE: solid phase extraction

SRT: solid retention time

TC: total carbon

TMP: transmembrane pressure

TN: total nitrogen

TP: total phosphorous

TSS: total suspended solids

UV: ultra-violet

WWTP: waste water treatment plant

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2. **T. Trinh**, B. van den Akker, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan, *Fate of pharmaceuticals during wastewater treatment by a membrane bioreactor*, ***GWF Wasser Abwasser***, *international issue 2011, pages 98-102.*
3. **T. Trinh**, B. van den Akker, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan, *Removal of endocrine disrupting chemicals and microbial indicators by a membrane bioreactor for decentralised water reuse*, ***Journal of Water Reuse and Desalination,*** 2012, *2* (2) , pages 67-73*.*
4. **T. Trinh**, B. van den Akker, R. M. Stuetz, H. M. Coleman, P. Le-Clech and S. J. Khan, *Removal of trace organic chemical contaminants by a membrane bioreactor*, ***Water Science and Technology,*** 2012, 66 (9), pages 1856-63.
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7. B. van den Akker, **T. Trinh**, H. Coleman, R. Stuetz, P. Le-Clech, J. Drewes, S. Khan, *Validation of a full-scale membrane bioreactor for water recycling*, paper in proceeding of ***OZwater Conference***, 8-10 May 2012 in Sydney.
8. **T. Trinh**, H. Coleman, R. Stuetz, P. Le-Clech, J. Drewes and S. Khan, Impacts of 2,4 dinitrophenol shock on membrane bioreactor performance, paper in proceeding of **Asian Pacific Water Recycling Conference**, 1-3 July 2013 in Brisbane.
9. **T. Trinh**, A. Branch, B. van den Akker, P. Le-Clech, J. Drewes, S. Khan, Chapter 7: Impacts of hazardous events on performance of membrane bioreactors, In: F. I. Hai, K. Yamamoto, C-H. Lee (Eds), *Membrane Biological Reactors*. IWA Publishing, London, 2014, pages 207-221.

CHAPTER 1. INTRODUCTION

1.1. BACKGROUND

In the past decades, water recycling has emerged as an important component of water management practices since pressures on water resources have increased. Decentralised wastewater treatment systems (or package plants) are becoming the preferred option for sewage treatment and recycling in regional and rural communities where connection to a centralised sewer networks is not possible or is economically unfeasible. Membrane bioreactors (MBRs) are becoming a favoured technology for decentralised water treatment and recycling due to their small footprint and the ability to produce high quality effluent over conventional activated sludge systems (Coleman et al., 2009, Le-Minh et al., 2010). MBRs comprise a combination of a conventional activated sludge process with microfiltration / ultrafiltration membrane separation, which enables these systems to produce effluents of suitable quality for a variety of reuse applications. MBRs can achieve excellent effluent qualities with respect to pathogens, suspended solids, organics and nitrogen (Yang et al., 2009).

Recently, interest in the ability of MBRs to eliminate trace organic chemical contaminants such as steroidal hormones, xenoestrogens, pesticides, pharmaceuticals and personal care products (PPCPs) has increased (Kimura et al., 2007, Coleman et al., 2009, Le-Minh et al., 2010, Sipma et al., 2010, Tambosi et al., 2010). Some of these trace organic chemical contaminants are known to have endocrine disrupting effects on aquatic organisms at low concentrations and others have been linked to ecological impacts due to acute and chronic toxicity mechanisms (Purdom et al., 1994, Farré et al., 2008, Hotchkiss et al., 2008, Okuda et al., 2008, Kasprzyk-Hordern et al., 2009, Radjenovic et al., 2009, Jury et al., 2011). The long-term effects of human exposure to most of these trace chemicals are still unknown but are currently the focus of much consideration. Investigating the removal of these trace chemical contaminants through treatment processes and assessing the risks associated with these chemicals to public health and surrounding environments are particularly important for water reuse applications.

In Australia, implementation of water treatment processes for recycling such as MBRs requires validation to demonstrate that the process is capable of achieving the required water quality objectives. In order to fully validate the performance of MBR systems, it is necessary to investigate their performance in terms of a range of parameters and under various operational conditions. Investigating the removal of trace organic chemical contaminants through MBRs during “hazardous events” is one important

aspect of this validation. Hazardous event a key aspect of the risk assessment philosophy adopted by the World Health Organisation (WHO) for the application of Water Safety Plans (WHO, 2009) and the Guidelines for Drinking Water Quality (WHO, 2011). The formalised consideration of hazardous events has been applied for a range of risk assessment and risk management applications including managing waterborne diseases (Mouchtouri et al., 2012), managing chemical accidents (Jang et al., 2011), preventing loss of containment of materials and energy of industrial processes (Dharmavaram and Klein, 2010), and probabilistic characterisation of possible future eruptions of a volcano (Neri et al., 2008). Hazardous events that may affect the operation of wastewater treatment systems can include sudden changes in source water composition, extreme weather events, human error and mechanical malfunctions. Until now, very little attention has been paid to the assessment of these hazardous events and their contribution to the risk of treatment failure or underperformance in MBRs. The research presented in this thesis addresses this knowledge gap for trace chemical contaminants and provides recommendations for how the impacts of such hazardous events may be identified and managed.

1.2. RESEARCH OBJECTIVES

This research on “process robustness and impacts of hazardous events on MBR treatment performance” was proposed with the following objectives:

- Investigate the fate and removals of a wide range of trace organic chemical contaminants comprising diverse physical and chemical properties (12 steroidal hormones, 5 xenoestrogens, 29 pharmaceuticals and personal care products, and 2 pesticides) through an onsite package MBR treating municipal wastewater during normal operating conditions.

- Investigate the impacts of a range of hazardous events on MBR performance as measured by key bulk water quality and operational parameters. Events to be considered included exposure to high concentrations of toxic substances (ammonia, salinity, 2,4-dinitrophenol), organic shocks, feed starvation, physical membrane damage and loss of power supply. The selected hazardous events for MBRs were identified through an expert workshop. Removals of trace chemicals were also monitored to identify which trace chemicals could provide useful roles as indicator chemicals to detect the impacts of the hazardous events on MBR performance.

- Assessment of the application of chemical indicators and surrogates for hazardous event identification and assessment.

1.3. THESIS STRUCTURE

The thesis is presented in 10 chapters.

Chapter 1 provides a brief introduction to the background, the research objectives and the thesis structure.

Chapter 2 reviews the available knowledge on the impacts of hazardous events on MBR operation and performance. Since MBR systems are relatively recent in their adoption for municipal wastewater treatment, and since the biological process is directly analogous to that of an AS system, literature on the impacts of hazardous events on both AS and MBR is reviewed.

Chapter 3 presents the analytical methods for water quality and MBR operational parameters including liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-tandem mass spectrometry (GC–MS/MS) methods for the analysis of trace chemical contaminants. Analytical methods used for key bulk water quality and MBR operational parameters are also presented.

Chapter 4 presents a baseline study under normal operating conditions at a full-scale package MBR in Wollumla, Bega Valley, NSW.

Chapter 5 describes the construction of four identical experimental MBRs in preparation for hazardous event simulation experiments. A background test with ultra-pure water and reproducibility experiments between the four pilot MBRs are also described in this chapter.

Chapter 6 describes the impacts of toxic shocks including ammonia, salinity and 2,4-dinitrophenol (DNP) on MBR performance. These impacts are measured by changes in key bulk water quality and operational parameters such as pH, chemical oxygen demand (COD), mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solid (MLVSS), trans membrane pressure (TMP) and capillary suction time (CST). Removals of trace chemicals were also monitored to identify which trace chemicals provide useful roles as indicator chemicals to detect the impacts of the hazardous events on MBR performance.

Chapter 7 presents the impacts of organic shock and feed starvation conditions on MBR performance measured by changes in key bulk water quality and operational parameters. Removals of trace chemicals were also studied to identify which trace chemicals are sensitive indicators for hazardous event identification and assessment.

Chapter 8 describes the impacts of physical membrane damage and loss of power supply on MBR performance as measured by changes in key bulk water quality and operational parameters. Removals of trace chemicals were also monitored to identify sufficient sensitive indicators to detect the impacts of the hazardous events on MBR performance.

Chapter 9 discusses the application of chemical indicators and surrogates for hazardous event identification and assessment. Risk assessment and management related to hazardous events are also included in this chapter.

Finally, chapter 10 summaries the conclusions from the study and presents recommendations for future research.

CHAPTER 2. IMPACTS OF HAZARDOUS EVENTS ON MBR AND ACTIVATED SLUDGE EFFLUENT QUALITY – A REVIEW

2.1. INTRODUCTION

MBRs have attracted a significant amount of interest as an alternative to conventional activated sludge (AS) systems for treating municipal wastewaters (Coleman et al., 2009, Le-Minh et al., 2010, Judd and Judd, 2011). MBRs comprise a combination of the conventional activated sludge biological process with microfiltration / ultrafiltration membrane separation, which enables these systems to produce high quality effluents within a relatively small physical footprint.

MBRs have attracted particular attention for a range of municipal water recycling applications. Such applications require validation to demonstrate that the treatment process is capable of achieving the required water quality objectives. In order to fully validate the performance of MBR systems, it is necessary to investigate their performance under various operational conditions. Investigating the removal of parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), total organic carbon (TOC), nutrients and trace organic chemical contaminants through MBRs during hazardous event situations is an important aspect of this validation process. Hazardous events may potentially include high and unsteady organic shock loads, feed starvation, toxic shock loads, loss of aeration or physical membrane damage. Evaluating the operational performance associated with these hazardous events will facilitate improved environmental and human health risk management for MBR systems. In order to fully characterize risks associated with hazardous events, an understanding of their frequency or likelihood is also required.

The purpose of this literature review is to examine the available knowledge on the impacts of hazardous events on MBR operation and performance, to identify the gaps in the knowledge and priorities for further research. Since MBR systems are relatively recent in there adoption for municipal wastewater treatment, and since the biological process is directly analogous to that of an AS system, literature on the impacts of hazardous events on both AS and MBR is reviewed.

2.2. IMPACTS OF HAZARDOUS EVENTS ON REMOVALS OF BULK ORGANIC MATTER AND NUTRIENTS

Much of the previous research on the impacts of hazardous events on the removal of bulk organic matter and nutrients in biological wastewater systems has been undertaken with a focus on AS systems and was published prior to the 1990s. These

studies are discussed in the following sub-sections along with the few more contemporary AS and MBR studies that have been reported.

During operation, wastewater treatment plants are often exposed to changing environmental conditions such as variations in the flow rate, concentration, and quality of the raw wastewater entering the process. In general, any rapidly occurring or immediate change in the chemical or physical environment might be classified as a system shock (Gaudy and Engelbrecht, 1961). These shocks including high and unsteady organic shocks, feed starvation, high and unsteady salinity shocks, high ammonia shocks, pH shocks, other toxicity shocks and hazardous events are reviewed in the following sections.

2.2.1. High and unsteady organic shock loads

Exposure to sudden organic shock loads has been an important area of research aimed at understanding the impacts of hazardous events to biological systems for more than 50 years (Gaudy and Engelbrecht, 1961). This is because of the common occurrence of challenging conditions with organic composition in influent wastewater often varying substantially over a single diurnal period as well as from day to day (Selna and Schroeder, 1979). However, much of the previously reported research on the impacts of organic shock loads has been undertaken on AS systems (Saleh and Gaudy, 1978, Normand and Perdrieux, 1981, Manickam and Gaudy, 1985, Mora et al., 2003, Thanh et al., 2009, Seetha et al., 2010). Comparatively very little research has been conducted using MBR systems (Al-Malack, 2007). Most of these studies on both AS and MBR were conducted on lab-scale reactors with synthetic wastewater including glucose (Saleh and Gaudy, 1978, Normand and Perdrieux, 1981, Manickam and Gaudy, 1985, Al-Malack, 2007, Thanh et al., 2009, Le-Minh, 2011) or molasses (Mora et al., 2003, Seetha et al., 2010) as the carbon source.

Results of AS organic shock load studies with glucose as a carbon source show that AS reactors, which were operated stably at influent organic concentrations around 100-500 $mg.L^{-1}$ COD can withstand influent shock concentrations of up to 1500 $mg.L^{-1}$ COD, even when the shock durations varied from hours to weeks (Gaudy and Engelbrecht, 1961, Saleh and Gaudy, 1978, Normand and Perdrieux, 1981). This can be explained by the reaction potential concept, which suggests that in steady-state, continuous flow, mass-culture biological oxidation systems, the microorganisms are generally operating at loadings below the reaction potential of the microorganisms (Eckhoff, 1969, McLellan and Busch, 1969, Normand and Perdrieux, 1981). When a

change in loading occurs, such as in the case of an organic shock load, and if the increase in microorganisms during the hydraulic retention time of the reactor is such that the reaction potential is not exceeded, no change in the substrate concentration flowing out of the reactor is expected (Eckhoff, 1969, McLellan and Busch, 1969, Normand and Perdrieux, 1981). At influent shock concentrations around 3000 $mg.L^{-1}$ COD, the change may exceed the maximum assimilation capacity of the biomass, so there is an increased deterioration of effluent quality caused by loss of biological solids, but recovery may still be rapid as reported by (Saleh and Gaudy, 1978, Manickam and Gaudy, 1985), who showed that the COD in an AS effluent returned to a low level within four to six days after a 3000 $mg.L^{-1}$ COD shock was applied..

A 3000 $mg.L^{-1}$ COD shock load to an AS system was reported to cause a rapid growth in biomass, a noticeable change in colour of the mixed liquor, a decrease in floc size, an increase in filamentous forms and a reduction in the number of protozoa (Saleh and Gaudy, 1978). Disruption in COD removal capacity and the change on colour of an AS system were observed to be correlated with changes in the biochemical composition of the sludge (Manickam and Gaudy, 1985). It was also reported that the dynamics of the biological treatment depended less on the growth rate of microorganisms than on other mechanisms, notably the storage of the carbon substrate in the cells (Normand and Perdrieux, 1981). This study also suggested that the impacts depended essentially on the magnitude of the perturbation imposed and not on the initial biomass concentration (Normand and Perdrieux, 1981). Sludge age also had a very distinct effect with lower response in time with older sludges (Normand and Perdrieux, 1981).

Previous AS and MBR studies on organic shock loads are summarised in Table 2.1.

Table 2.1 Summary of AS and MBR studies on organic shock loads

Process information	Feed concentrations: at steady state → at shock load *(shock load period)* → after shock load	Impacts on removal of organics and nutrients	Impacts on biomass	Reference
Continuous flow AS (2 L) Mixed liquor suspended solid (MLSS): 2 $g.L^{-1}$	500 → ***1500 (17 d)*** →500 ($mg.L^{-1}$ glucose)	No significant impact.	Increased cell growth, biomass concentration, gradual subsidence to a new steady-state within 3.5 d.	(Saleh and Gaudy, 1978)
	500 → ***3000 (15 d)*** → 500 ($mg.L^{-1}$ glucose)	Loss of biomass causing deterioration of effluent quality, recovery rather rapid.	Colour changed, floc size decreased, filametous forms increased and number of protozoa reduced.	
	Cyclic shock load 500 ***→ 1500 (12 h) →500 (12 h) → 1500 (12 h)→500 (12 h) …continue for 18 d*** ($mg.L^{-1}$ glucose)	No significant effect.	Cyclic rise and fall in biomass concentration but its amplitude slightly diminished over time.	
Continuous flow AS (2L) MLSS: 2 $g.L^{-1}$	500 → ***3000 (10 d)*** →500 ($mg.L^{-1}$ glucose)	Filtrate COD increased. Transient response lasted 4 d.	Protein and carbohydrate composition of biomass changed.	(Manickam and Gaudy, 1985)
Continuous flow AS (25L) Hydraulic retention time (HRT): 3 h Solid retention time (SRT): 5-15 d MLSS: 1-4 $g.L^{-1}$	60→ ***480 (∞)***→ 60 → ***1345 (1 h)*** → 60→ ***1930 (0.5 h)*** → 60→ ***1843 (0.5 h)*** ($mg.L^{-1}$ TOC, glucose is a carbon source)	At 480 $mg.L^{-1}$ TOC shock: no significant impact. At 1345 $mg.L^{-1}$ TOC shock: slightly higher substrate concentration in effluent. At 1930 and 1843 $mg.L^{-1}$ TOC shock: greater substrate concentration in effluent.	Impacts depended essentially on magnitude of perturbation imposed, not on initial biomass concentration.	(Normand and Perdrieux, 1981)

Process information	**Feed concentrations: at steady state → at shock load *(shock load period)* → after shock load**	**Impacts on removal of organics and nutrients**	**Impacts on biomass**	**Reference**
Two sequencing batch airlift reactors (2.5 L) HRT: 3 h Cultivated aerobic granules with two types of supports including bivalve shell carrier (BSC) and anaerobic granules (ANG) MLVSS: 4-5 $g.L^{-1}$	600 ***→ 1200 (3 wk)→ 2400 (3 wk)→ 4800-9600 (3 wk)*** ($mg.L^{-1}$ COD, glucose is a carbon source)	Organic removal efficiency >96%. Higher organic loading applied causing higher suspended solid concentration in effluent.	When influent COD increased to 1200 $mg.L^{-1}$, BSC reactor work as usual while ANG experienced significant wash out of biomass and granular size decreased but it developed back to initial size at the end of 2400 $mg.L^{-1}$ COD shock load.	(Thanh et al., 2009)
Three 9L sequential batch reactors (SBRs) operated with various aeration periods of 0, 15, and 30 min HRT: 24h (one cycle per day) SRT: 70 d MLVSS: 3-4 $g.L^{-1}$	200 ***→ 500*** ($mg.L^{-1}$ COD, molasses is a carbon source)	SBR reactors can withstand the shock loading. Denitrification rate decreased when increasing aeration time.	not available	(Mora et al., 2003)
Continuous two-stage AS-biofilm reactor (35 L)	350-500 ***→ 808 (6 h)*** → 350-500 ***→1170 (6 h)*** → 350-500 ***→ 1358 (6 h)*** → 350-500 ***→1900 (6 h)*** → 350-500 ($mg.L^{-1}$ COD,	Effluent COD increased with duration of shock load and recovered to steady state	Bacterial type changed from gram positive rods to gram-negative oval shaped	(Seetha et al., 2010)

Process information	**Feed concentrations: at steady state → at shock load *(shock load period)* → after shock load**	**Impacts on removal of organics and nutrients**	**Impacts on biomass**	**Reference**
HRT: 6 h	molasses is a carbon source)	conditions in about 7, 17, 20, 27 h after removing shock loads of 808, 1170, 1358 $mg.L^{-1}$ COD. Nitrification unaffected during a shock load of 808 $mg.L^{-1}$ COD but inhibited after shock load of 1170 $mg.L^{-1}$ COD.	bacteria. Autotrophs likely outcompeted by heterotrophs and washed out the system	
Immersed MBR (20 L) HRT: 12-15 h SRT: 2-74 d MLSS: 10-15 $g.L^{1}$	4998 → ***8024 (2 d)→12100 (2 d) →16000 (2 d)→ 2000*** ($mg.L^{-1}$ COD, glucose is a carbon source)	COD removal not significantly affected. COD removal efficiency increased with increasing MLSS concentration.	not available	(Al-Malack, 2007)

Sequential batch reactors have been shown to be able to withstand influent molasses shock concentration from 200 $mg.L^{-1}$ COD (at steady state) to 500 $mg.L^{-1}$ COD (shock load) (Mora et al., 2003). Similarly, two-stage AS-biofilm reactors have recovered quickly from organic shock load by molasses at concentrations of 808, 1170, 1358 and 1900 $mg.L^{-1}$ COD and recovery time was proportional to the magnitude of the shock loads (Seetha et al., 2010). Shock loads have been observed to change the dominant bacterial type in bioreactor from gram-positive rods to gram-negative oval shaped bacteria. It is likely that autotrophs were outcompeted by heterotrophs and washed out of the system (Seetha et al., 2010). In general, high organic concentration in influent wastewater is known to inhibit nitrification as it supports the growth of heterotrophic bacteria, which compete with autotrophic nitrifying bacteria for oxygen, nutrients and space (Sharma and Ahlert, 1977, Hanaki et al., 1990, Ohashi et al., 1995, van Benthum et al., 1997, Zhu and Chen, 2001, Chen et al., 2006b).

Multiple organic shocks of equal magnitude administered cyclically have been shown to be well accommodated by an AS reactor, leading to minimal cyclic disturbance in effluent quality (Saleh and Gaudy, 1978). A cyclical change from 500 $mg.L^{-1}$ glucose feed for 12 hours to 1500 $mg.L^{-1}$ glucose feed for 12 hours, continuously for 18 days, resulted in a corresponding rise and fall in biomass concentration but its amplitude was diminished over time (Saleh and Gaudy, 1978). Cultivated aerobic granular sludge can also withstand various organic shock loads from 1200 to 9600 $mg.L^{-1}$ COD (Thanh et al., 2009).

In addition to the quantitative organic shock loads caused by an immediate increase in influent concentration, a similar shock load may be caused by a rapid change in hydraulic load. One study reported that shock loads of different types, -hydraulic, quantitative, or in combinations - did not produce equivalent disruptions of effluent quality for equal increases in mass organic loading rate (Manickam and Gaudy, 1985). A pure hydraulic and a hydraulic-quantitative shock at the same mass loading produced equal increases in total effluent COD, resulting from suspended solids in the hydraulic shock and soluble COD in the combined shock. A quantitative shock caused a much higher increase in effluent COD than a combined hydraulic-quantitative shock did at the same mass loading (Manickam and Gaudy, 1985).

The impacts of quantitative organic shock loads on immersed MBRs were also investigated (Al-Malack, 2007) as summarized in Table 2.1. Results show that influent organic shock loadings from 5-16 $g.L^{-1}$ COD removal did not significantly affect the MBR process, which was operated with synthetic wastewater at very high MLSS concentration of 15 $g.L^{-1}$ (Al-Malack, 2007).

2.2.2. Feed starvation

Wastewater treatment systems are usually designed for a uniform flow regime. However, in extreme conditions, some treatment plants exhibit feed starvation periods during which no appreciable wastewater feeds the systems. This discrepancy between the conceptual design and the practical situation may lead to process upsets and unsatisfactory system performance, resulting in potential violation of discharge standards (Beler Baykal et al., 1990).

Previous studies have shown that biomass concentrations in AS decreased sharply during the first four days of the starvation period and then reduced more slowly after that (Urbain et al., 1993, Coello Oviedo et al., 2003). In addition, the bacteria cell size was also found to be reduced, which was described as one of the adaptative responses to starvation conditions (Kjelleberg et al., 1987, Urbain et al., 1993, Coello Oviedo et al., 2003). These responses were related to the degradation of both proteins and polysaccharides contents of the sludge (Urbain et al., 1993) and led to a decrease in respiratory activity of the microorganisms (Urbain et al., 1993, Coello Oviedo et al., 2003). After 3-4 days under starvation conditions, the biomass drastically lost its ability to biodegrade exogenous nutrients reactions (Urbain et al., 1993).

Starvation shocks have been found to cause a noticeable decline in activated sludge settleability and dewaterability (Horan and Shanmugan, 1986). Starvation shocks also resulted in disappearance of some of the typical microbial groups usually found in an AS, and appearance of other opportunistic microorganisms (Coello Oviedo et al., 2003). The aerobic granulation in SBR was found to be initiated by starvation phase (Li et al., 2006).

Mixed liquor pH was slightly increased in the first few days of the starvation shock, which could be a result of ammonia release in the liquid, as a result protein hydrolysis (Urbain et al., 1993, Coello Oviedo et al., 2003). Following this step, pH of mixed liquor was gradually decreased, which might be explained either by a hydrolytic metabolism (volatile fatty acids production), or by nitrification reactions (Urbain et al., 1993, Coello Oviedo et al., 2003).

The removal of DOC and nitrogen by lab-scale MBRs was reported not to be affected under a feed starvation period of 2 days (Le-Minh, 2011) whereas, the removal efficiencies of COD, TOC, total suspended solid (TSS), total kjeldhal nitrogen (TKN) and phosphate by a MBR were reduced significantly during a starvation period of 5 days (Yogalakshmi et al., 2007). In addition, a large fraction of biomass wash off and a reduction in microbial activity inside the bioreactor was observed (Yogalakshmi et al., 2007). The removal of organics and nutrients

recovered to 90% after 3 days and was fully recovered to steady state conditions after 6 days of normal operation. However, it took nearly a month of continuous operation to regain the amount of biomass lost during feed starvation shock load (Yogalakshmi et al., 2007). The maintenance of an aerobic storage period under starvation conditions before recycling the sludge in the aeration tank has been suggested as an option to reduce the sludge production in AS (Urbain et al., 1993, Coello Oviedo et al., 2003).

2.2.3. High and unsteady salinity shock loads

Sea water infiltration and discharging of salty wastewater from industries such as tanning, seafood processing, vegetable processing can cause high salinity in the sewage in many regions (Panswad and Anan, 1999, Dan et al., 2003). Salinity shock is classified as toxic shock as it involves an influx of organics or inorganic elements, radicals or compounds, which wholly or partially inhibit and/or damage the existing metabolic pathways or disrupt the established physiological condition of the microbial population (Gaudy and Engelbrecht, 1961). High or changing salinity concentrations in influent wastewater have been reported to reduce organic, nutrients removal efficiencies and biomass settleability in biological treatment processes (Dan et al., 2003, Ng et al., 2005, Hong et al., 2007). This is most likely due to the salty conditions produce a high osmotic pressure on the bacteria cells that inhibit bacterial growth and floc formation (Dan et al., 2003). Additionally, high salt concentration conditions also reduce gravity separation due to lower density difference between water and biomass (Ng et al., 2005). Impacts of salinity shock load on AS and MBR performance have been investigated in some previous studies (Stewart et al., 1962, Ludzack and Noran, 1965, Kincannon and Gaudy, 1966, Kincannon and Gaudy, 1968, Panswad and Anan, 1999, Ng et al., 2005, Reid et al., 2006, Yogalakshmi and Joseph, 2010). Particular emphasis has been placed on the study of the effect of high concentrations of NaCl since it is one of the more common salts found in large amounts in some carriage waters and is a major inorganic constituent of wastes from several industrial processes (Kincannon and Gaudy, 1968). These studies are summarised in Table 2.2.

It has generally been observed that significant change in AS performance is not encountered while chloride concentrations in influent wastewater are maintained below 10 $g.L^{-1}$ (Stewart et al., 1962, Ludzack and Noran, 1965, Ng et al., 2005). However, with influent salt shock load concentrations ≥ 20 $g.L^{-1}$, COD removal efficiency has been found to be severely reduced (Ludzack and Noran, 1965, Kincannon and Gaudy, 1966, Kincannon and Gaudy, 1968, Ng et al., 2005). A decrease in COD removal efficiency was found almost linearly with increasing influent NaCl concentrations between 20 to 60 $g.L^{-1}$ (Ng et al., 2005). Nitrification

during high-chloride operation was found to achieve only about 10% of that expected for the same operation at low chloride concentrations. In this case, several hours of high-chloride operation were required to inhibit nitrification (Ludzack and Noran, 1965).

Activated sludge developed in low-salt wastewater was found to be able to withstand shock loadings of high salinity concentration more effectively than sludge developed in a high-salt medium could withstand a rapid decrease in salt concentration (Kincannon and Gaudy, 1966). The treatment efficiency was severely affected when fresh-water sludge was shocked with 45 $g.L^{-1}$ NaCl, and nearly total destruction of treatment efficiency occurred when sludge acclimated to 45 $g.L^{-1}$ NaCl was subjected to fresh-water (Kincannon and Gaudy, 1966).

The responses of AS to salinity shock loads include a reduction in biosolid concentrations (Kincannon and Gaudy, 1968, Panswad and Anan, 1999), changes in population variety and activity (Stewart et al., 1962, Ludzack and Noran, 1965, Ng et al., 2005) and a decrease in sludge settleability (Ludzack and Noran, 1965). Sludges grown in high salt concentrations have low carbohydrate and protein contents and abnormally high lipid and ribonucleic acid (RNA) contents (Kincannon and Gaudy, 1966). Sustained high chlorides generally depresses biomass respiration (Ludzack and Noran, 1965).

Previous studied on immersed MBRs found that COD and nutrient removal efficiencies decreased at the following increasing shock loads of 5, 10, 20, 30, 50, 60 $g.L^{-1}$ NaCl (Reid et al., 2006, Yogalakshmi and Joseph, 2010). Nitrification was completely inhibited at the highest shock load of 60 $g.L^{-1}$ and the removal of COD and total nitrogen was lowest at this shock load. It took 4-9 days for the system to recover to the steady state conditions (Yogalakshmi and Joseph, 2010). The properties of the biomass were significantly affected by high salinity and thus the permeability was also influenced. In high salinity conditions, the soluble microbial product (SMP) and extracted extracellular polymeric substance (EPS) concentration of the mixed liquor increased, those parameters linked to physical parameters of the AS such as particle size, capillary suction time and turbidity (Reid et al., 2006). In addition, a decrease in settleability of the biomass was observed to be correlated with increasing NaCl concentrations (Yogalakshmi and Joseph, 2010). For a shock load of 5 $g.L^{-1}$ NaCl, at the flux of 8 $L.m^{-2}.h^{-1}$, the permeability decreased instantly after the salinity shock and recovered to 85-90% of the initial permeability after 9 days and remained at this level for the next 3 weeks, whereas at a flux of 16 $L.m^{-2}.h^{-1}$, after the salinity shock, the permeability decreased similarly but no recovery was observed during the operational period (Reid et al., 2006).

Table 2.2 Summary of AS and MBR studies on salinity shock loads

Process information	Feed concentrations: at steady state → **at shock load *(shock load period)*** → after shock load	Impacts on removal of organics and nutrients	Impacts on biomass	Reference
Two identical extended-aeration continuous flow AS (113.5 L) run in parallel. Wastewater made up of fresh and Pacific Ocean water plus organic substances HRT: 1.5 d SRT: ∞ MLVSS: 0.3-3.7 $g.L^{-1}$	Run 1 system I: fresh water sewage **→ 5.8 $g.L^{-1}$ chloride (first 20 d with BOD = 250 $mg.L^{-1}$ and next 9 d with BOD = 750 $mg.L^{-1}$)** Run 1 system II: fresh water sewage 50 $mg.L^{-1}$ chloride Run 2: fresh water **→ 18-20 $g.L^{-1}$ chloride and 4.5 folds increase in flow rate (20.5 h) → 5.8 $g.L^{-1}$ chloride and normal flow rate.** Run 3: 5.8 **→ 18-20 $g.L^{-1}$ chloride (4 d)** → fresh water Run 4: fresh water **→ 18-20 $g.L^{1}$chloride (12 d)** → fresh water	At normal hydraulic and organic loading, effluent quality not significant affected by salinity shock. Under abnormally severe changes in salinity, combined with unusually heavy hydraulic and organic loadings, treatment efficiency reduced temporally, recovery time depending on duration and magnitude of the shock.	Run1: MLVSS concentrations increased from 0.3 to 1.5-1.7 $g.L^{-1}$ in both systems. Run 2: immediately after severe shock, protozoa reduced significantly. However, after that, protozoa increased fairly rapid. Run 3: MLVSS concentrations increased from 1.4 to 3.6 $g.L^{-1}$. Run 4: after the shock, protozoa population turned over rapidly but total population grew steadily. The reverse occurred on returning to fresh water feed.	(Stewart et al., 1962)
Continuous flow AS (4.8 L), fed 5 d per wk and rested on weekends. Wastewater made up	Experiment 1: 0.1 → 4 (1 d)→6 (1 d) → 7.3 (1 d) → altered each d to 20 $g.L^{-1}$ chloride in 3 wk. Experiment 2: 0.1 → **20 (20 wk, BOD load decreased 50% from 9th**	Chloride concentration < 8 $g.L^{-1}$: no significant impact. Nitrification during high-chloride operation reduced significantly	Population variety and activity changed significantly. Sustained high chlorides generally depressed respiration.	(Ludzack and Noran, 1965)

Process information	**Feed concentrations:** at steady state → **at shock load** ***(shock load period)*** → after shock load	**Impacts on removal of organics and nutrients**	**Impacts on biomass**	**Reference**
of fish-meal slurry. HRT: 8 h MLVSS: 2 $g.L^{-1}$	**to 14th wk)** → 0.4 $g.L^{-1}$ chloride. Experiment 3: cyclic shock of 0.1 (1 wk) → **20 (1 wk)** → 0.1 (1 wk) → **20 (1 wk)** $g.L^{-1}$ chloride …continue for 20 wk		Poor sludge settling caused clarification problems.	
Continuous flow AS reactors (2.4 L) fed synthetic wastewater HRT: 8 h MLVSS: 3.2 $g.L^{1}$	0 → **30 (1 wk)** →0 $g.L^{-1}$ NaCl	COD removal efficiency decreased rather severely. It took 2 d for the system to attain a new steady state.	biomass concentration decreased rather severely and there was possible change in species predominant.	(Kincannon and Gaudy, 1968)
Batch AS units (1.5 L) Daily feed with synthetic wastewater SRT = 3 d	Using fresh water activated sludge: Experiment 1: 0 →**30 $g.L^{-1}$ NaCl** Experiment 2: 0 →**45 $g.L^{-1}$ NaCl** Using glucose-acclimated sludge grown in synthetic waste containing 30 $g.L^{1}$ NaCl and 45 $g.L^{1}$ NaCl: Experiment 3: 30 → **0 $g.L^{-1}$ NaCL** Experiment 4: 45 → **0 $g.L^{-1}$ NaCL**	When a fresh-water sludge shocked with 30 $g.L^{-1}$ NaCl, COD removal efficiency reduced 30%. However, when an activated sludge acclimated to 30 $g.L^{-1}$ NaCl dosed with fresh-water, COD removal efficiency decreased by 75 %. Treatment efficiency severely affected when fresh-water sludge shocked with 45 $g.L^{-1}$ NaCl, and nearly total destruction of efficiency occurred when sludge acclimated	In both cases, immediate response involves a release of cellular components indicative of lysis. Sludges grown in high salt concentrations have low carbohydrate and protein contents and abnormally high lipid and RNA contents.	(Kincannon and Gaudy, 1966)

Process information	Feed concentrations: at steady state → **at shock load** ***(shock load period)*** → after shock load	Impacts on removal of organics and nutrients	Impacts on biomass	Reference
		to 45 $g.L^{1}$ NaCl subjected to fresh-water.		
SBR (10 L) fed synthetic wastewater followed 6 h 40 min cycle. MLVSS: 2.5 $g.L^{-1}$	Tapwater + NaCl feed at 0, 5, 10, 20, 30, 40, 60 $g.L^{-1}$ NaCl Seawater: 20 $g.L^{-1}$ NaCl, 0.3 mg COD.mg $VSS^{-1}.d^{-1}$ 20 $g.L^{-1}$ NaCl, 0.6 mg COD.mg $VSS^{-1}.d^{-1}$	NaCl ≤ 10 $g.L^{-1}$: no significant impact. NaCl > 20 $g.L^{-1}$: treatment efficiency deteriorated. NaCl > 30 $g.L^{-1}$: effluent turbidity increased significantly.	When NaCl increased, protozoa and rotifers eliminated. Ciliates became dominant microorganisms at NaCl of 5 $g.L^{-1}$. However, ciliates were absent when NaCl > 10 $g.L^{-1}$.	(Ng et al., 2005)
Two anaerobic/anoxic/aerobic processes fed synthetic wastewater run in parallel. HRT: 2 + 2+ 12 h SRT: 10 d MLSS: 2.5 $g.L^{-1}$	NaCl acclimated sludge system: 5 → **70 (4 d)** → 5 10 → **70 (4 d)** → 10 20 → **70 (4 d)** → 20 30 → **70 (4 d)** 30 Non-acclimated sludge system: 0 → **70 (4 d)** → 0 5 → **70 (4 d)** → 5 10 → **70 (4 d)** → 10 20 → **70 (4 d)** → 20 30 → **70 (4 d)** 30 (**$g.L^{-1}$ NaCl)**	In both non-acclimated and acclimated systems, when NaCl increased from 0 to 30 $g.L^{-1}$, COD, nitrogen and phosphorous removal efficiency reduced around 10-40%. Time required to reach steady state was higher (10±20 d) for the non-acclimated system than that with acclimation (8±15 d).	When NaCl increased from 0 to 30 $g.L^{-1}$, MLSS in non-acclimated system decreased from 2670 to 1600 $mg.L^{-1}$ whereas MLSS in acclimated system not varied significantly.	(Panswad and Anan, 1999)
Immersed MBR (3.2 m3), fed municipal	0.35 → **5 $g.L^{1}$ NaCl**	COD removal affected immediately and it took 1 wk to recover, where	Biomass properties were significantly affected. SMP	(Reid et al., 2006)

Process information	**Feed concentrations:** at steady state → **at shock load *(shock load period)*** → after shock load	**Impacts on removal of organics and nutrients**	**Impacts on biomass**	**Reference**
sewage HRTs: 36-72 h SRT: 64 d MLSS: 9-17 g.L^{-1}		as nitrification less profoundly affected. At flux of 8 L.m^{-2}.h^{-1}, permeability was decreased and recovered 90% after 9 d. For the flux of 16 L.m^{-2}.h^{-1}, permeability decreased similarly but no recovery was observed.	and EPS concentration of mixed liquor increased.	
Immersed MBR (6L), fed with synthetic wastewater HRT: 8 h MLSS: 10-15 g.L^{-1}	0 → **5 (1 d)** → 0 (3-4 d) → **10 (1 d)** → 0 (3-4 d) → **20 (1 d)** → 0 (3-4 d) → **30 (1 d)** → 0 (3-4 d) → **50 (1 d)** → 0 (3-4 d) → **60 (1 d)** g.L^{-1} NaCl	COD and nutrient removal efficiencies decreased in all shock loads. At highest shock load of 60 g.L^{-1}, removal of COD and total nitrogen was lowest with nitrification being completely inhibited. It took 4-9 d for system to recover.	Biomass settleability decreased with increasing NaCl concentration.	(Yogalakshmi and Joseph, 2010)

2.2.4. High ammonia shock loads

Ammonia is a waste product of human, animal and microbial metabolism. Ammonia is also released from industrial wastewater such as fertilizer production, food processing, mining, pulp and paper mills that are collected by sewerage collection systems for biological wastewater treatment. Ammonia shock is also classified as toxic shock (Gaudy and Engelbrecht, 1961). Sudden increase in ammonia concentration in biological treatment process can be due to increase ammonia concentration in raw sewage or inhibition of nitrification in the biological treatment process (Hart et al., 2003). Previous research has shown that at the ammonium shock concentrations of 70, 190, 390 $mg.L^{-1}$ nitrogen (approximately 2, 5 and 10 times the average ammonia in the influent, respectively), COD removal efficiency by AS was not significantly affected (Henriques et al., 2007). The shock loads did not produce strong deflocculation events or long recovery times from initial increase in effluent total suspended solid. The ammonia shock conditions have some inhibitory effect on biomass specific oxygen uptake rate (SOUR), but those effects were modest and/or short-lived (Henriques et al., 2007). Another study on AS fed mixed wastewater containing glucose and leachate showed that when the ammonia-N concentration increased from 50 $mg.L^{-1}$ to 800 $mg.L^{-1}$, COD removal efficiency decreased from 98 to 78% and SOUR reduced from 68 to 45 $mgO_2.gMLSS^{-1}$ (Li and Zhao, 1999). A nitrifying AS reactor operated efficiently at an ammonia loading rate of 4 g $N.L^{-1}.d^{-1}$ was found to become completely inhibited when inlet salt concentration was increased above 525 mM (13.7 $g.L^{-1}$ NaCl, 19.9 $g.L^{-1}$ $NaNO_3$ and 8.3 $g.L^{-1}$ Na_2SO_4) (Campos et al., 2002). High ammonium concentration in wastewater was also found to deteriorate AS settling and dewatering properties (Novak, 2001) as high concentration of monovalent cations like ammonium could replace divalent cations in the flocs, weakening the binding biopolymers and causing weaker and less-dense flocs (Higgins and Novak, 1997, Novak, 2001). Following this, the settling properties of the sludge improved slowly and recovery seemed to have been due to replacement with new flocs rather than alteration of the existing biomass (Novak, 2001).

2.2.5. pH shock loads

pH in biological treatment processes can be varied due to pH variation in raw sewage or due to failure of denitrification process within the biological treatment units. Rapid changes in pH of the wastewater are also considered to be in the class of toxic shock loading although they are more easily controlled and may be of less significance than

other toxicity shock loads (Gaudy and Engelbrecht, 1961). Previous studies have found that AS systems may be resilient to pH shock loadings as low as 4.0 and as high as 10.4, without serious disruption of biological solids concentration and filtrate COD (Yang and Thavinpipatkul, 1978, Henriques et al., 2007). However, at pH 4.0 shock loading, filamentous organisms were reported to be predominant, which may cause negative affect to the settling characteristics of biomass (Yang and Thavinpipatkul, 1978). Alkaline pH 11 was found to inhibit COD removal ability, flocculation ability, biomass growth, respiration rates, settleability, and dewaterability of activated sludge (Henriques et al., 2007) as well as obstruct nitrification in sequencing batch reactors (Kelly et al., 2004).

2.2.6. Other toxicity shock loads

Previous studies have shown that high concentrations of phenol, 1-octanol, 1-chloro-2,4-dinitrobenzene (CDNB), pentachlorophenol, acetone, cadmium, chromium and cyanide affect the performance of MBR and AS systems significantly (Yang and Thavinpipatkul, 1978, Rozich and Gaudy, 1985, Grau and Da-Rin, 1997, Bott et al., 2001, Bott and Love, 2001, Love and Bott, 2002, Kelly et al., 2004, Al-Malack, 2007, Henriques et al., 2007). The effects were found to be correlated with the magnitudes of these shocks (Yang and Thavinpipatkul, 1978, Bott et al., 2001, Al-Malack, 2007) and included poor removal of soluble COD and BOD, high effluent volatile suspended solid concentrations, low respiration rates, slow growth of biomass, population dynamic irregularities, flocculation irregularities, poor or completely inhibited nitrification, and enormous abundance of flagellates (Yang and Thavinpipatkul, 1978, Grau and Da-Rin, 1997, Bott et al., 2001, Bott and Love, 2001, Kelly et al., 2004, Al-Malack, 2007, Henriques et al., 2007). It took at least a week (Al-Malack, 2007) to six months (Grau and Da-Rin, 1997) for the biological processes to recovery from these shock loads depend on the magnitude of the shocks.

AS was reported to be able to handle temperature shock loads from 23 to 36°C without any increase of effluent filtrate COD (Yang and Thavinpipatkul, 1978). However, with a 46°C temperature shock, COD removal efficiency by AS dropped to 85% (Yang and Thavinpipatkul, 1978). The NH_3-N and COD removal efficiencies by a package MBR were found to be significantly reduced when temperature suddenly dropped from 12 - 26°C to ≤ 4°C due to a dormancy state of the sludge bacteria and physical blockage of the membrane (Sun et al., 2009).

Electron inhibitors such as 2,4 dinitrophenol (DNP) can also inhibit the performance of biological treatment systems. DNP has been used commercially for a number of purposes such as a dye, a wood preservative and a pesticide (Rich and Yates, 1955). In addition, DNP is also referred to as an "uncoupler" because at low $ng.L^{-1}$ concentrations, it has been shown to considerably reduce sludge yield in AS, but did not significantly affect COD removal rate or settleability of activated sludge (Rich and Yates, 1955, Chen et al., 2008a). Thus it is suggested to use at low $ng.L^{-1}$ concentrations to reduce the sludge production at wastewater treatment plant (Mayhew and Stephenson, 1998, Chen et al., 2008a). As DNP has been used widely in various applications, the chance that this chemical entering sewage system accidentally or even intentionally increases (Rich and Yates, 1955). DNP is an electron inhibitor that is very toxic and can persist for extended periods in the environment because of the presence of nitrite groups on the phenolic parent compound ,which deter enzyme attack (Bruhn et al., 1987). DNP concentrations in sewage have been reported at up to 41 $ng.L^{-1}$ (Loos et al., 2003).

Previous studies found that at low concentration, uncouplers such as DNP has been found to stimulate electron transfer and respiration rate (Mitchell and Moyle, 1967, Henriques et al., 2005). However, at high concentrations, the respiration stimulation effects changed to respiration inhibition (Henriques et al., 2005). At high concentration, uncouplers like DNP can inhibit bacterial metabolic process including interfering with amino acid and nutrient transportation into bacteria cells (Brummett and Ordal, 1977, Decker and Lang, 1977, Nicholas and Ordal, 1978, Bakker and Randalll, 1984, Henriques et al., 2005) and hindering protein translocation into cytoplasmic membrane (Enequist et al., 1981, Geller, 1991, Henriques et al., 2005). A stress protein was found to be induced in bacteria in response to DNP shock (Bott et al., 2001). This stress protein induction was hypothesised as a cause in BOD removal efficiency reduction during biological treatment processes due to the temporary redirection of energy away from growth to protein biosynthesis (Love and Bott, 2002). In addition, during DNP shock condition, significant potassium (K+) efflux was induced as a physical bacterial stress response mechanism, resulting in biomass deflocculation (Bott and Love, 2002, Love and Bott, 2002).

Literature (Henriques et al., 2005, Chen et al., 2006a, Henriques et al., 2007) reported a large variation in DNP concentrations that inhibited COD removal efficiency of AS. A study on batch AS reactor fed with synthetic wastewater found that at 20 $mg.L^{-1}$ DNP, COD removal of an AS reduced from 90% to 53% (Chen et al., 2006a) while another

study on SBR fed with domestic wastewater reported no affect on COD removal efficiency at DNP concentration up to 107 $mg.L^{-1}$ (Henriques et al., 2007). It has been hypothesised that some variation may be explained by variable endogenous concentrations of DNP (or other chemicals with similar properties) in municipal wastewaters, and hence, variable populations of DNP-degrading bacteria in wastewater treatment plants (Jo and Silverstein, 1998).

2.2.7. Other hazardous events

Beside the sudden changes in influent flow and concentration, other potentially hazardous events including physical membrane damage and loss of aeration are also expected to affect the MBR treatment process performance. The available literature related to this topic is discussed in this section.

Physical membrane damage

The membranes used in MBRs have a limited lifespan and are known to suffer from various modes of failure (Ayala et al., 2011, Cote et al., 2012). The key failure modes for flat sheet membranes include mechanical failure of the membrane weld and loss of permeability (Ayala et al., 2011). Similarly, the failure modes of hollow fibre membranes include hollow fibre breakage, mechanical module and cassette failure, weakening of the potting resin-membrane fibre bond and increase in cleaning frequency to meet flow throughput (Cote et al., 2012). However, these failure modes tend to be gradual rather than sudden and are easily identified by long-term changes in flux or operating pressures. Accordingly, their relevance as 'hazardous events' leading to sudden deterioration in water quality appears low. Nonetheless, there is some evidence to suggest that events such as chemical membrane cleaning and accidental exposure to excessive chlorine concentrations may physically harm some types of water treatment membranes leading to reduced performance (Simon et al., 2009, Beyer et al., 2010).

Loss of aeration performance

Temporary interruptions to aeration of MBR (or AS) systems would be expected to have a detrimental impact on the aerobic metabolic degradation of chemical contaminants and potentially lead to change within the microbial community. Loss of aeration may also lead to loss of suspension of the MLSS, potentially causing damage to MBR membranes. However, no reports of investigation of the precise performance impacts of aeration loss could be identified.

2.3. IMPACTS OF HAZARDOUS EVENTS ON REMOVALS OF TRACE ORGANIC CHEMICAL CONTAMINANTS

The impacts of starvation and phenolic shocks on degradation of phenolic compounds by moving bed biofilm SBRs have been reported in several studies (Moreno-Andrade et al., 2009, Buitrón and Moreno-Andrade, 2011). Shock loads of a single compound (4-chlorophenol) at 500 and 1050 $mg.L^{-1}$ were found to cause minimal impact on 4-cholorophenol degradation rate, with losses of 6% and 8%, respectively (Moreno-Andrade et al., 2009). However, a decrease in the degradation rate of 62% and 95% was observed for the shock load of a mixture of phenolic compounds (phenol, 4-clorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol) at 500 and 750 $mg.L^{-1}$, respectively. This suggests that a mixture may have a synergistic effect, increasing the inhibitory capacity (Buitrón and Moreno-Andrade, 2011). Insignificant impacts to SBR performance for removing phenolic compounds were observed after a starvation period of up to 24 hours. When the starvation period was extended for more than 24 hours, negative impacts on removals of phenolic compounds were detected (Moreno-Andrade et al., 2009, Buitrón and Moreno-Andrade, 2011). Nevertheless, the observed effects of starvation and shock loads were only transient and in the following cycle the previous activity was recovered (Buitrón and Moreno-Andrade, 2011).

Several studies have investigated the impacts of hazardous events on the removal of trace organic chemical contaminants by MBRs with a focus on impacts of sudden pH changes (Urase et al., 2005, Bo et al., 2009) and feed starvation shock (Le-Minh, 2011) on the removal of endocrine disrupting chemicals (EDCs) (Urase et al., 2005) and pharmaceuticals (Urase et al., 2005, Bo et al., 2009, Le-Minh, 2011). pH changes were found to affect the removal of ionisable trace organic contaminants (Urase et al., 2005, Bo et al., 2009, Tadkaew et al., 2010) while the removal of non-ionisable compounds were relatively independent of the mixed liquor pH (Tadkaew et al., 2010). This impact does not only depend on sudden shock but is also observed to occur under gradual changes in pH (Tadkaew et al., 2010), resulting in changes in adsorption of the trace chemical contaminants to activated sludge as well as changes of microorganism activity due to pH variations (Urase et al., 2005, Bo et al., 2009, Tadkaew et al., 2010). A previous study found that the removal of sulfonamide and trimethoprim antibiotics by MBRs was not affected by a feed starvation shock load of 2 days (Le-Minh, 2011).

A close link between the application of nitrification and effective removal of EDCs in AS and MBR treatment processes has been observed in previous studies (Kim et al.,

2007a, Yi and Harper, 2007, Pholchan et al., 2008, Cajthaml et al., 2009, De Gusseme et al., 2009, Forrez et al., 2009, Silva et al., 2012). When nitrification is inhibited artificially by adding inhibitors such as allylthiourea (ATU) or Hg_2SO_4, degradation of EDCs was reported to be considerably reduced (Kim et al., 2007a, Yi and Harper, 2007, Pholchan et al., 2008, Cajthaml et al., 2009, De Gusseme et al., 2009, Forrez et al., 2009, Silva et al., 2012). Nitrification is often inhibited under high organic shock loads (Esfandi and Kincannon, 1981, Ohashi et al., 1995).

2.4. CONCLUSIONS

Reported studies on impacts of hazardous events including organic shock, starvation, salinity shock, ammonia shock, pH shock and other toxic shock loads on removal of bulk organic matter and nutrients by AS date back a number of decades, revealing a lack of recent attention to this topic. Accordingly, few studies have reported investigations focusing on the more contemporary concerns of the fate of trace chemical contaminants. Similarly, there has been limited studies on impacts of hazardous events on removal of bulk organic matter and nutrients by MBRs, for which common use is a relatively recent development compared to conventional AS systems. The literature regarding the impact of physical membrane damage and loss of aeration on MBR process performance was very limited.

As literature on impacts of hazardous events on MBRs is very limited, the results of hazardous event studies on AS systems can be used to anticipate impacts on MBRs based on logical interpretation of the operational mechanisms of AS and MBR systems and comparative differences between these types of treatment processes. The impacts of organic, pH, ammonia, salinity and other toxicity shocks and loss of aeration on MBRs may be expected to be less pronounced than for AS because the biological degradation process is similar for AS and MBRs but MBRs have higher MLSS concentrations and extra membrane barriers.

Overall, the topic of hazardous events and their impacts on the performance of MBRs is a sparsely investigated field. In particular, very little attention has been paid to the assessment of likelihoods or frequencies of specific hazardous events. These are all important gaps in the knowledge required to fully understand and characterise the operational robustness of these systems. Further work in this area is crucial to improve current practices of performance validation, risk assessment and management of MBRs for wastewater treatment.

CHAPTER 3. ANALYTICAL METHODS FOR WATER QUALITY AND MBR OPERATIONAL PARAMETERS

This chapter has been published in part in the following journal paper:

T. Trinh, N. B. Harden, H. M. Coleman, S. J. Khan, Simultaneous determination of estrogenic and androgenic hormones in water by isotope dilution gas chromatography-tandem mass spectrometry, **Journal of Chromatography A**, 2011, 1218, pages 1668-1676.

3.1. INTRODUCTION

In this chapter, the analytical methods for 48 trace organic chemical contaminants of concern are described. These trace chemicals were selected in this study considering the following factors: their potential adverse impacts to human health and the environment, their high annual consumption in Australia (Khan and Ongerth, 2004), their diversity in terms of physio-chemical characteristics (e.g. neutral, acidic, ionic, hydrophobic and hydrophilic) and their perceived potential to be used as effective indicator chemicals for MBR performance. In addition, the analytical methods for key bulk water quality and MBR operational parameters are also presented. These parameters are analysed to provide a clear picture of the performance of the MBRs used in this study.

Among various analytical techniques for determining trace organic chemical contaminants, chromatographic-mass spectrometric methods are considered a standard technique due to its high selectivity, specificity and accuracy. In this chapter, a simple, reliable and sensitive gas chromatography tandem mass spectrometry (GC-MS/MS) method was developed for the simultaneous determination of the most common 7 steroidal estrogens (17α-estradiol, 17β-estradiol, estrone, estriol, 17α-ethynylestradiol, levonorgestrel, mestranol) and 5 androgens (testosterone, etiocholanolone, androstenedione, androsterone, dihydrotestosterone) in aqueous environmental matrices. The details of this method are presented and discussed in section 3.2. The pharmaceutical and personal care products (PPCPs), xenoestrogens and pesticides of interest in this study are analysed by a liquid chromatography tandem mass spectrometry (LC-MS/MS) method adapted from Vanderford and Snyder (2006). The details of this LC-MS/MS method are presented in section 3.3. The analytical methods for key bulk water quality and MBR operational parameters are also described in section 3.4.

3.2. DEVELOPMENT OF A GC-MS/MS METHOD FOR ANALYSIS OF STEROIDAL HORMONES

This section presents the development of a GC-MS/MS method for analysis of steroidal hormones. The hormones in the method including the most common 7 steroidal estrogens (17α-estradiol, 17β-estradiol, estrone, estriol, 17α-ethynylestradiol, levonorgestrel, mestranol) and 5 androgens (testosterone, etiocholanolone,

androstenedione, androsterone, dihydrotestosterone). The sources of these hormones are presented in Table 3.1.

Table 3.1 Sources of steroidal hormones

Hormones	Sources
Estrogens	
17α-Estradiol	Natural estrogen excreted by human and animal
17β-Estradiol	Natural estrogen excreted by human and animal
Estrone	Metabolite of estradiol
Estriol	Metabolite of estradiol and estrone
17α-Ethynylestradiol	Main component of contraceptive pill
Mestranol	Main component of contraceptive pill
Levonorgestrel	Main component of contraceptive pill
Androgens	
Androstenedione	Natural androgens excreted by human and animal
Testosterone	Natural androgens excreted by human and animal
Dihydrotestosterone	Metabolites of testosterone
Etiocholanolone	Metabolites of testosterone
Androsterone	Metabolites of testosterone

3.2.1. Materials

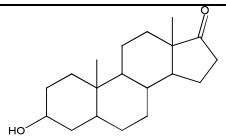
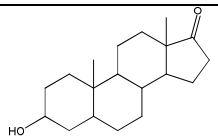
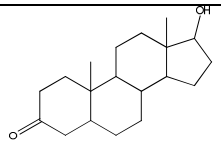
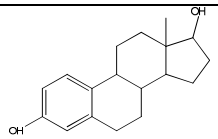
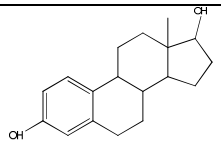
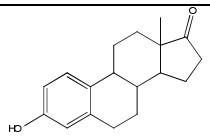
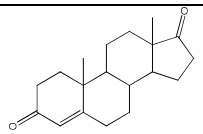
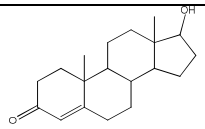
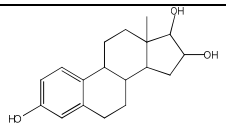
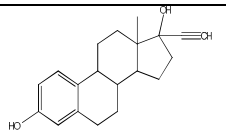
17α-estradiol, 17β-estradiol, estrone, estriol, 17α-ethynylestradiol, levonorgestrel, mestranol, testosterone, etiocholanolone, androstenedione, androsterone, dihydrotestosterone, pyridine and 99% N, O-bis(trimethylsilyl)trifluoro-acetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (all analytical grade), Whatman glass fibre filters and filtering system were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). D3-estriol, D3-dihydrotestosterone, D2-testosterone, D4-17α-ethynylestradiol, D4-estrone, D4-17β-estradiol, D2-etiocholanolone were purchased from CDN isotopes Inc., Canada and D3-androstenedione was purchased from National Measurement Institute, Australia.

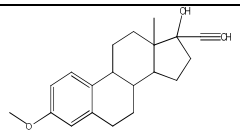
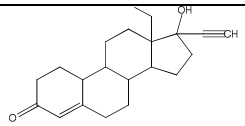
Acetonitrile and methanol (anhydrous spectroscopy grade) were purchased from Ajax Finechem (Tarron Point, NSW, Australia). Ultrapure water was produced using a Driec-Q filtering system from Millipore (North Ryde, NSW, Australia). Kimble culture tubes (13 mm I.D. x 100 mm) and a Thermo Speedvac concentrator (model No. SPD121P)

were purchased from Biolab (Clayton, VIC, Australia). Oasis hydrophilic lipophilic balance (HLB) solid phase extraction cartridges (6 mL, 500 mg) were purchased from Waters (Rydalmere, NSW, Australia).

Stock standard solutions of steroidal hormones and isotope labelled steroidal hormones were initially prepared in acetronitrile (500 $mg.L^{-1}$, 20 mL) in amber vials and then further serial diluted with acetonitrile to obtain working standard solutions of lower concentrations. All standard solutions were stored at -18^{o}C and prepared freshly every three months. Working solutions of steroidal hormones and isotope labelled steroidal hormones at lower concentrations were stored at 4^{o}C and freshly prepared from concentrated stock standards monthly. Chemical structures of target analytes and their isotope labelled standards used in this study are presented in Table 3.2.

Table 3.2 Chemical structures of target analytes and their corresponding isotope labelled standards

Target analytes (Corresponding isotope labelled standards)	**MW of target analytes (MW of corresponding isotope labelled standards)**	**Structure of target analytes**
Androsterone (16,16-D2-Etiocholanolone)	290.4 (292.5)	
Etiocholanolone (16,16-D2-Etiocholanolone)	290.4 (292.5)	
Dihydrotestosterone (16,16,17-D3-Dihydrotestosterone)	290.4 (293.5)	
17α-Estradiol (2,4,16,16-D4-17β-Estradiol)	272.4 (276.4)	
17 β-Estradiol (2,4,16,16-D4-17β-Estradiol)	272.4 (276.4)	
Estrone (2,4,16,16-D4-Estrone)	270.4 (274.4)	
Androstenedione (19,19,19-D3-Androstenedione)	286.4 (289.4)	
Testosterone (1,2-D2-Testosterone)	288.4 (290.4)	
Estriol (2,4,17-D3-Estriol)	288.4 (291.4)	
17α-Ethynylestradiol (2,4,16,16-D4-17α-Ethynylestradiol)	296.4 (300.4)	

Target analytes (Corresponding isotope labelled standards)	MW of target analytes (MW of corresponding isotope labelled standards)	Structure of target analytes
Mestranol (2,4,16,16-D4-17α-Ethynylestradiol)	310.4 (300.4)	
Levonorgestrel (2,4,16,16-D4-17α-Ethynylestradiol)	312.5 (300.4)	

3.2.2. Sample preparation

Sample collection

All samples were collected in 500 mL amber glass bottles. Ultrapure water was produced using a Driec-Q filtering system from Millipore. Drinking water was collected from a regular potable water tap at UNSW. Membrane Bioreactor (MBR) effluent was collected from a laboratory-scale MBR treating a synthetic feed solution. The design characteristics, operational parameters and synthetic feed solution of this MBR have been previously described (Metzger et al., 2007). Surface water was collected from a pond in a large municipal park in Sydney. Tertiary treated effluent was a disinfected final effluent from a municipal wastewater treatment plant in western Sydney. The dissolved organic carbon (DOC) and total suspended solids (TSS) of each of these water matrices are presented in Table 3.3.

Table 3.3 DOC and TSS of water matrices used for method validation

Matrices	DOC ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Ultrapure water	0.1	not applicable
Drinking water	2	not applicable
MBR effluent	8	not applicable
Surface water	16	14
Tertiary treated effluent	15	3

Samples were spiked with stock solutions of all analytes for method recovery and detection level determination. The target concentrations of analytes were dependent on the specific experiments as described in the method validation studies (Section 3.2.5)

below. All samples were then further spiked with isotopically labelled standards for accurate isotope dilution quantification. The target concentrations of the isotope standards were selected to be within an order of magnitude of the spiked analyte concentrations.

Spiked ultrapure water, drinking water and synthetic MBR effluents were extracted without any further treatment or processing. Surface water samples and tertiary treated effluent samples were filtered by 0.75 µm Whatman filter paper prior to extraction. All samples were extracted within 24 hours of collection and spiking.

Solid phase extraction (SPE)

The Oasis HLB SPE cartridges were pre-conditioned prior to extraction with methanol (5 mL), followed by ultrapure water (5 mL). SPE cartridges were loaded by drawing through 500 mL of the aqueous samples under vacuum, maintaining a consistent loading flow rate of less than 5 $mL.min^{-1}$. The SPE cartridges were rinsed with 10 mL of ultrapure water before drying by passing through a flow of nitrogen gas until visibly dry (approximately 1 hour). If required, dried cartridges were stored at -18^{o}C prior to elution and quantitative analysis. Analytes were eluted from the cartridges with methanol (2 x 5 mL) into Kimble culture tubes. The extracts were centrifugally evaporated under vacuum at 35^{o}C using a Thermo Speedvac (Biolab) concentrator. The concentrator was set to an 'auto vacuum' run, with a final pressure of 0.5 Torr. This evaporation process took approximately 1-4 hours, depending on the number of samples and the types of matrices (a maximum of 32 samples can be dried in a single batch). The evaporated samples were reconstituted with anhydrous acetonitrile (1 mL) and transferred to amber GC autosampler vials and dried under a gentle nitrogen stream until visibly dry (approximately 3-15 minutes depending on the types of matrices) .

Trimethylsilyl derivatisation

In preparation for GC-MS/MS analysis, all samples underwent chemical derivatisation. 50 µL of BSTFA (99%)-TCMS (1%), 50 µL of pyridine and 400 µL of acetonitrile (anhydrous grade) were added to the dried samples, then the vials were sealed and heated at 60^{0}C for 30 minutes. The derivatised samples were then allowed to cool to room temperature.

It should be noted that this derivatisation process is sensitive to the presence of any moisture. Accordingly, it is important to ensure that the samples are fully dried (as described in the previous section) before the addition of the derivatising reagents and

anhydrous acetonitrile. Furthermore, the smallest commercially available bottles of pyridine (100 mL) and anhydrous acetonitrile (100 mL) were used to avoid long storage times of these moderately hygroscopic solvents. Similarly, the mixed derivatising reagent was purchased in 1mL packs and used only on the same day that they were opened.

3.2.3. Gas chromatography-tandem mass spectrometry

Samples were analysed on an Agilent 7890A gas chromatograph (GC) coupled with an Agilent 7000B triple quadrupole mass spectrometer (MS/MS). The GC injection port was operated in splitless mode. The inlet temperature and the gas chromatograph mass spectrometer (GC/MS) interface temperature were maintained at 250˚C. An injection volume of 1µL was used. The inlet was used in splitless mode with a purge time of 1.5 minutes. Analytes were separated on an Agilent HP5-MS (30 m x 250 µm x 0.25 µm) column using a 0.8 $mL.min^{-1}$ helium flow. The GC oven temperature was initiated at 130˚C and held for 0.5 minute, then increased by 40˚$C.min^{-1}$ to 240˚C, and increased by 5˚$C.min^{-1}$ to 280˚C and held at 280˚C for 3.75 minutes. The total run time was 15 minutes.

Mass spectrometric ionisation was undertaken in electron impact (EI) ionisation mode with an EI voltage of 70 eV and a source temperature of 280˚C. The triple quadrupole MS detector was operated in multiple reaction monitoring (MRM) mode with the gain set to 100 for all analytes. In order to identify the most suitable transitions for MRM, analytical standards were initially analysed in scan mode to identify suitable precursor ions in MS1 with a scan range of *m/z* 30 to *m/z* M+10 (where M is the derivatised mass of the compound of interest). Fragmentation of the precursor ions in the collision cell was assessed by performing a product ion scan using the same mass range and scan time. All samples were run with a solvent delay of 5 minutes and the analytes were separated into 3 discrete time segments for MRM monitoring with dwell times ranging from 3 to 25 ms, depending on the time segment, to achieve 10-20 cycles across each peak for good quantification. All ions were monitored at wide resolution (1.2 amu at half height).

The ion transitions monitored for all analytes and isotope standards, as well as the specific dwell times and collision energies for the method are presented in Table 3.4. The first MRM transition shown for each molecule was used for quantification, while the second transition shown was monitored only for confirmation of molecular identification.

Table 3.4 Optimal analyte dependent parameters for tandem mass spectrometry

Segment start time	Analytes and isotope labelled standards	MRM transitions	Retention time (min)	Dwell time (ms)	Optimum collision energy (V)
7.00 min	Androsterone	347.2→ 271.2	8.58	25	6
		347.2→ 175.1		25	8
	Etiocholanolone	347.2→ 271.2	8.70	25	6
		347.2→ 175.1		25	8
	D2-Etiocholanolone	349.2→ 273.3	8.68	25	6
		349.2→ 175.0		25	8
9.20 min	Dihydrotestosterone	347.2→ 213.2	9.70	3	10
		347.2→ 271.2		3	10
	D3-Dihydrotestosterone	350.1→ 215.1	9.67	3	10
		350.1→ 273.2		3	10
	17α-Estradiol	416.0→ 285.1	9.79	3	10
		416.0→ 326.2		3	5
	17ß-Estradiol	416.0→ 285.1	10.25	3	10
		416.0→ 326.2		3	5
	D4-17ß-Estradiol	420.0→ 287.2	10.23	3	10
		420.0→ 330.3		3	5
	Estrone	342.1→ 257.1	9.82	3	15
		342.1→ 243.9		3	15
	D4-Estrone	346.3→ 261.2	9.79	3	15
		346.3→ 246.2		3	15
	Androstenedione	286.1→ 109.1	10.10	3	5
		286.1→ 124.1		3	5
	D3-Androstenedione	289.3→ 110.0	10.07	3	5
		289.3→ 127.0		3	5
	Testosterone	360.2→ 174.1	10.41	3	11
		360.2→ 162.1		3	11
	D2-Testosterone	362.1→ 176.1	10.40	3	11
		362.1→ 164.1		3	11
	Mestranol	367.0→ 193.2	10.82	3	17
		367.0→ 173.1		3	17
11.15 min	17α-Ethynylestradiol	425.0→ 193.1	11.45	9	20
		425.0→ 231.2		9	20
	D4-17α-Ethynylestradiol	429.1→ 195.1	11.43	9	20
		429.1→ 233.1		9	20
	Levonorgestrel	355.0→ 167.0	12.13	9	20
		355.0→ 193.0		9	20
	Estriol	504.2→ 324.3	12.58	9	11
		504.2→ 386.3		9	9
	D3-Estriol	507.3→ 327.0	12.55	9	11
		507.3→ 389.4		9	9

A chromatogram showing quantifier peaks of 12 analytes in tertiary treated effluent matrix at a spiking concentration o f 10 ng.L^{-1} is presented in Figure 3.1.

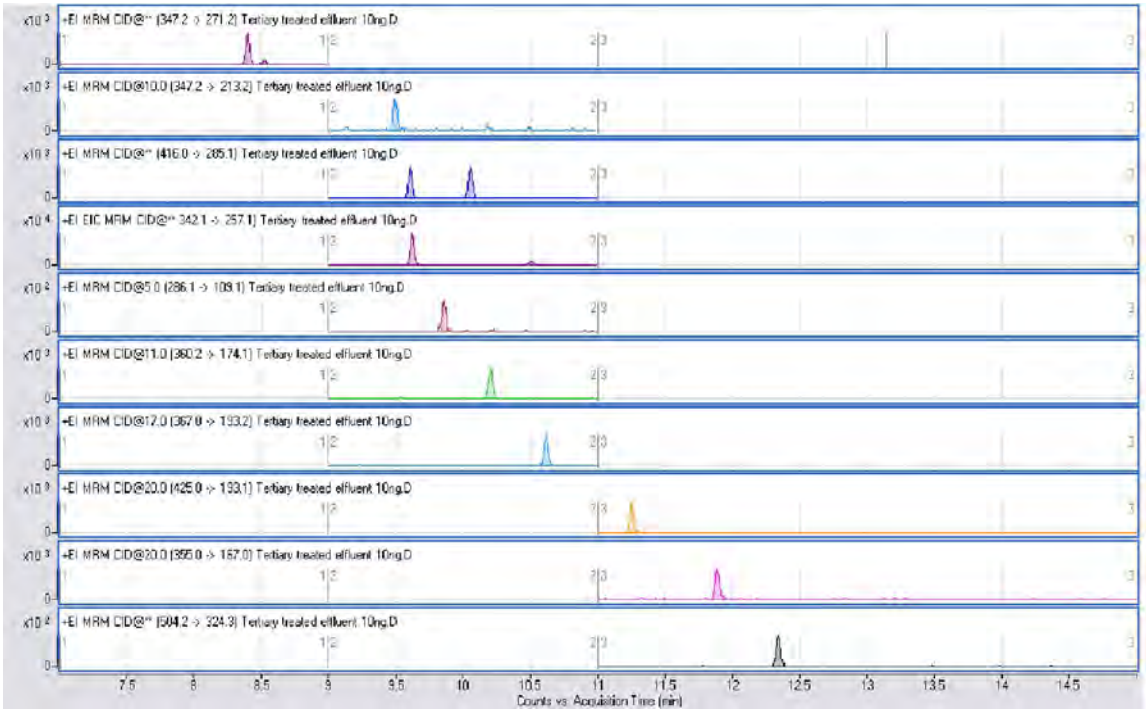


Figure 3.1 A chromatogram showing quantifier peaks of 12 analytes in tertiary treated effluent matrix (on column mass = 10 pg)

3.2.4. Identification and quantification

As described in the previous section, two MRM transitions of a single precursor ion were monitored for each target compound. Analysis of the acquired data was undertaken using Agilent MassHunter software. The confirmed identification of a target compound was only established once the analysis met all of the identification criteria. These included the observed presence of the two expected transitions at the same retention time, the area ratio of two transitions within a range of 20% variability with respect to the mean area ratio of all calibration solutions, and a consistent analyte-surrogate relative retention time as that of calibration solutions with relative standard deviation of less than 0.1 min.

3.2.5. Method validation studies

Isotope labelled compounds were used as surrogate standards to correct for matrix effects, SPE recovery variabilities and instrumental variations for the steroid analytes. Direct analogue isotopic standards were used for etiocholanolone, dihydrotestosterone, 17β-estradiol, estrone, androstenedione, testosterone, estriol and 17α-ethynylestradiol.

However, for four of the target analytes, alternative isotope standards were used based on their structural similarity and confirmed suitability (see Section 3.1.Analyte recovery experiments in Results and Discussion). Accordingly, D2-etiocholanolone was selected for its stereoisomer androsterone, and D4-17β-estradiol was selected for its stereoisomer 17α-estradiol, and D4-17α-ethynylestradiol was selected as the isotopic standard for mestranol and levonorgestrel. Method recoveries of the target analytes were validated in a variety of matrices including ultrapure water, drinking water, synthetic MBR effluent, natural surface water and tertiary treated effluent. The method recoveries of target analytes in various matrices are presented in Table 3.5.

Table 3.5 Method recoveries of analytes in various water matrices from a spiking concentration of 100 ng.L^{-1}, μ (± σ) %

Analytes	Method recoveries				
	Ultrapure water n = 9	Drinking water n= 9	MBR effluent n = 9	Surface water n = 9	Tertiary treated effluent n=3
Androsterone	110 (±10)	104 (±3)	105 (±4)	103 (±6)	114 (±4)
Etiocholanolone	101 (±5)	98 (±3)	97 (±3)	106 (±5)	100 (±7)
Dihydrotestosterone	98 (±5)	97 (±8)	92 (±7)	93 (±7)	95 (±7)
17α-Estradiol	102 (±2)	101 (±2)	102 (±2)	106 (±5)	96 (±4)
Estrone	116 (±5)	100 (±4)	96 (±2)	100 (±4)	96 (±4)
Androstenedione	104 (±3)	105 (±3)	103 (±3)	105 (±7)	104 (±7)
17β-Estradiol	100 (±2)	98 (±2)	94 (±3)	98 (±7)	98 (±6)
Testosterone	101 (±2)	100 (±4)	100 (±3)	104 (±4)	106 (±7)
Mestranol	90 (±15)	90 (±4)	84 (±2)	80 (±10)	86 (±10)
17α-Ethynylestradiol	112 (±5)	88 (±4)	83 (±2)	81 (±5)	90 (±3)
Levonorgestrel	100 (±15)	100 (±8)	99 (±7)	107 (±7)	120 (±10)
Estriol	101 (±3)	92 (±5)	98 (±3)	94 (±5)	96 (±5)

SPE absolute recoveries were assessed using the spiked ultrapure water, surface water and tertiary treated effluent samples at both a high concentration (100 ng.L^{-1}) and a low concentration (10 ng.L^{-1} except for dihydrotestosterone, which was spiked at 20 ng.L^{-1} since it has a method detection limit (MDL) of 15.8 ng.L^{-1}). Because the aim was to assess the loss of the target analytes during SPE extraction, the isotope standards (50 ng) were added to the SPE extracts only after the elution step for direct relative comparison to the analytes. The results of this experiment are presented in Table 3.6.

Table 3.6 SPE absolute recoveries of analytes from low spiking concentration (10 ng.L^{-1}) and high spiking concentration (100 ng.L^{-1}), μ (± σ) %

Analytes	SPE recoveries 100 ng.L^{-1} spiked			SPE recoveries, 10 ng.L^{-1*} spiked	
	Ultrapure water n=3	Surface water n=3	Tertiary treated effluent n=3	Surface water n=3	Tertiary treated effluent n=3
Androsterone	90 (±3)	101 (±1)	106 (±3)	107 (±3)	102 (±3)
Etiocholanolone	87 (±5)	89 (±7)	102 (±3)	106 (±1)	100 (±3)
Dihydrotestosterone	92 (±7)	92 (±7)	100 (±5)	100 (±3)	104 (±5)
17α-Estradiol	97 (±3)	92 (±7)	93 (±1)	98 (±4)	87 (±5)
Estrone	95 (±2)	95 (±6)	99 (±5)	105 (±4)	104 (±4)
Androstenedione	86 (±7)	92 (±7)	97 (±2)	103 (±7)	109 (±7)
17β-Estradiol	95 (±2)	95 (±5)	92 (±2)	98 (±5)	102 (±4)
Testosterone	95 (±6)	96 (±6)	102 (±6)	96 (±7)	97 (±7)
Mestranol	52 (±6)	95 (±2)	96 (±3)	96 (±2)	97 (±5)
17α-Ethynylestradiol	98 (±2)	92 (±5)	99 (±2)	92 (±5)	98 (±6)
Levonorgestrel	68 (±2)	105 (±7)	109 (±6)	104 (±7)	107 (±7)
Oestriol	97 (±3)	98 (±6)	95 (±2)	91 (±7)	93 (±4)

** Except for dihydrotestosterone, which was spiked at 20 ng.L^{-1} since it has an MDL of 15.8 ng.L^{-1}*

To assess potential analyte losses occurring specifically during the drying by Speedvac concentrator and reconstitution steps, 3 centrifuge tubes containing 10 mL anhydrous grade methanol were spiked with 100 ng of the target analytes before being vacuum dried for 3 hours and reconstituted in anhydrous acetonitrile. The results of this assessment are presented in Table 3.7. Further potential losses after reconstitution in anhydrous acetonitrile and during drying under nitrogen gas were also assessed with various drying times of 5 minutes, 30 minutes and 1 hour. The results of these assessments are also presented in Table 3.7.

Table 3.7 Recoveries during drying/reconstituting by Speedvac concentrator and drying by nitrogen gas from a spiking concentration of 100 ng.L^{-1}, μ (± σ) %

Analytes	Recoveries during drying & reconstituting by Speedvac concentrator	Recoveries during drying by nitrogen gas		
	Dry 3 h n = 3	Dry 5 min n=3	Dry 30 min n=3	Dry 60 min n=3
Androsterone	99 (±2)	97 (±4)	100 (±2)	90 (±5)
Etiocholanolone	96 (±5)	97 (±6)	93 (±5)	90 (±7)
Dihydrotestosterone	105 (±3)	110 (±8)	112 (±2)	104 (±9)
17α-Estradiol	99 (±4)	105 (±3)	108 (±3)	107 (±5)
Estrone	97 (±2)	100 (±4)	110 (±5)	106 (±3)
Androstenedione	100 (±7)	95 (±5)	103 (±9)	105 (±7)
17β-Estradiol	100 (±2)	106 (±4)	104 (±4)	106 (±3)
Testosterone	107 (±9)	106 (±9)	107 (±8)	108 (±6)
Mestranol	99 (±5)	108 (±5)	110 (±3)	102 (±3)
17α-Ethynylestradiol	100 (±4)	107 (±4)	104 (±3)	101 (±5)
Levonorgestrel	104 (±7)	90 (±10)	91 (±11)	89 (±9)
Estriol	101 (±5)	102 (±3)	109 (±5)	105 (±9)

Finally, the impact of any potential sample volume-specific effects, such as SPE breakthrough, was assessed by extracting larger sample volumes (1 L, 2 L, 3 L and 4 L) of tertiary treated effluent, each spiked with 20 ng of each analyte, and comparing the recoveries.

MDLs were determined in each of the matrices described above according to Method 1030C from Standard method for examination of water and wastewater (APHA, 2005). For each matrix, seven samples of 500 mL were spiked with target analytes at concentrations close to the expected MDLs. The samples were then spiked with isotopic standards, extracted and analysed through all of the above sample processing and data quantification steps. The seven samples were not analysed sequentially, but were divided into two batches and processed independently on different days to better represent day-to-day variability. MDLs were calculated by multiplying the standard deviation of seven replicates by Student's T value of 3.14 (one-side T distribution for

six degrees of freedom at the 99% level of confidence). Where the calculated MDLs were greater than the actual spiked concentration of any target analytes, a further seven replicates spiked with higher concentrations were analysed to calculate revised MDLs for those analytes. Alternatively, where the calculated MDLs were 5 or more times smaller than the actual spiked concentrations, a further seven replicates spiked with lower concentrations were analysed to calculate revised MDLs. This procedure was repeated until MDLs of all target analytes were determined with a signal-to-variability ratio within the bounds of the above criteria. Final MDL values are presented in Table 3.8.

Table 3.8 MDLs of target analytes in various water matrices

Analytes	**MDLs ($ng.L^{-1}$)**				
	Ultrapure water n = 7	**MBR effluent n = 7**	**Drinking water n = 7**	**Surface water n = 7**	**Tertiary treated effluent n=7**
Androsterone	1.0	1.0	1.0	1.2	1.4
Etiocholanolone	5.0	5.0	5.0	5.8	6.4
Dihydrotestosterone	8.9	11.3	15.2	15.8	15.0
17α-Estradiol	0.8	0.9	1.2	0.9	1.0
Estrone	0.7	0.7	0.7	0.7	0.8
Androstenedione	5.0	5.0	5.0	5.0	5.5
17β-Estradiol	1.1	1.3	1.2	1.2	1.3
Testosterone	5.0	5.0	5.0	5.0	6.0
Mestranol	1.0	1.3	1.0	1.0	1.2
17α-Ethynylestradiol	1.0	1.0	1.0	1.3	1.2
Levonorgestrel	5.0	6.0	7.5	5.0	7.0
Estriol	2.5	2.5	2.5	2.6	3.0

Note: injection volume is 1 µL, thus 1 $ng.L^{-1}$ is equal to 1 pg on column mass

Instrument stability was assessed on an intra-day and inter-day basis by injecting a standard solution containing all analytes (100 ng.mL^{-1}) onto the column three times per day over two separate days and comparing the variation in the signal intensity of each analyte standard from these injections. This variation was expressed at the coefficient of variation (C_v) determined as the ratio of the standard deviation (σ) to the mean (μ). The absolute stability of the whole method for measuring surface water and tertiary treated effluent samples was also assessed by processing three samples of each matrix at various times within a day and three additional samples for each matrix on a different day. The coefficients of variation for these samples are presented in Table 3.9. Note that the instrument stability calculation does not include correction by isotope dilution, but the method stability does.

Table 3.9 Coefficient of variation C_v = σ/ μ for instrument stability and method stability of target analytes in various water matrices.

Analytes	**Instrument stability[1]**		**Method stability[2]**			
	Standard 100 ng.mL^{-1}		**Surface water 100 ng.L^{-1}**		**Tertiary treated effluent 100 ng.L^{-1}**	
	Intra-day n = 3	**Inter-day n = 6**	**Intra-day n = 3**	**Inter-day n = 6**	**Intra-day n = 3**	**Inter-day n = 6**
Androsterone	0.08	0.11	0.06	0.07	0.05	0.07
Etiocholanolone	0.03	0.12	0.04	0.04	0.04	0.04
Dihydrotestosterone	0.08	0.10	0.04	0.07	0.02	0.02
17α-Estradiol	0.02	0.08	0.03	0.02	0.02	0.05
Estrone	0.04	0.10	0.01	0.06	0.01	0.06
Androstenedione	0.10	0.12	0.02	0.07	0.01	0.07
17β-Estradiol	0.02	0.09	0.02	0.04	0.04	0.05
Testosterone	0.06	0.11	0.02	0.05	0.02	0.05
Mestranol	0.05	0.12	0.03	0.06	0.06	0.10
17α-Ethynylestradiol	0.04	0.12	0.01	0.06	0.03	0.04
Levonorgestrel	0.07	0.13	0.05	0.09	0.05	0.10
Estriol	0.05	0.10	0.01	0.05	0.01	0.05

[1] *Instrument stability not corrected by isotope dilution*

[2] *Method stability includes correction by isotope dilution*

Matrix assessment was undertaken by spiking all of the target analytes (and isotopic standards) into extracted and reconstituted surface water and tertiary treated effluent matrix samples. These spiked matrix samples were then derivatised and analysed by the GC-MS/MS. The absolute signal of each analyte was compared to a standard solution (prepared in acetonitrile) of the same concentration in order to calculate a percentage signal enhancement or suppression. The mean values and standard deviations for triplicate samples are presented in Table 3.10. Note that these experiments did not include correction of measured ion intensities by isotope dilution.

Table 3.10 Signal enhancement/suppression in surface water and tertiary treated effluent matrices from a spiking concentration of 20 ng.L^{-1}, μ (±σ) %

Analytes	**Surface water matrix n=3**	**Tertiary treated effluent matrix n=3**
Androsterone	-4 (±9)	-13 (±7)
Etiocholanolone	-18 (±10)	-25 (±8)
Dihydrotestosterone	+9 (±9)	+15 (±2)
17α-Estradiol	-5 (±10)	-8 (±8)
Estrone	-8 (±6)	-1 (±5)
Androstenedione	-10 (±9)	+19 (±10)
17β-Estradiol	-8 (±10)	-7 (±9)
Testosterone	+15 (±11)	+24 (±10)
Mestranol	-3 (±9)	+5 (±9)
17α-Ethynylestradiol	-5 (±7)	+12 (±7)
Levonorgestrel	-9 (±10)	+11 (±10)
Estriol	-1 (±7)	+9 (±9)

Quantitative determination of the target analytes was undertaken using external calibration principles combined with the isotope dilution technique. Calibration curves were comprised of at least 5 points out of nine calibration points for the non-labelled standards (1.0, 2.5, 5.0, 10, 25, 50, 100, 250, 500 ng.mL^{-1} in GC autosampler vials). The lowest calibration point used for each analyte was that corresponding to the lowest concentration above the analyte-specific MDL as shown in Table 3.8. Isotope standards were added to all calibration solutions in a mass equivalent to the mass of isotope standards added to the samples to be analysed.

3.2.6. Analyte recovery

The calculated method recoveries of the target compounds in ultrapure water, drinking water, synthetic MBR effluent, surface water and tertiary treated effluent matrices are shown in Table 3.5. It was observed that the use of isotope dilution satisfactorily corrected for any loss during sample processing, matrix effects and instrument variation leading to accurate quantification in all tested matrices. D2-etiocholanolone and D4-17β-estradiol were confirmed to be suitable isotope standards for the quantification of their stereoisomers androsterone and 17α-estradiol, respectively, with method recoveries in all tested matrices between 96-114% (max σ = 10%). Similarly, D4-17α-ethynylestradiol was confirmed to be a reasonable isotopic standard for quantification of mestranol and levonorgestrel with method recoveries from 80% to 120% (max σ = 15%).

The results of SPE absolute recoveries of the target compounds from low concentration (10 $ng.L^{-1}$) and high concentration (100 $ng.L^{-1}$) spiking tests are presented in Table 3.5. In surface water and tertiary treated effluent matrices, the absolute SPE recoveries ranged from 89% to 109% when spiked at 100 $ng.L^{-1}$ and from 87% to 109% when spiked at 10 $ng.L^{-1}$. Interestingly, the absolute recoveries from ultrapure water spiked at 100 $ng.L^{-1}$, were somewhat lower (52% to 97%) suggesting that dissolved organic carbon in the matrix may enhance the SPE recovery. These results emphasize the importance of isotope dilution for SPE recovery correction among diverse matrices.

The mean analyte recoveries from spiked methanol samples after drying by the Speedvac concentrator are shown in Table 3.7. This table also shows the recoveries of the analytes from evaporation of anhydrous acetonitrile samples after evaporation under nitrogen with various drying times (5 min, 30 min and 60 min). The results of these two experiments confirm that negligible losses of all analytes occurred under all of the tested drying conditions.

The results of the recovery experiments from larger sample volumes of tertiary treated effluent (not shown) indicate that recovery efficiencies for all analytes were not detrimentally affected for sample volumes up to 1 L. This suggests that the MDLs may be driven somewhat lower by the use of 1 L samples instead of 0.5 L samples in some circumstances. However, recoveries of most of the analytes were diminished by up to 50 % for sample volumes of 2 L or greater.

3.2.7. Method detection levels

The MDLs in the different water matrices are presented in Table 3.8. These results show that in ultrapure water, drinking water, synthetic MBR effluent, surface water and tertiary treated effluent, MDLs typically ranged between 1-5 $ng.L^{-1}$. However, slightly higher MDLs were observed for etiocholanolone (up to 6.4 $ng.L^{-1}$), androstenedione (up to 5.5 $ng.L^{-1}$), testosterone (up to 6.0 $ng.L^{-1}$), levonorgestel (up to 7.5 $ng.L^{-1}$) and dihydrotestosterone (up to 15.8 $ng.L^{-1}$) in some aqueous matrices. Numerous previous studies have reported the presence of estrogenic hormones in effluents of sewage treatment plants at concentrations of 1-70 $ng.L^{-1}$ (Ternes et al., 1999, Kolodziej et al., 2003, Quintana et al., 2004). Furthermore, estrogenic hormones have been reported at up to 6 $ng.L^{-1}$ in impacted surface waters (Belfroid et al., 1999). Much fewer data are available for androgenic hormones, but some have been reported in surface water at concentrations up to 12 $ng.L^{-1}$ (Vanderford et al., 2003).

Dihydrotestosterone was the least sensitive target compound with generally higher MDLs in ultrapure water (8.9 $ng.L^{-1}$), synthetic MBR effluent (11.3 $ng.L^{-1}$), drinking water (15.2 $ng.L^{-1}$), surface water (15.8 $ng.L^{-1}$) and tertiary treated effluent (15.0 $ng.L^{-1}$). However, these elevated MDLs were the consequence of a decision to base the quantification of this analyte on the most specific (but not most intense) ion transition at m/z 347.2 → m/z 213.2. This decision was made in order to facilitate the clear distinction of dihydrotestosterone from androsterone and etiocholanolone. If required, reduced MDLs for dihydrotestosterone can be achieved by alternatively basing the quantification on the more intense m/z 347.2 → m/z 271.2 transition.

The fact that the MDLs were not significantly reduced from ultrapure water to more complex matrices highlights an important advantage of this method compared to HPLC-MS (or HPLC-MS/MS) methods incorporating electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI).

Some previous studies have quoted lower detection limits for some of the analytes presented in this paper. While the approach taken to determine these detection limits has been variable (and often not explicitly stated), the most common procedure has been to identify an analyte concentration for which a signal-to-noise ratio (S/N) of 3 can be obtained. The concentration obtained by this approach is most correctly termed the 'lower level of detection' (LLD) or the 'level of detection' (LOD) (APHA, 2005). This approach is intended to set the probability of both false positives and false negatives at 5%. However, the LLD method is not well suited to GC-MS/MS analysis since it is

commonly not possible to observe any 'noise' (for example, see Figure 3.1). A more robust (but somewhat more conservative) approach for defining detection limits has been adopted for this study, referred to as the 'method detection level' (MDL). The MDL is used to describe the analyte concentration that, when processed through the complete method, produces a signal with a 99% probability that it is different from the blank (APHA, 2005).

The better sensitivity of the estrogens compared to the androgens is assumed to be largely due to differences in EI fragmentation at 70 eV. Fragmentation of estrogens generally resulted in the production of around 6-10 highly stable ion fragments (as observed in full scan mass spectra, not shown). However, the androgens were typically fragmented into a much larger number of ion fragments, thus the overall signal was distributed (or diluted) between a larger number of *m/z* values. The stable ion formation for many of the estrogens (with the exception of mestranol) may be partially due to the TMS-derivatised phenol group, which the androgens lack.

3.2.8. Instrument stability, matrix effects and calibration range

The results of instrument and method stability assessments are presented in Table 3.9. The coefficients of variability ($C_v = \sigma/\mu$) for instrument variability on an intra-day basis ranged from 0.02-0.10. Slightly greater coefficients of variability for instrument variability were observed on an inter-day basis, from 0.08 to 0.13. However, the coefficients of variability for the full method analysis of spiked surface water and tertiary effluent samples, on both an intra-day and inter-day basis were observably lower. These varied from 0.01 to 0.07 for analytes with direct isotope labelled analogue correction and up to 0.10 for analytes with alternative isotope labelled analogue correction. This observation emphasises the importance of the isotope dilution process to ensure a high level of analytical reproducibility.

The results of the signal enhancement/suppression assessment in surface water and tertiary treated effluent matrices are presented in Table 3.10. These data represent the means and standard deviations of three samples assessed in each of the two matrices. Some degree of signal suppression may be evident for a few analytes (eg. etiocholanolone) and enhancement for others (eg. testosterone). However, these results reveal a high degree of variability between samples, thus obscuring any real trends. This variability again reinforces the importance of isotope dilution for accurate quantification in real sample matrices.

Blank (unspiked) matrix samples were run to assess background concentrations of the analytes in ultrapure water, drinking water, synthetic MBR effluent, surface water and tertiary treated effluent. The only observed analyte in these matrix samples was estrone, which was measured in tertiary treated effluent at a concentration of 1 $ng.L^{-1}$. Accordingly, all validation experiments on this matrix were calculated after correcting for a background concentration of 1 $ng.L^{-1}$ estrone.

The linear calibration range for the target compounds was determined to be from their identified MDLs to 500 $ng.L^{-1}$, thus the upper quantification limit is 500 $ng.L^{-1}$ for all analytes. The calibration points for each of the analytes were fitted to linear regressions and the calibration curve regression correlation coefficients were always at least 0.99 for all sample batches.

3.3. LC-MS/MS METHOD FOR ANALYSIS OF XENOESTROGENS, PESTICIDES, PHARMACEUTICALS AND PERSONAL CARE PRODUCTS

The LC-MS/MS method for analysis of PPCPs, xenoestrogens and pesticides of interest in this study was adapted from a method previously developed by a leading research laboratory at Southern Nevada Water Authority (USA) (Vanderford and Snyder, 2006). Target compounds analysed by LC-MS/MS in this study include 36 chemicals. The uses of xenoestrogens, PPCPs and pesticides of concern are summarized in Table 3.11.

Table 3.11 List of xenoestrogens, pesticides, PPCPs of concern and their uses

Group/Chemical	Use
Xenoestrogens	
Bisphenol A	to produce plastic
Nonylphenol	breakdown product of the chemical used in detergents and personal care products
2-Phenylphenol	agriculture fungicide and household disinfectant
Propylparaben	preservative in water-based cosmetics, such as creams, lotions and some bath products
4-tert-Octylphenol	breakdown product of octylphenol ethoxylate used in detergents, emulsifiers, solubilizers, wetting agents and dispersants

Group/Chemical	Use
Pharmaceuticals	
Atenolol	beta-adrenoceptor blocking
Amitriptyline	anti-depressant
Atorvastatin	cholesterol lowering
o-Hydroxyatorvastatin	atorvastatin metabolite
p-Hydroxyatorvastatin	atorvastatin metabolite
Carbamazepine	anti-convulsant and mood stabilizing
Diazepam	anti-anxiety and muscle relaxant
Diclofenac	anti-inflammatory/analgesic
Dilantin	anti-convulsant
Enalapril	treatment of hypertension and some types of chronic heart failure
Gemfibrozil	lipid-lowering
Hydroxyzine	relief of anxiety and tension
Ibuprofen	anti-inflammatory/analgesic
Ketoprofen	anti-inflammatory/analgesic
Meprobamate	anti-anxiety
Metformin	an antidiabetic
Naproxen	anti-inflammatory/analgesic
Omeprazole	treatment of peptic ulcer, gastroesophageal reflux
Paracetamol	anti-inflammatory/analgesic
Risperidone	treat schizophrenia
Simvastatin	lipid-lowering drugs
Simvastatin hydroxy acid	simvastatin metabolite
Sulfamethoxazole	antibiotic
Triamterene	treatment of hypertension and edema
Trimethoprim	antibiotic
Personal care products	
Caffeine	stimulant
DEET	insect repellents
Triclosan	antibacterial agent used in disinfectants, soaps and other household products.
Triclocarban	antibacterial agent used in disinfectants, soaps and other household products.

Group/Chemical	Use
Pesticides	
Atrazine	herbicide
Linuron	herbicide

(Staples et al., 1998, Staples et al., 1999, Vanderford et al., 2003, Tumah, 2005, Vanderford and Snyder, 2006, Regueiro et al., 2009)

3.3.1. Materials

15N13C-paracetamol and D5-diazepam were purchased from Cambridge Isotope Laboratories Inc., USA. D4-sulfamethoxazole, D6-trimethoprim, D5-atorvastatin, D5-p-hydroxyatorvastatin, D5-o-hydroxyatorvastatin, D4-risperidone, D5-enalapril, D6-simvastatin, D6-simvastatin hydroxy acid, D3-triclosan, D5-triamterene, D3-meprobamate and D8-hydroxyzine were purchased from Toronto Research Chemicals Inc., Canada. D6-amitriptyline, D7-atenolol, D5-atrazine, D7-bisphenol A, D9-caffeine, D10-carbamazepine, D4-DEET, D4-diclofenac, D10-dilatin, D6-gemfibrozil, D5-fluoxetine, D5-norfluoxetine, D3-ibuprofen, D3-ketoprofen, D6-linuron, D6-metformin, D3-naproxen, D3-omeprazole and D4-triclocarban were purchased from Dr. Ehrenstorfer GmbH, Germany. Atorvastatin, fluoxetine, norfluoxetine, o-hydroxyatorvastatin, p-hydroxyatorvastatin, risperidone, simvastatin hydroxy acid were purchased from Toronto Research Chemicals Inc., Canada and other analytes were purchased from Sigma Aldrich. Methanol and ammonium acetate was also purchased from Sigma Aldrich.

3.3.2. Sample preparation

The sample preparation procedure for analysis of xenoestrogens, pesticides, PPCPs was similar with that for the steroidal hormones. The aqueous samples were collected, spiked with isotopically labelled standard, underwent SPE, eluted with methanol and reconstituted in acetonitrile. Then the samples was analysed for xenoestrogens, pesticides and PPCPs using isotope dilution LC-MS/MS method before being derivatised and analysed by GC-MS/MS for steroidal hormones.

3.3.3. Liquid chromatography-tandem mass spectrometry

The target xenoestrogens, pesticides, pharmaceuticals and personal care products were analysed by two different LC-MS/MS methods using positive mode electrospray ionisation (ESI+) and negative mode electrospray ionisation (ESI-) following an

adaptation of a previous published method (Vanderford and Snyder, 2006). The analytical instrument includes the Agilent series 1200 liquid chromatography (LC) system coupled with an Applied Biosystems QTrap API 4000 mass spectrometer. LC separation was carried out with a Luna C18, 5 µm, 150 mm x 4.6 mm, 100A column with a security guard cartridge C18, 5 µm, 4mm x 3 mm, 100A (Biolab). Mobile phases were high performance liquid chromatography (HPLC) grade methanol (100%) and ultrapure water with 5mM ammonium acetate.

Direct isotopically labelled analogues were used for 33 compounds included 28 PPCPs (amitriptyline, atenolol, atorvastatin, carbamazepine, diazepam, DEET, diclofenac, dilantin, enalapril, gemfibrozil, hydroxyzine, ibuprofen, ketoprofen, meprobamate, metformin, naproxen, omeprazole, o-hydroxyatorvastatin, p-hydroxyatorvastatin, paracetamol, simvastatin, simvastatin hydroxy acid, sulfamethoxazole, triamterene, triclocarban, triclosan, trimethoprim, risperidone), 2 pesticides (atrazine, linuron), 2 xenoestrogens (bisphenol A, nonylphenol) and caffeine. For other 3 xenoestrogens 4-tert-octylphenol, 2-phenylphenol and propylparaben, no direct isotopically labeled was able to be found, therefore D17-n-octylphenol was used for quantification of 4-tert-octylphenol and D6-bisphenol A was used for quantification of propylparaben and 2-phenylphenol.

3.3.4. Identification and quantification

Similar with GC-MS/MS method, two MRM transitions of a single precursor ion were monitored for each target compound. Analysis of the acquired data was undertaken using Analyst software. The confirmed identification of a target compound was only established once the analysis met all of the identification criteria. These included the observed presence of the two expected transitions at the same retention time, the area ratio of two transitions within a range of 20% variability with respect to the mean area ratio of all calibration solutions, and a consistent analyte-surrogate relative retention time as that of calibration solutions with relative standard deviation of less than 0.1 min.

3.3.5. Limit of quantification

The limit of quantification (LOQ) for analytes by LC-MS/MS method was determined by the second lowest concentration lay in the calibration curve with signal/noise (S/N) ratio > 10. The LOQ of bisphenol A was 10 $ng.mL^{-1}$ due to high background concentration of this chemical. The LOQ for other chemicals varied from 2.5 to 5 $ng.mL^{-1}$. These LOQ

will be converted to ng.L^{-1} according to the extraction volume and presented in coming chapters.

3.4. ANALYTICAL METHODS FOR KEY BULK WATER QUALITY AND MBR OPERATIONAL PARAMETERS

The analytical methods for key bulk water quality and MBR operational parameters including pH, chemical oxygen demand (COD), dissolved organic carbon (DOC), total nitrogen (TN), total phosphorous (TP), turbidity, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), Fluorescence Excitation-Emission Matrix (EEM), mixed liquor capillary suction time (CST) and transmembrane pressure (TMP) are described in this section.

3.4.1. PH

pH was measured by the 5-Star portable pH meter from Thermo Scientific Orion. The pH meter was calibrated every week.

3.4.2. COD

COD was measured by The HACH method 8000 (reactor digestion method). 2 mL of permeate sample (or 0.5 mL of influent sample plus 1.5 mL of ultrapure water for dilution) was added into a COD digestion reagent vial. The COD concentration range of the vial was 0 to 150 mg.L^{-1}. The cap of the vial was replaced tightly and the vial was gentle mixed. The vial was heated for two hours at 150 °C and then cooled to room temperature (about 20 minutes) before analysis by the HACH spectrometer at program number of 430.

3.4.3. DOC and TN

The samples were pre-filtered with a 0.45um Millipore filter paper. 30 mL of each permeate sample (or 10 mL of influent sample plus 20 mL of ultra pure water for dilution) was added to an acid washed DOC vial and was acidified by 70 uL of 2M HCl. The sample was then analysed for total carbon (TC) and inorganic carbon (IC) on a TOC-5000A Analyser (Shimadzu, Australia). The DOC was calculated by difference between TC and IC. The instrument was calibrated prior to each series of analyses using a series of potassium hydrogen phthalate standards and each result was

obtained as a calculated average of three measurements. TN concentrations were also obtained from the TOC-5000A Analyser.

3.4.4. TP

The HACH method 8190 (PhosVer 3 with Acid Persulfate Digestion) was used for TP analysis in this study. 1 mL of sample and 4 mL of ultra pure water (for dilution) was added into a TP vial. Potassium persulfate powder was added into the vial and the vial was heated for 30 min at 150 °C. The vial was cooled to room temperature. 2 mL of NaOH solution was added into the vial and the vial was recorded as a blank. After that, PhosVer 3 sachet was added into the vial. The color of the vial was changed from clear to blue color within 2 minutes. The vial was then measured by the HACH spectrometer at program number of 490.

3.4.5. Turbidity

Turbidity of permeate samples was analysed by 2100N turbidity meter supplied by HACH.

3.4.6. MLSS and MLVSS

MLSS and MLVSS was measured followed the Standard Method for Examination of Water and Wastewater (APHA, 2005). 15 mL of mixed liquor sample was filtered through a pre-weighed (m_0) Millipore glass fibre filter paper. The filter paper was dried at 105°C for at least 1 hour. The filter paper was cool to room temperature in a desiccator and the mass of the paper was determined (m_{105}). MLSS concentration was determined as (m_{105}- m_0)/15 mL. The same filter paper was then put in a 550 °C oven for at least 1 hour. The filter paper was cool to room temperature again in a desiccator and the mass of the paper was determined (m_{550}). MLVSS concentration was determined as (m_{105}- m_{550})/15 mL.

3.4.7. EEM

This method consists of exciting molecules by making them absorb photons and measure the specific radiation emitted when those molecules come back to their stable low energy level. Each molecule has a specific fluorescence spectrum, and consequently this method can allow the identification of molecules within an unknown sample by comparing its Fluorescence Excitation-Emission Matrix with another one of identified molecules. Raw EEM spectra were obtained using a Cary Eclipse

Fluorescence Spectrophotometer (Varian, Australia). EEMs were acquired from samples in 4 mL capacity quartz cuvettes with 1 cm pathlength (Starna, Australia). Sample temperature was controlled at 25°C using a Julabo F34-ME circulating water bath (Julabo Labortechnik GmbH, Germany). Fluorescence EEMs were obtained between excitation wavelengths of 200-400 nm (at 5 nm increments), and emission wavelengths of 280- 500 nm, at a scan speed of 9600 $nm.min^{-1}$, with a photomultiplier tube (PMT) voltage of 800 V.

3.4.8. CST

The CST test determines the rate of water release from the mixed liquor. It provides a quantitative measure (reported in sec), of how readily a mixed liquor releases its water. The results can be used to assess the filterability of the mixed liquor through the membrane. CST was measured by a Triton Type 319 multipurpose CST. The test consisted of placing a mixed liquor sample in a small cylinder on a sheet of chromatography paper. The paper extracted water from the mixed liquor by capillary action. The time required for the water to travel a specified distance was recorded automatically by monitoring the conductivity change occurring at two contact points appropriately spaced and in contact with the chromatography paper. The elapsed time was indicative of the water drainage rate. The CST measurement of each sample was done in duplicate, if the variation between the duplicate was more than 0.4 sec, the measurement was repeated a third time. The result presented was the average of the duplicate or triplicate measurements. In order to compare the CST results of mixed liquor between the 4 MBRs, the CST results were normalised to MLSS concentrations so the unit was changed to $s.gMLSS^{-1}.L$.

3.4.9. TMP

The pressure needed to press water through a membrane is called TMP. TMP is equal to the pressure of the feed minus pressure of the filtrate (permeate). TMP is an important MBR operational parameter that needs to be monitored in MBR treatment processes.

The pressure transducers in the experimental MBRs was set up to measure the pressure of the permeate. When the permeate pump was stopped, the feed pressure was equal to the permeate pressure. Thus, before operating the systems, the permeate pump had been stopped and when the pressure had stabilised, the initial pressure value (P_0) was recorded. The pump was then set to operate at nominal fluxes (J) of 10

$L.m^{-2}.h^{-1}$ and the pressure (P_t) was then recorded online continuously during the experiments. TMP was calculated as follows:

TMP (kPa) = P_0- P_t (Equation 3.1)

3.5. CONCLUSIONS

An analytical method was developed for the simultaneous analysis of 12 natural and synthetic hormones in aqueous matrices. No previous gas chromatography mass spectrometry (GC-MS) method is known that encompasses this full range of estrogenic and androgenic analytes. Furthermore, the use of GC-MS/MS has enabled unambiguous identification and non-interfering quantification of closely eluting chromatographic peaks in a very short analysis time of only 15 minutes. The use of isotope dilution for all analytes ensures the accurate quantification, accounting for analytical variabilities that may be introduced during sampling, extraction, derivatisation, chromatography, ionisation or mass spectrometric detection. Direct isotopically labelled analogues were used for 8 of the 12 hormones. However, satisfactory isotope standards were determined for the remaining 4 hormones, based on structural similarity and observed method recoveries of 80-120% in all sample matrices.

The established MDLs for most steroid hormones were 1-5 $ng.L^{-1}$ in a variety of aqueous matrices. However, slightly higher MDLs were observed for etiocholanolone, androstenedione, testosterone, levonorgesterol and dihydrotestosterone in some aqueous matrices. Sample matrices were observed to have only a minor impact on MDLs indicating that interferences such as ion suppression, which is a common problem for HPLC-MS (or HPLC-MS/MS) methods, did not have a significant impact on sensitivity for this method. The method validation confirmed very good method stability over intra-day and inter-day analyses.

LC-MS/MS method was selected for analysis of PPCPs, xenoestrogens and pesticides of interest in this study due to its availability and high sensitivity. The LC-MS/MS method was adapted from a method previously developed by Vanderford and Snyder (2006).

CHAPTER 4. REMOVALS AND FATE OF TRACE ORGANIC CHEMICAL CONTAMINANTS BY A FULL-SCALE PACKAGE MBR IN WOLUMLA, BEGA VALLEY

This chapter has been published in part in three journal papers and one conference paper below:

T. Trinh, B. van den Akker, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan, Fate of pharmaceuticals during wastewater treatment by a membrane bioreactor, **GWF Wasser Abwasser**, international issue 2011, pages 98-102.

T. Trinh, B. van den Akker, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan, Removal of endocrine disrupting chemicals and microbial indicators by a membrane bioreactor for decentralised water reuse, **Journal of Water Reuse and Desalination**, 2012, 2(2) , pages 67-73.

T. Trinh, B. van den Akker, R. M. Stuetz, H. M. Coleman, P. Le-Clech and S. J. Khan, *Removal of trace organic chemical contaminants by a membrane bioreactor*, ***Water Science and Technology,*** 2012, 66(9), pages 1856-63.

T. Trinh, B. van den Akker, H. M. Coleman, R. M. Stuetz, P. Le-Clech, J. E. Drewes and S. J. Khan, *Fate of endocrine disrupting chemicals during wastewater treatment by a membrane bioreactor*, **proceeding of *OZwater Conference***, 8-10 May 2012 in Sydney.

4.1. INTRODUCTION

MBRs have emerged as an important technology for water recycling as they are capable of transforming wastewater to high quality effluent suitable for various reuse applications (Yang et al., 2009). Recently, interest in the ability of MBRs to eliminate trace organic contaminants such as endocrine disrupting chemicals (EDCs), pesticides, pharmaceuticals and personal care products (PPCPs) has increased – particularly for decentralised systems in regional water reclamation schemes (e.g. direct or indirect potable reuse) (Coleman et al., 2009, Le-Minh et al., 2010). The removal mechanisms for trace organic chemical contaminants through MBRs are complex and include biodegradation/transformation, sorption to biomass, volatilisation and physical retention by the membrane (Stevens-Garmon et al., 2011). Given that the molecular weight cut off for ultra-filtration membranes is about 100-200 kDa, they are not expected to retain trace organic chemicals, unless the chemicals adsorb to larger particles (de Wever et al., 2007). In addition, the low Henry's constant ($H < 10^{-4}$) for the targeted trace chemicals in this study suggests that volatilisation is an insignificant removal mechanism for these compounds (Stevens-Garmon et al., 2011, US EPA, 2011). Biodegradation/transformation and sorption to biomass are therefore the two most important removal pathways for these trace chemicals. Biodegradation/transformation are grouped together since it is often difficult to distinguish between processes of chemically or biologically mediated transformation or degradation processes. This is largely due to current analytical limitations for the analysis of metabolites and other transformation products.

Previous work (Kimura et al., 2005, Kim et al., 2007b, Kantiani et al., 2008, Bo et al., 2009, Le-Minh et al., 2010, Tadkaew et al., 2011) has involved the analysis of trace chemicals in the aqueous phase alone and therefore the removal by transformation/biodegradation or by adsorption to biomass can not be distinguished. To better understand the fate and removal mechanisms of trace chemicals through MBRs, both the aqueous and the solid phases of the MBR need to be investigated.

This chapter presents the results on the removal of 48 trace organic chemical contaminants through a full-scale package MBR plant in Wollumla, Bega Valley, NSW Australia under normal operating conditions. This research is novel because it includes a wide range of studied trace chemical contaminants covering steroidal hormones, xenoestrogens, pesticides, PPCPs. In addition, the investigation was undertaken at a full-scale package MBR plant treating real municipal wastewater and both the aqueous

(influent and effluent) and the biomass samples were analysed. A full mass balance was calculated to estimate the contribution of biodegradation/transformation and sorption to biomass to the overall removals of the trace chemicals by the MBR. Key operation parameters such as pH, dissolved organic carbon (DOC), total nitrogen (TN), total phosphorous (TP) and NH_3 were also analysed to provide information about treatment process performance.

4.2. MATERIALS AND METHODS

4.2.1. Description of the package MBR

Samples were collected from a full-scale package MBR plant (800 equivalent persons) located in Wolumla, Bega Valley, New South Wales, Australia. A schematic diagram of the MBR is presented in Figure 4.1, which summarises the key components, flow direction and sample sites. The treatment process comprises of a fine screen (3 mm), a bioreactor tank, two parallel-submerged membrane modules and a medium pressure ultra-violet (UV) disinfection unit. The sludge retention time (SRT) of the bioreactor was 10-15 days, the hydraulic retention time (HRT) was 1 day and the mixed liquor suspended solids (MLSS) concentration was 7.5 - 8.5 $g.L^{-1}$. The bioreactor tank was intermittently aerated in 10 minute cycles (dissolved oxygen set-point of 1 $mg.L^{-1}$) to achieve simultaneous nitrification and denitrification. The submerged membrane modules were made of hollow fibre membranes (Koch Puron, Stafford, UK) which have an effective pore size of 0.1–0.2 µm and a surface area of 235 m^2 (each). For cleaning, scour air was applied to the membranes using a positive displacement blower and backwashing occurred every 360 seconds for a period of 60 seconds. Chemical backwashing occurred automatically every three weeks, in accordance with the manufacturer's recommendations, to maintain a transmembrane pressure (TMP) of <20 kPa. The membrane unit was designed to achieve an average flux of 25 $L.m^{-2}.h$.

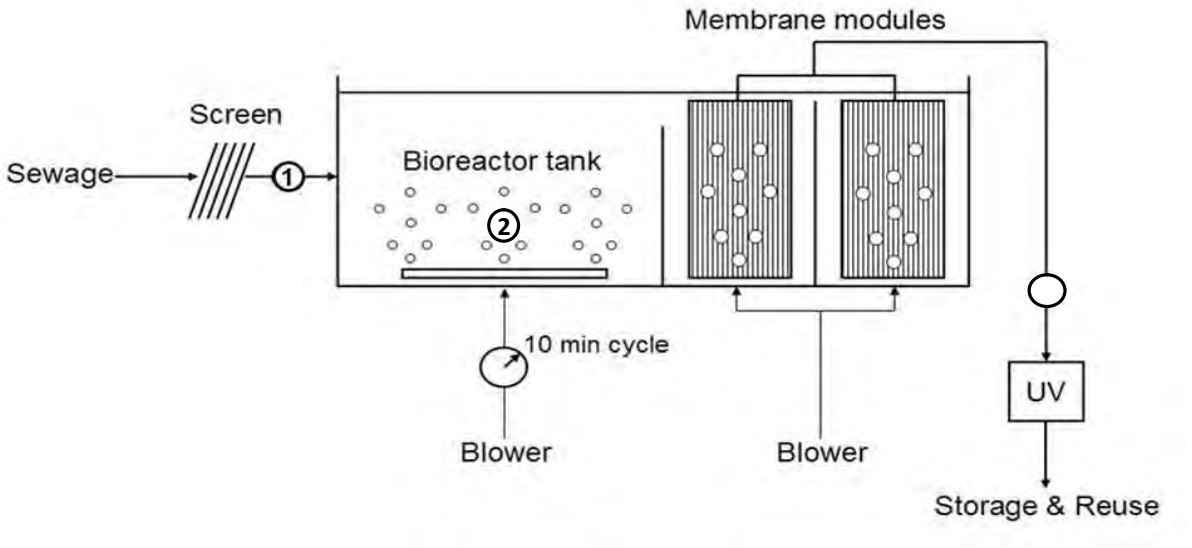


Figure 4.1 Schematic diagram of the full-scale membrane bioreactor summarising the key components, flow directions and sample sites: (1) raw sewage, (2) mixed liquor and (3) permeate

A medium pressure UV disinfection unit was installed after the membrane units to provide an extra barrier for removal of pathogens to ensure that high quality effluent standards are met. All of the final effluent (approximately 40 $L.d^{-1}$) is used for irrigation. The water quality values in raw sewage and MBR permeate are presented in Table 4.1.

Table 4.1 Quality of raw sewage and MBR permeate (mean ± stdev, n=10)

Quality parameters	Raw sewage	MBR permeate
DOC ($mg.L^{-1}$)	132 (± 18)	14 (± 2)
NH_3 ($mg.L^{-1}$)	38 (± 12)	0.1 (± 0.1)
Total N ($mg.L^{-1}$)	76 (± 8)	3 (± 2)
Total P ($mg.L^{-1}$)	Unavailable[1]	4 (± 2)
pH	7.0 (± 0.4)	8 (± 0.4)

[1]*Total P was not measured in raw sewage since the colourimetric method used onsite was not suitable for such coloured samples.*

4.2.2. Sample collection

Daily composite aqueous samples of raw sewage (0.5 L), MBR permeate (1 L) and grab samples of mixed liquor (0.5 L) were taken in triplicates over a 5-day-period in September 2010 (winter sampling) and a 5-day-period in March 2011 (summer sampling), giving a total of 30 raw sewage samples, 30 permeate samples and 30 mixed liquor samples. Temperature of the bioreactor during these sampling events is

presented in Figure 4.2. Winter sampling was undertaken when temperature in the bioreactor was lowest, around 15^0C and summer sampling was undertaken when temperature in the bioreactor was in the highest range, round 25^0C. After collection, the raw sewage was immediately filtered through 0.7 µm Millipore glass fibre prefilters. All aqueous samples were then spiked with isotopically labelled standards of trace chemicals of interest for accurate isotope dilution quantification. The samples were stored in ice and extracted onsite using solid phase extraction (SPE) within 24 hours of collection.

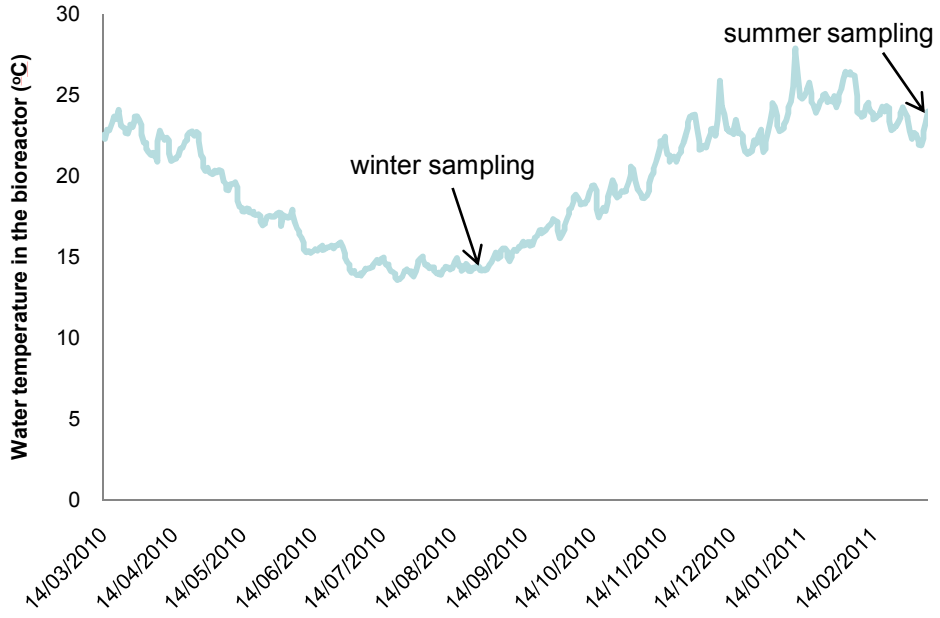


Figure 4.2 Temperature in the bioreactor of the MBR during sampling events

4.2.3. Extraction of biomass

After collection, mixed liquor samples were immediately filtered through 0.7 µm Millipore glass fibre prefilters (Millipore-North Ryde, NSW, Australia). The solid biomass was then stored in a 70 mL plastic container and frozen for at least 1 day. Frozen biomass samples were then freeze dried over a period of up to 4 days. The freeze-dried samples were then subjected to ultrasonic solvent extraction following an adaptation of a method previously reported for extraction of sewage sludge samples (Ternes et al., 2005, Coleman et al., 2009) as outlined below. Freeze dried samples were ground to fine powder using mortar and pestle. Ground samples were then weighed (0.5 g) into 13 mL glass culture tubes. The isotope standard stock solutions (1 $mg.L^{-1}$) were added to the culture tube (100-200 µL). Methanol (5 mL) was then added and the solution thoroughly mixed using a vortex mixer. Each sample was then

ultrasonicated (Unisonics, Australia) for 10 minutes at 40°C followed by gravity settling and decanting of the supernatant. The ultrasonication step was repeated with addition of 5 mL of acetone and the two supernatants combined in an acid-washed 500 mL bottle. The combined supernatant was then diluted with ultrapure water (500 mL) and filtered through 0.7 µm Whatman filter paper in preparation for SPE.

4.2.4. Solid phase extraction

The Oasis HLB SPE cartridges (Waters-Rydalmere, NSW, Australia) were pre-conditioned with methanol (5 mL), followed by ultrapure water (5 mL). SPE cartridges were loaded by drawing through 500 mL (for raw sewage samples) or 1000 mL (for MBR permeate samples) of the aqueous samples under vacuum, maintaining a consistent loading flow rate of less than 10 $mL.min^{-1}$. The SPE cartridges were rinsed with 10 mL of ultrapure water before drying with air for approximately 30 minutes. The dried cartridges were stored at -18°C prior to elution and quantitative analysis. Analytes were eluted from the cartridges with methanol (2 x 5 mL) into Kimble culture tubes. The extracts were centrifugally evaporated under vacuum at 35°C using a Thermo Speedvac (Biolab-Clayton, VIC, Australia) concentrator. The evaporated samples were reconstituted with anhydrous methanol (0.5 mL) and transferred to amber autosampler vials before analysis.

4.2.5. Analysis of trace chemicals

The 48 trace chemicals of interest were analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and gas chromatography tandem mass spectrometry (GC-MS/MS) methods as described in Chapter 3. Take in to account the volume of sample extracted, the limit of quantifications (LOQs) of the trace chemicals in this chapter are presented in Table 4.2 below.

Table 4.2 LOQs of trace chemicals analysed

Chemical	**LOQ**		
	Raw sewage ($ng.L^{-1}$)	**Permeate ($ng.L^{-1}$)**	**Biomass ($ng.g^{-1}$)**
Steroidal hormones			
17α-Estradiol	1.0	0.5	1.0
17β-Estradiol	1.3	0.7	1.3
Estrone	0.8	0.4	0.8
Estriol	3.0	1.5	3.0
17α-Ethynylestradiol	1.2	0.6	1.2
Mestranol	1.2	0.6	1.2
Levonorgestrel	7.0	3.5	7.0
Testosterone	6.0	3.0	6.0
Dihydrotestosterone	15	7.5	15
Etiocholanolone	6.4	3.2	6.4
Androsterone	1.4	0.7	1.4
Androstendione	5.5	2.8	5.5
Xenoestrogens			
Bisphenol A	20	10	20
4-Nonylphenol	5.0	2.5	5.0
2-Phenylphenol	10	5.0	10
Propylparaben	10	5.0	10
4-tert-Octylphenol	10	5.0	10
Pharmaceuticals			
Atenolol	5.0	2.5	5.0
Amitriptyline	10	5.0	10
Atorvastatin	10	5.0	10
o-Hydroxyatorvastatin	10	5.0	10
p-Hydroxyatorvastatin	10	5.0	10
Carbamazepine	5.0	2.5	5.0
Diazepam	5.0	2.5	5.0
Diclofenac	5.0	2.5	5.0
Dilantin	5.0	2.5	5.0
Enalapril	10	5.0	10
Gemfibrozil	5.0	2.5	5.0

Chemical	LOQ		
	Raw sewage ($ng.L^{-1}$)	Permeate ($ng.L^{-1}$)	Biomass ($ng.g^{-1}$)
Hydroxyzine	5.0	2.5	5.0
Ibuprofen	10	5.0	10
Ketoprofen	5.0	2.5	5.0
Meprobamate	5.0	2.5	5.0
Metformin	5.0	2.5	5.0
Naproxen	5.0	2.5	5.0
Omeprazole	5.0	2.5	5.0
Paracetamol	5.0	2.5	5.0
Risperidone	10	5.0	10
Simvastatin	10	5.0	10
Simvastatin hydroxy acid	10	5.0	10
Sulfamethoxazole	5.0	2.5	5.0
Triamterene	5.0	2.5	5.0
Trimethoprim	5.0	2.5	5.0
Personal care products			
Caffeine	10	5.0	10
DEET (N,N-Diethyl-meta-toluamide)	5.0	2.5	5.0
Triclosan	5.0	2.5	5.0
Triclocarban	5.0	2.5	5.0
Pesticides			
Atrazine	5.0	2.5	5.0
Linuron	5.0	2.5	5.0

4.2.6. Mass balance calculation

The concentrations of trace chemicals in raw sewage, MBR permeate and biomass were used together with the aqueous and biomass flow data to establish a mass balance for the fate of each chemical. These mass balances were calculated based on Equation 4.1:

Influent load = effluent load + biomass load + biodegradation load (Equation 4.1)

It is noted that the raw sewage was filtered through 0.7 μm before further analysis and the concentrations of trace chemicals in suspended solid of raw sewage was assumed as negligible. If the concentration of the trace chemical in permeate or in biomass is under the LOQ, the LOQ value is used to calculate the mass balance.

4.3. RESULTS AND DISCUSSION

4.3.1. Concentrations of EDCs in the raw sewage

The concentrations of EDCs, which include the steroidal hormones and xenoestrogens, in the raw sewage are presented in Figure 4.3. The main components of the contraceptives pills (17α-ethynylestradiol, mestranol and levonorgestrel), and the breakdown product of nonylphenol ethoxylates used in detergents and personal care products (4-nonylphenol) were not detected. The natural estrogens that were detected included 17α-estradiol, 17β-estradiol and its metabolised products estrone and estriol. The results show that the androgenic hormones were detected at higher concentrations than estrogenic hormones, which may be due to the higher excretion rates of androgens compared to estrogens in humans (Leusch et al., 2006). Testosterone and its metabolised products, androsterone, etiocholanolone and dihydrotestosterone were all detected. The concentration of testosterone in raw sewage was highly variable between different sampling dates, varying from LOQ< (<6 $ng.L^{-1}$) to 533 $ng.L^{-1}$. In general, the concentrations of steroidal hormones are consistent with previous Australian studies; with the exception of dihydrotestosterone, which were one to two orders of magnitude higher than values reported in the literature (Coleman et al., 2010, Le-Minh et al., 2010). This may be due to higher sensitivity (LOQ = 15 $ng.L^{-1}$) of the analytical method used here compared to other studies (LOQ = 25 – 30 $ng.L^{-1}$) (Coleman et al., 2010, Le-Minh et al., 2010).

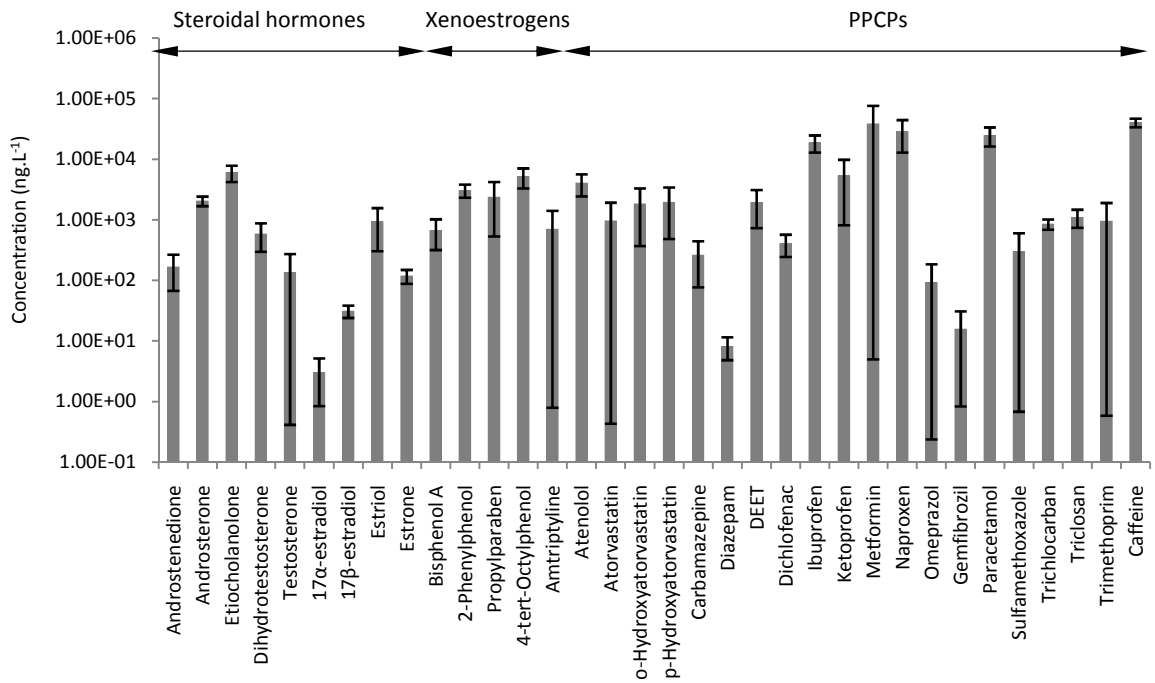


Figure 4.3 Concentrations of trace organic chemical contaminants in the raw sewage

The detected estrogenic phenolic compounds included bisphenol A, 2-phenylphenol and 4-tert-octylphenol. Bisphenol A is used to produce polycarbonate plastic and epoxy resins (Staples et al., 1998) and 2-phenylphenol is used as an agriculture fungicide and household disinfectant (Tumah, 2005) whereas 4-tert-octylphenol is the breakdown product of octylphenol ethoxylate that is widely used in detergents, emulsifiers, solubilizers, wetting agents and dispersants (Staples et al., 1999). The level of bisphenol A detected was comparable to previous studies (Lee et al., 2005, Cases et al., 2011) while the level of 4-tert-octylphenol detected was one order of magnitude higher than values reported in previous research (Coleman et al., 2009, Cases et al., 2011), this may be due to discharge of industrial wastewater into sewage. Previous studies suggest that the higher the industrial fraction of the sewage, the higher the concentration of 4-tert-octylphenol (Clara et al., 2005, Lee et al., 2005). Literature on the level of 2-phenylphenol in raw sewage is limited but a previous study reported similar values to what have been found in this study (Lee et al., 2005).

Propylparaben is a preservative typically found in many water-based cosmetics, such as creams, lotions and some bath products. The concentration of this compound in raw sewage was very variable from 443 to 5260 $ng.L^{-1}$, but in general, the concentration is comparable with previous reported values in the raw sewage (Regueiro et al., 2009).

4.3.2. Concentrations of PPCPs and pesticides in the raw sewage

The concentrations of PPCPs that were detected in raw sewage are shown in Figure 4.3. Of the 36 studied PPCPs and pesticides, 10 were not detected in the raw sewage. These included 8 pharmaceuticals (dilantin, enalapril, hydroxyzine, meprobamate, simvastatin, simvastatin hydroxy acid, triamterene, risperidone) and 2 pesticides (atrazine, linuron). Gemfibrozil was only detected in a single sampling date during the two sampling events at a concentration of 60 $ng.L^{-1}$. Caffeine was found at concentrations of up to 40 $\mu g.L^{-1}$ which was 4 times higher than values reported in raw sewage in the literature (Kim et al., 2007b), indicating high consumption of caffeine in the area. Pharmaceuticals including ibuprofen, metformin, naproxen and paracetamol were all detected in the raw sewage at average concentrations in the range of 18.8 $\mu g.L^{-1}$ - 38.1 $\mu g.L^{-1}$, which was not surprising given that these pharmaceuticals are used extensively in Australia (Khan and Ongerth, 2004). The concentrations of carbamazepine, diclofenac and sulfamethoxazole are consistent with published Australian data while ketoprofen was found to be 5 times higher (Al-Rifai et al., 2007, Le-Minh et al., 2010). The population of the area has a high proportion of old people. The median age of the area is 48 years old which is 10 year older than median age of whole Australia. This feature may contribute to higher consumption of pharmaceuticals in this area compared to other areas. High day-to-day variability in concentrations of some chemicals, including amitriptyline, atorvastatin, omeprazole, sulfamethoxazole and trimethoprim was observed. Such variability may be the expected result for relatively low prescription rate drugs in a very small wastewater catchment (800 equivalent persons).

4.3.3. Removals of EDCs by the MBR

The percentage removals of EDCs through the package MBR relative to the influent load are presented in Figure 4.4. 2-phenylphenol and 4-tert-octylphenol were not analysed in winter sampling as these compounds were added to the analytical method after the winter sampling. 17α-estradiol was not detected in winter sampling so only summer data of this chemical is presented in Figure 4.4. Estradiol is excreted from human mainly in the form of 17β-estradiol while it is excreted from animal in the form of 17α-estradiol (Shore and Shemesh, 2003, Prokai-Tatrai et al., 2010). The present of 17α-estradiol in the raw sewage in the summer sampling may be due to run-off from animal farm to the sewage.

Results from this study show that EDCs including 17α-estradiol, 17β-estradiol, estrone, estriol, dihydrotestosterone, androsterone, etiocholanolone, bisphenol A, propylparaben, 2-phenylphenol and 4-tert-octylphenol were effectively removed by the MBR, with the over removal efficiencies including removal by biodegradation/transformation and adsorb to biomass in the order of 90% – almost 100%. These results are consistent with previous studies on MBRs (Kim et al., 2007b, Spring et al., 2007, Lee et al., 2008, Coleman et al., 2010, Le-Minh et al., 2010). The high removal efficiencies noted here can be attributed to the high SRT and MLSS concentration in the MBR (Clara et al., 2005, Chen et al., 2008b, Coleman et al., 2009).

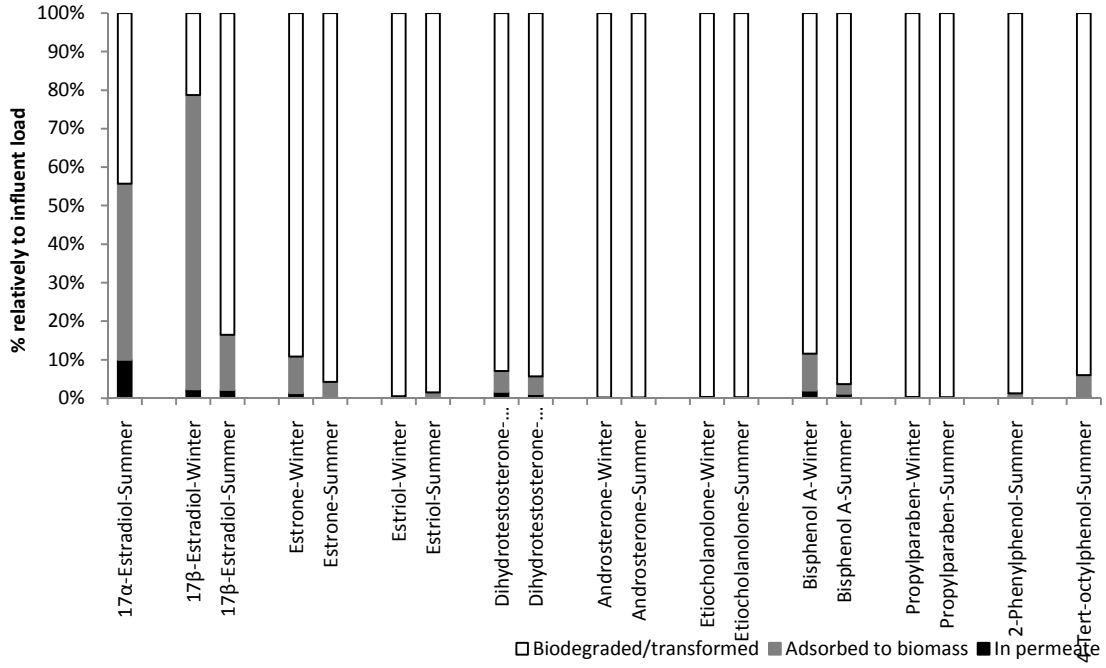


Figure 4.4 Removal of EDCs by the MBR

The percentage removal of trace chemicals by different removal mechanisms (biodegraded/transformed or adsorbed to biomass) relative to the influent load is also presented in Figure 4.4. It is noted that the concentration of 17α-estradiol, estriol, dihydrotestosterone, androsterone and etiocholanolone in biomass was under the LOQs, and the concentration of 17α-estradiol, 17β-estradiol, estriol, dihydrotestosterone, androsterone, etiocholanolone, bisphenol A and propylparaben in permeate was under the LOQs. Thus the LOQs for biomass and permeate samples was used to calculate the mass balance for these chemicals.

Results from Figure 4.4 show that removal via adsorption to biomass was significant for 17α-estradiol and 17β-estradiol. For 17β-estradiol, removal via adsorption to biomass was 76% in winter sampling and 14% in summer sampling. This variation in percentage

of adsorption to biomass of 17β-estradiol may be due to the different temperature between winter and summer sampling. The temperature in the bioreactor in summer was 25°C and 15°C in winter. This higher temperature in the bioreactor in summer may enhance biological degradation process in the reactor and thus the fraction of 17β-estradiol that adsorbed to biomass degrades more quickly and is at lower concentration in the biomass. A previous study also found that degradation of 17β-estradiol in wastewater treatment plants increased significantly when temperature increase by 15°C (Layton et al., 2000). The concentration of 17α-estradiol in the biomass was less than LOQ in the summer sampling so the LOQ was used to calculate the mass balance and the result shows that removal via adsorption to biomass contributed < 46% to the overall removal of this chemical. In the winter sampling, 17α-estradiol in the raw sewage was also below the LOQ so no conclusion about the variation in percentage of adsorption to biomass of 17α-estradiol between summer and winter samplings could be drawn.

Removal via adsorption to biomass was 10% of the overall removal of estrone and bisphenol A. A previous study also identified that both sorption to biomass and biodegradation are important removal mechanisms for bisphenol A (Hu et al., 2007). For the other EDCs - estriol, dihydrotetosterone, androsterone, etiocholanolone, propylparaben, 2-phenylphenol and 4-tert-octylphenol, biodegradation/transformation was a dominant removal mechanism while removal via adsorption to biomass contributed less than 5% to the overall removal. Removal via adsorption to biomass of these compounds can be predicted based on their solid-liquid partition coefficients, which are ≤ 0.5 $L.g^{-1}$ MLSS (Xue et al., 2010, Stevens-Garmon et al., 2011) as a previous study concluded that sorption is an insignificant removal pathway for compounds with Kd ≤ 0.5 $L.g^{-1}$ MLSS (Ternes et al., 2004).

Similar to 17β-estradiol, the percentage removal via adsorption to biomass of other EDCs in general was lower in summer than that in winter. This can be explained by higher biodegradation rate due to higher temperature in the bioreactor in summer (25°C) than that in winter (15°C). The fraction of EDCs that adsorbed to biomass degrades quicker and thus is at lower concentration in the biomass. This finding is consistent with results from a previous study (Layton et al., 2000).

4.3.4. Removals of PPCPs by the MBR

The removal of PPCPs through the MBR is presented in Figure 4.5. Most of PPCPs were effectively removed by the MBR. Overall removal efficiencies of atenolol, amitriptyline, atorvastatin, o-hydroxyatorvastatin, p-hydroxyatorvastatin, DEET, ibuprofen, ketoprofen, metformin, naproxen, paracetamol, sulfamethoxazole, triclosan, triclocarban and caffeine were between 82 – almost 100 %. Previous studies on MBRs have reported similar removal efficiencies for bisphenol A, ibuprofen, triclosan and caffeine (Clara et al., 2005, Kim et al., 2007b, Radjenovic et al., 2007, Coleman et al., 2009, Radjenovic et al., 2009). Studies have shown that atenolol, ibuprofen, naproxen, paracetamol and caffeine are readily biodegradable (Abegglen et al., 2009, Radjenovic et al., 2009) while triclosan can adsorb to biomass (Coleman et al., 2009). The high removal efficiencies noted here can be attributed to the high SRT and MLSS concentration in the MBR (Clara et al., 2005, Chen et al., 2008b, Coleman et al., 2009). Conversely, diazepam and diclofenac were not effectively removed through the MBR with the removal efficiencies varying from 21% to 54%. The overall removal efficiencies of omeprazole and trimethoprim were very variable between winter and summer sampling. The overall removal of omeprazole was 89% in winter sampling and 62% in summer sampling. The overall removal of trimethoprim was 42% in winter sampling and 96% in summer sampling. Diazepam, diclofenac, omeprazole and trimethoprim have also been identified as persistent compounds that are difficult to be removed through MBRs with various removal efficiencies in literature ranging from 0% to 50%. This most likely due to these compounds been not easily biodegraded and poorly adsorb to the biomass (Clara et al., 2005, de Wever et al., 2007, Kim et al., 2007b, Radjenovic et al., 2007, Radjenovic et al., 2009).

Carbamazepine had overall removal of 24% in winter sampling and -94% in summer sampling. Previous studies also found that carbamazepine was persistent to biological degradation (Clara et al., 2004, Joss et al., 2005, Vieno et al., 2007). These studies also found higher concentrations of carbamazepine in treated water than in influent, up to twice as much in the influent (Clara et al., 2004, Joss et al., 2005, Vieno et al., 2007). The negative removal of carbamazepine may be due to the enzymatic cleavage of the glucuronic conjugate of carbamazepine and release of the parent compound during the treatment process (Vieno et al., 2007). The trace organic chemical contaminants that are partially removed through MBRs in normal operating conditions could be used as potential indicators for assessing MBR performance as these

chemicals are usually sensitive to changes in MBR treatment process performance (Drewes et al., 2008).

Results from Figure 4.5 show that removal via adsorption to biomass was significant for amitriptyline, triclosan and triclocarban. Removal via adsorption to biomass contributed 73-86% to the overall removal of amitriptyline, 51-52% to the overall removal of triclosan and 35-40% to the overall removal of triclocarban. Previous studies have also shown that sorption to biomass was a significant removal mechanism for triclosan and triclocarban during wastewater treatment (Heidler et al., 2006, Coleman et al., 2009). A previous study revealed that triclosan is quickly sorbed onto biomass and then, direct biodegradation of sorbed triclosan is achieved (Stasinakis et al., 2007). Adsorption to biomass was a moderate removal mechanism for carbamazepine, diazepam, diclofenac and omeprazole with the percentage removal by adsorption to biomass of 8%, 11-16%, 6-9% and 15-22% for carbamazepine, diazepam, diclofenac and omeprazole, respectively. In contrast, adsorption to biomass was an insignificant removal mechanism for the remaining chemicals which are atorvastatin, *o*-hydroxyatorvastatin, *p*-hydroxyatorvastatin, DEET, ibuprofen, ketoprofen, naproxen, paracetamol and caffeine, with the percentage of removal by adsorption to biomass contributed less than 2% to the overall removal. This result is comparable with previous studies which found that the percentage removal by adsorption to biomass of ibuprofen, ketoprofen, naproxen, sulfamethoxazole and trimethoprim by MBR processes was from 0 to < 6% (Clara et al., 2005, Joss et al., 2005, Göbel et al., 2007, Kimura et al., 2007, Abegglen et al., 2009).

Figure 4.6 presents Log $D_{pH=8}$ versus % removal via adsorption to biomass of the trace chemicals in order to determine if there is a relationship between log $D_{pH=8}$ and percentage removal via adsorption to biomass. Results show that chemicals having log $D_{pH=8}$ < 3.2 were insignificantly removed by adsorption to biomass (<6%). Chemicals that have log $D_{pH=8}$ > 3.2, adsorption to biomass which can be a significant or an insignificant removal mechanism with percentage removal via adsorption to biomass varied from 0.2% to 86%. The varying percentage adsorption to biomass of chemicals with log $D_{pH=8}$ > 3.2 may depend on their biodegradability. Unfortunately, the biodegradability constants of most of these chemicals are not available so no general trend could be concluded.

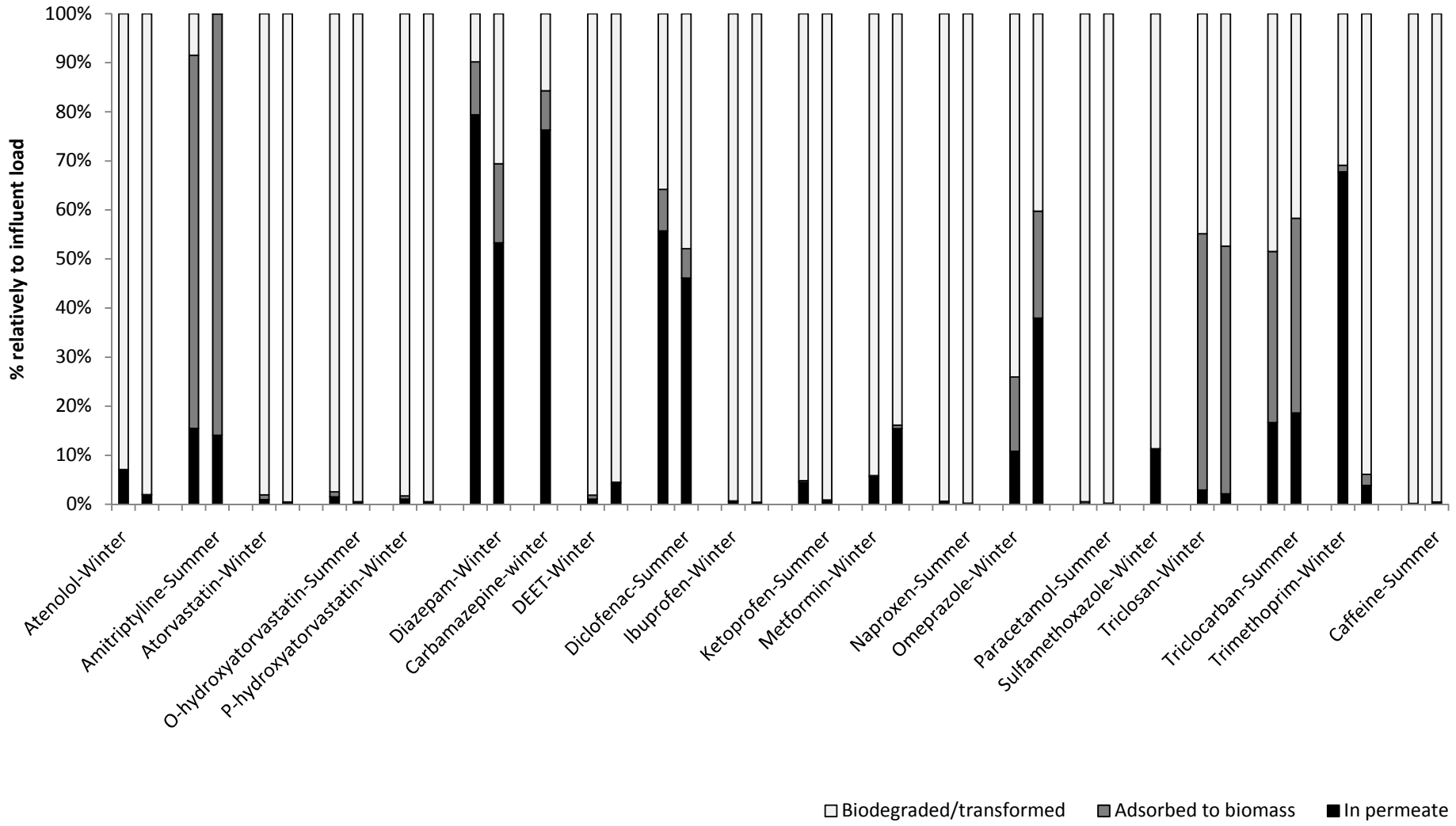


Figure 4.5 Removal of PPCPs and pesticides by the MBR

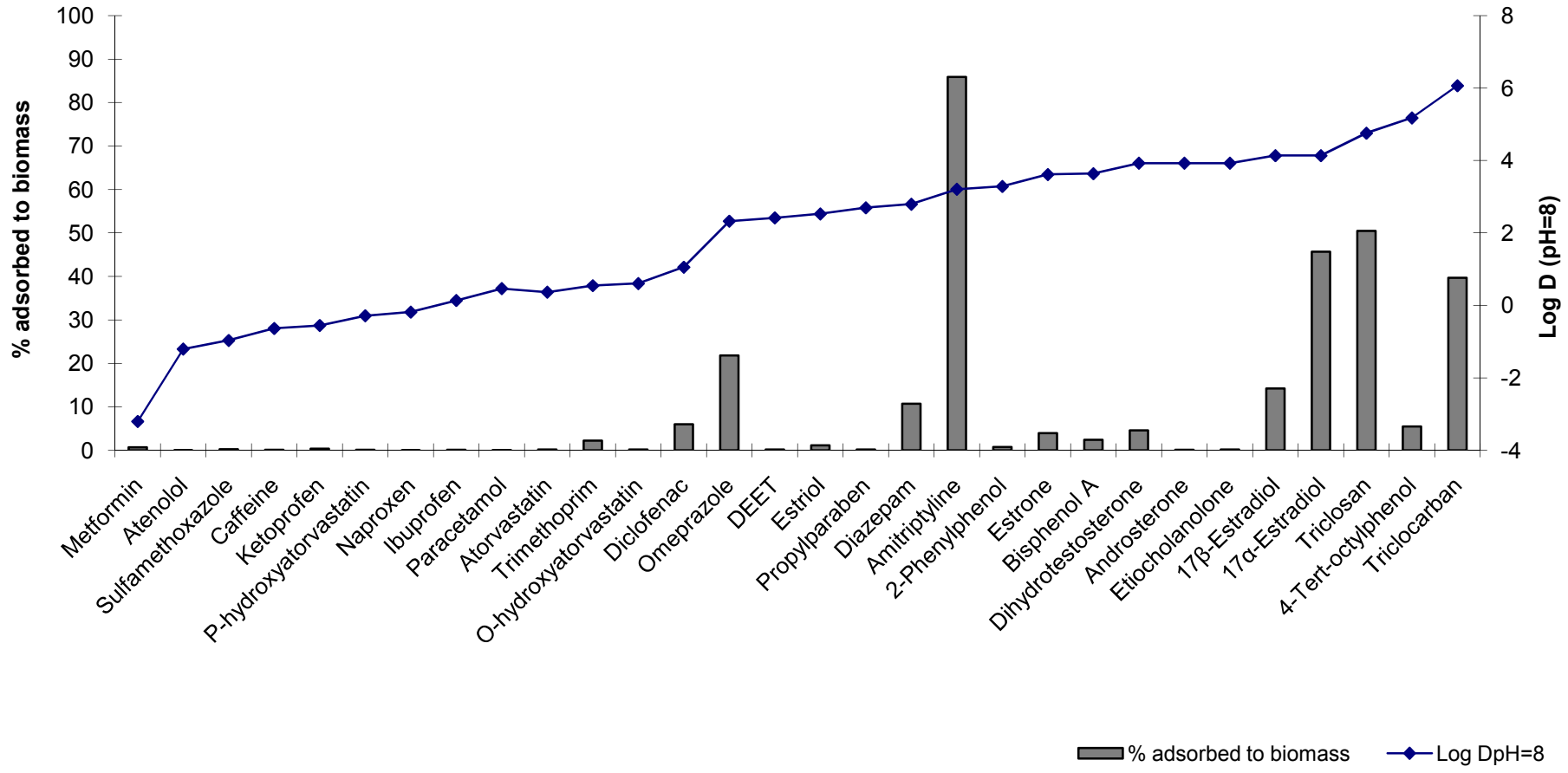


Figure 4.6 Log $D_{pH=8}$ versus % removal via adsorbed to biomass

4.3.5. Concentrations of trace chemical contaminants in the MBR permeate

The concentrations of trace chemical contaminants detected in the MBR permeate are presented in Figure 4.7. As diazepam, diclofenac, omeprazole and trimethoprim were only partially removed by the MBR, they were remained in the MBR permeate at concentrations varied from 5.3 to 205 ng.L^{-1}. Carbamazepine was detected at a concentration of 288 ± 131 ng.L^{-1} in the MBR permeate. The enzymatic cleavage of the glucuronic conjugate of carbamazepine could occur lead to the release of the parent compound during the treatment process (Vieno et al., 2007). Other chemicals including bisphenol A, 2-phenylphenol, 4-tert-octylphenol, atenolol, amitriptyline, atorvastatin, o-hydroxyatorvastatin, p-hydroxyatorvastatin, DEET, ibuprofen, ketoprofen, naproxen, paracetamol, sulfamethoxazole, triclosan, triclocarban and caffeine presented in the raw sewage at high concentrations, thus despite their high removal efficiencies (82 to almost 100%), they were still detected in the MBR permeate at concentrations around 5.7-263 ng.L^{-1}. Similarly, metformin was detected at a high concentration (38 µg.L^{-1}) in the raw sewage, so although 94% of metformin was removed by the MBR, it was still detected at a concentration up to 3.3 µg.L^{-1} in the MBR permeate. Estrone was the only steroidal hormone detected in the MBR permeate with an average concentration of 0.9 ng.L^{-1}.

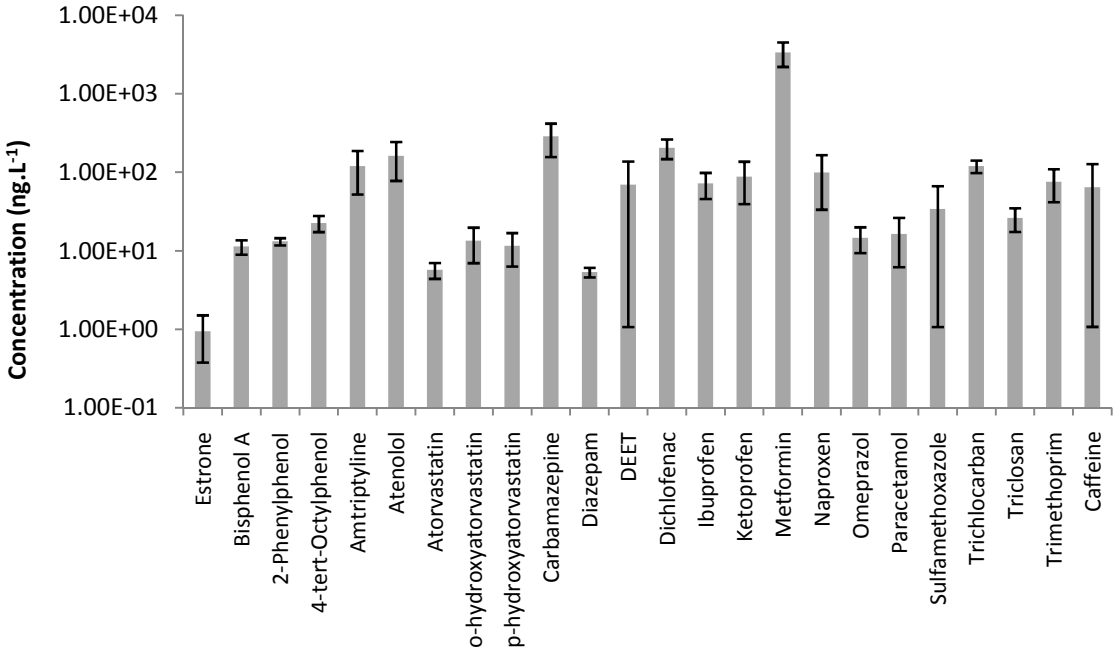


Figure 4.7 Concentrations of trace chemical contaminants detected in the MBR permeate

The concentrations of chemicals detected in the MBR permeate was compared with maximum Australia guideline values for augmentation of drinking water supplies (NHMRC & EPHC, 2008) as presented in Table 4.3. Triclosan was detected at a concentration of 263 ± 8.79 $ng.L^{-1}$, which was lower than the guideline value of 350 $ng.L^{-1}$. The concentrations of other chemicals detected in the MBR permeate were 1 to 5 orders of magnitude lower than Australian guideline values for water recycling.

Table 4.3 Concentrations of trace chemical contaminants detected in the MBR permeate and Australia guideline values for water recycling

Chemical	Concentration of trace chemical contaminants ($ng.L^{-1}$)	
	MBR permeate (mean ± stdev)	**Australian guideline values for augmentation of drinking water supplies[1]**
Estrone	0.95 ± 0.57	3×10^1
Bisphenol A	11.3 ± 2.4	20×10^4
2-Phenylphenol	13.2 ± 1.4	10×10^5
4-tert-Octylphenol	22.6 ± 5.3	50×10^3
Amitriptyline	120 ± 68	70×10^3
Atenolol	161 ± 83	Not available, but values for other β-blockers are $0.35=40 \times 10^3$)
Atorvastatin	5.73 ± 1.31	5×10^3
o-hydroxyatorvastatin	13.4 ± 6.4	Not available, but expected in similar range with atorvastatin
p-hydroxyatorvastatin	11.6 ± 5.3	Not available, but expected in similar range with atorvastatin
Cabamazepine	288 ± 131	100×10^3
Diazepam	5.34 ± 0.75	2.5×10^3
DEET	69.3 ± 128	25×10^5
Diclofenac	295 ± 58	180×10^1
Ibuprofen	72.3 ± 26.4	40×10^4
Ketoprofen	88.3 ± 48.7	35×10^2
Metformin	3373 ± 1165	2500×10^2
Naproxen	99.8 ± 66.3	220×10^3
Omeprazole	14.7 ± 5.3	Not available
Paracetamol	16.4 ± 10.1	17.5×10^4
Sulfamethoxazole	34.1 ± 43.2	35×10^3
Triclocarban	120 ± 22	Not available
Triclosan	263 ± 9	350
Trimethoprim	75.9 ± 33.9	70×10^3
Caffeine	64.5 ± 87.7	35×10^2

[1] *(NHMRC & EPHC, 2008)*

4.4. CONCLUSIONS

Emerging wastewater treatment processes such as MBRs have attracted a significant amount of interest internationally due to their ability to produce high quality effluent suitable for water recycling. It is therefore important that the efficiency of MBRs in

removing hazardous trace chemical contaminants be assessed. Accordingly, this study investigated the removal of trace organic chemical contaminants through a full-scale, package MBR plant in New South Wales, Australia. This study was unique in the context of MBR research as it characterised the removal of 48 trace organic chemical contaminants, which included steroidal hormones, xenoestrogens, pesticides, caffeine, PPCPs. The investigation was also undertaken at a full-scale package MBR plant treating real municipal wastewater with both aqueous (influent and effluent) and biomass samples analysed. A full mass balance was calculated to estimate the contribution of biodegradation/transformation and sorption to biomass to the overall removal of the trace chemicals by the MBR.

Results show that the removal of most trace organic chemical contaminants through the MBR was high (> 82%). However, diazepam and diclofenac were only partially removed through the MBR with removal efficiencies of 21% - 54%. The overall removal efficiency of omeprazole and trimethoprim was also variable between winter and summer sampling, varying from 42 to 96%. Carbamazepine had an overall removal of 24% in winter sampling and -94% in summer sampling. The negative removal of carbamazepine was atributed to the enzymatic cleavage of the glucuronic conjugate of carbamazepine and the release of the parent compound during the treatment process. Trace chemical contaminants that are partially removed through MBRs in normal operating conditions could act as potential indicators for assessing MBR performance as these chemicals are usually sensitive to changes in the treatment system.

Overall, biodegradation was a dominant removal mechanism for most trace chemicals. However, removal via adsorption to biomass was a significant removal mechanism for amitriptyline, 17α-estradiol, triclosan and triclocarban with the percentage of removal via adsorb to biomass ranging from 35 to 86%. Removal via adsorption to biomass was reduced from 76% to 14% for 17β-estradiol from winter to summer sampling. This was attributed to the higher temperature in the bioreactor in summer, which enhanced biodegradation of 17β-estradiol. Adsorption to biomass was a moderate removal mechanism for bisphenol A and estrone with the percentage of removal via adsorption to biomass being 10%. Adsorption to biomass was not a significant removal mechanism for the other monitored chemicals including estriol, dihydrotetosterone, androsterone, etiocholanolone, propylparaben, 2-phenylphenol and 4-tert-octylphenol, atorvastatin, *o*-hydroxyatorvastatin, *p*-hydroxyatorvastatin, DEET, ibuprofen,

ketoprofen, naproxen, paracetamol and caffeine with the percentage removal via adsorption to biomass representing less than 5%.

Triclosan was detected at a concentration of 263 ± 8.79 $ng.L^{-1}$, which was lower than the guideline value of 350 $ng.L^{-1}$. The concentrations of other chemicals detected in the MBR permeate were 1 to 5 orders of magnitude lower than Australian guideline values for water recycling. The results of this study enhance the understanding of the levels, fate and removal of a comprehensive list of 48 trace chemical contaminants of concern through MBR systems.

This chapter gives a background on the variations of trace organic chemical concentrations in the raw sewage and their removals by the full scale package MBR over a long period under different weather conditions (winter vs. summer). Thus, these results provide useful information for assessing impacts of hazardous events on MBR performance in subsequent chapters (chapter 5 to 8).

CHAPTER 5. EXPERIMENTAL MBRs - CONSTRUCTION, CLEAN WATER TESTS AND REPRODUCIBILITY EXPERIMENTS

5.1. INTRODUCTION

In order to study the impacts of hazardous events on MBR performance, four identical experimental MBRs were built. The design, construction process and operational conditions of the experimental MBR systems are described in this chapter. After the experimental MBRs were constructed, tests were undertaken to determine whether their system components themselves may be a source of chemical contaminants. These tests were undertaken by monitoring water quality while operating with ultrapure water as a feed source. The four experimental MBRs were then relocated to the site of a full-scale wastewater treatment plant (WWTP) and operated with primary settled municipal wastewater for the subsequent reproducibility experiments. For these experiments, the four MBRs were operated in parallel, treating identical source water, under the same operational conditions. Samples from these MBRs were taken for analysis of trace chemicals, key bulk water quality and operational parameters in order to assess the reproducibility of treatment performance between the four systems.

5.2. CONSTRUCTION OF EXPERIMENTAL MBRS

The design, construction process and operational conditions of the experimental MBR systems are described in this section.

5.2.1. Materials and parts

An influent tank (200 L) was constructed from a high density polyethylene ice box, purchased from Techniice (Frankston, VIC, Australia). Four MBR tanks (each 30 L) were constructed from synthetic polymer methyl methacrylate (Perspex) sheets. Four effluent tanks were constructed from polypropylene tubs (each 80 L), purchased from Bunnings Warehouse (Mascot, NSW, Australia). Scaffolding framework was also purchased from Bunnings Warehouse. Master flex peristaltic pumps (L/S® computer-compatible/programmable digital drive, 0.1 to 600 rpm, 115/230 VAC) were purchased from John Morris Scientific Pty Ltd (Chatswood, NSW, Australia). HP 80 Blowers (capacity 80 $L.min^{-1}$) were purchased from Pumpserv (Hornsby, NSW, Australia). Arrow Engineering mixers (Arrow Engineering, model 1750) were purchased from Arrow Engineering Co., Inc (Hillside, New Jersey, USA). Tempress control compact pressure transducers (-1 to 1.5 Bar, 0-5 VDC output) were purchased from Tempress Control (Gosford, NSW, Australia). NI USB-6009 data logger (14-Bit, 48 kS/s low-cost multifunction DAQ) was purchased from National Instruments Australia (North Ryde,

NSW, Australia). Horizontal float switches (model LS-803-21), ball valves 3/4 inches and 3/8 inches were purchased from Cole Parmer (Chatswood, NSW, Australia).

The membranes used in this experiment were polyvinyllidene-difluoride membranes that were manufactured by Siemens Water Technology (North Ryde, NSW, Australia). Each membrane has an internal diameter of 800 µm and an external diameter of 1300 µm. The pore size of the membrane is 0.04 µm. Operating limits of the membrane are temperature 0- ~45 °C, TMP 150 kPa, and pH 2-~10. As the membrane is very fragile, it required careful handling. Epoxy resin Part A and Part B glue (Selleys) used for membrane potting were purchased from Bunnings Warehouse.

5.2.2. Design and construction of the system

The overall experimental system was comprised of four identical experimental MBRs as presented in Figure 5.1. These MBR tanks were constructed from synthetic polymer methyl methacrylate, each with an operating liquid volume of approximately 30 L. The MBRs were each designed to operate at hydraulic retention time (HRT) of 1 day, solid retention time (SRT) of 30 days and a flux of 10 $L.m^{-2}.h^{-1}$. This HRT was selected for consistency with the package MBR plant described in Chapter 4. The SRT was the same with the larger pilot MBR from which these 30 L systems would be seeded (see description in Section 5.4). Although the selected operational flux was relatively low (10-50 $L.m^{-2}.h^{-1}$ is typical (Judd and Judd, 2011)), it was selected to minimise biofouling since other common biofouling control measures (eg. incorporation of an aeration 'relaxation' period) were not feasible with this simple system design. Each MBR was constructed with an aerobic chamber and a membrane chamber. Each air blower (80 $L.min^{-1}$) was connected to two MBRs to supply air for the chambers of reactors. A valve was placed in the air tube supplying air to each chamber so that the air flow can be adjusted if needed. The air diffusion system for these chambers was made with perforated rubber hosing. The four parallel MBRs were fed by gravity from a single well-mixed influent tank. A fine-screen mesh (1 mm) was placed inline of the source water to the tank. The influent tank was designed to hold sufficient feedwater volume to supply the four bioreactor tanks for a period of one day. Since the experimental hydraulic flow rate was intended to be 1 day, a total feed volume of 120 L per day would be required. As such, the influent tank was sized at 200 L, in order to ensure that sufficient volume would be available. The mixers were placed in the influent tank and in each MBR to ensure effective mixing within these tanks. A horizontal float switch was installed in each MBR. This horizontal float switch was connected to the permeate

pump so that, in cases where the water level in the reactor may fall below a set level, the pump would be switched off to protect the membrane modules from drying. A computer was connected to the MBRs for continuous measurement of transmembrane pressure (TMP). Data was collected using Labview 2012 software (National Instruments, North Ryde, NSW, Australia). The overall system was configured using scaffolding framework (Figure 5.1).

The system design included the ability to backwash membrane modules in circumstances where the TMP was observed to exceed a specified maximum value (set at 50 kPa). This backwashing involved transfer of the membranes to separate tanks filled with tapwater. The backwashing process was to be undertaken at twice the normal flux for a period of 20 minutes. A simple schematic diagram of the MBR system is presented in Figure 5.2.

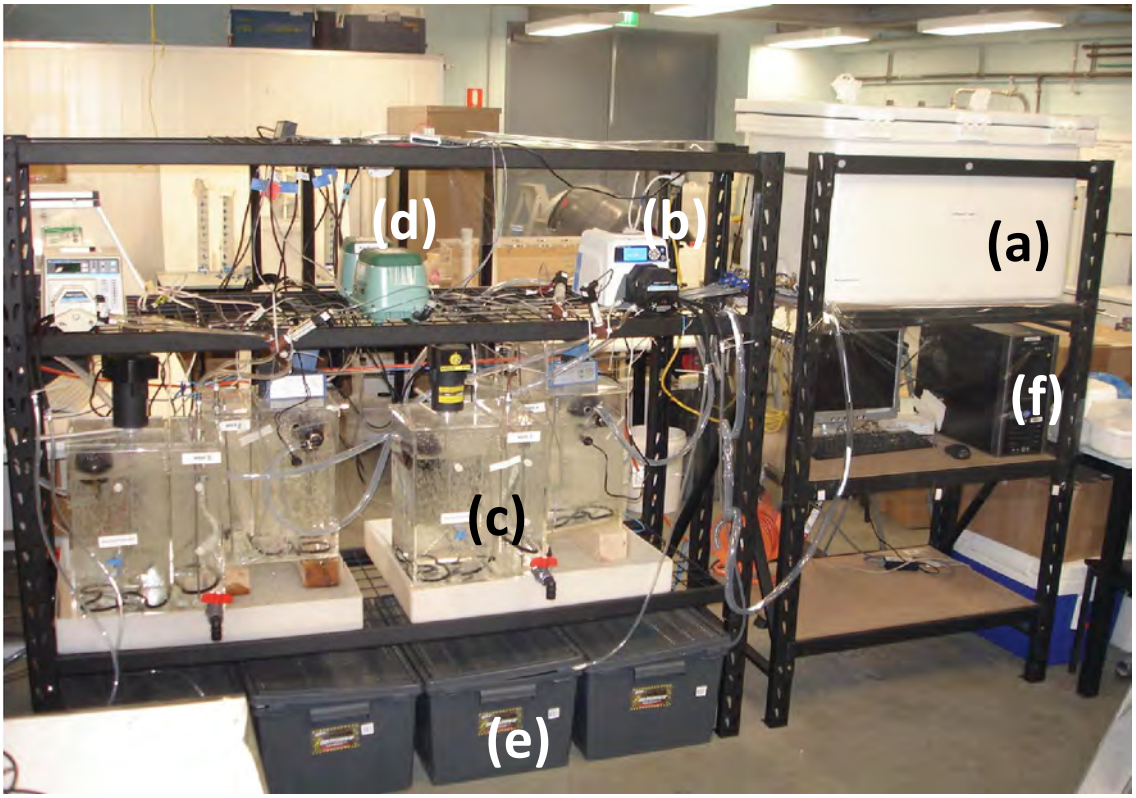
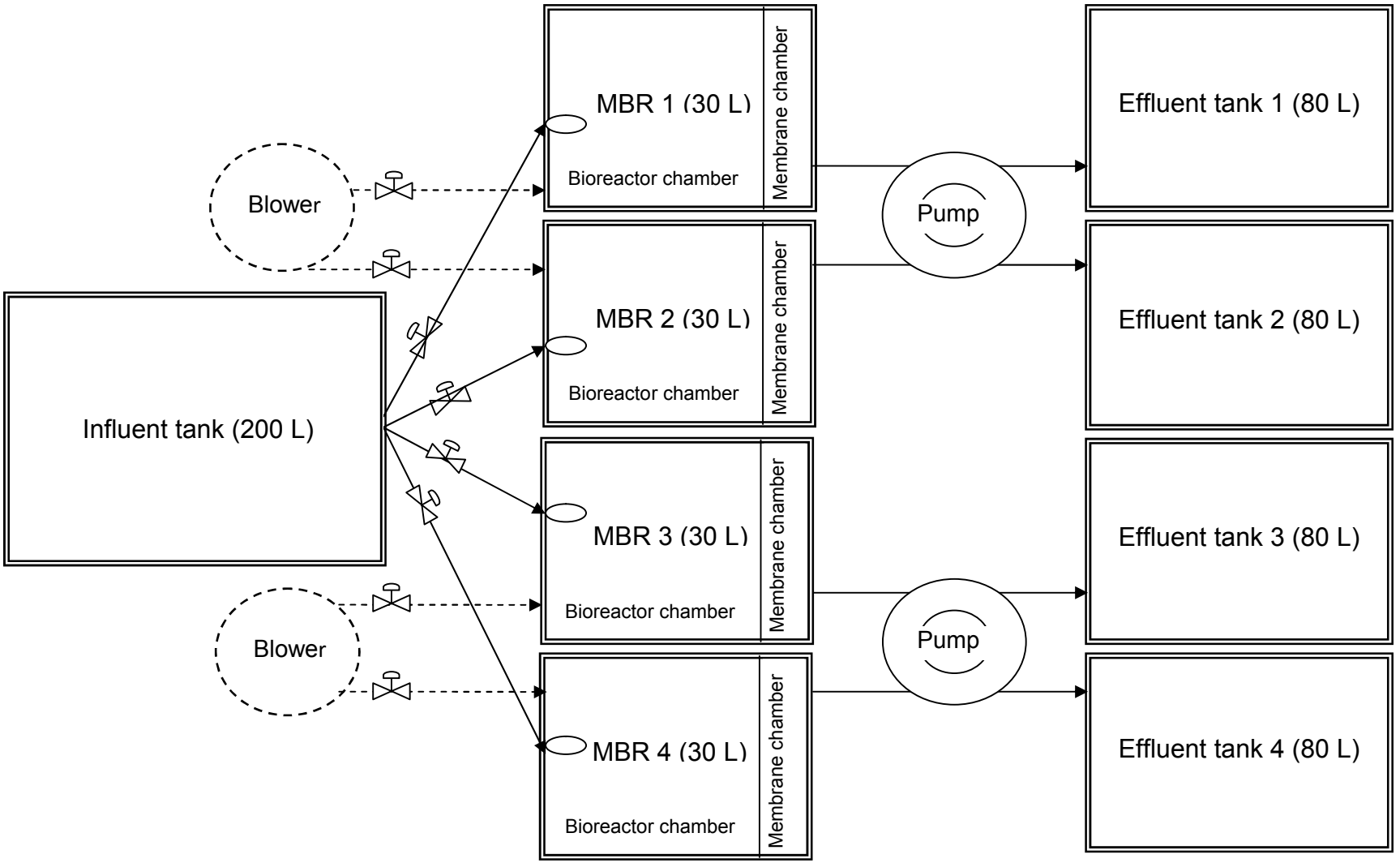


Figure 5.1 Experimental MBR set up: (a) influent (primary settled effluent) tank, (b) peristaltic permeate pumps, (c) membrane bioreactor, (d) blower, (e) effluent tank, (f) computer for data acquisition



Note: cistern valve: ⬭; two way ball valve: ⋈ ; water flow: ⟶ ; air flow: ⇢

Figure 5.2 A simple schematic diagram of the MBR system (not to scale)

After completing the construction of the tanks, including transfer lines, valves, pumping and aeration configuration. The next step was the preparation of the membrane modules. Approximately 40 membrane modules were prepared for hazardous event simulation experiments. This procedure involved cutting the membrane to the appropriate length and potting the membrane fibres together manually in each module. The number of fibres in each module was calculated based on the dimensions of the reactor and the design flux for the experiment. In these experiments, each membrane module had 30 fibres with an average working length of each fibre of 28 cm.

The membrane fibre was first checked to make sure there was no damage. Then, the fibre was cut into the designed length (28 cm + 10 cm extra for gluing both sides). After that, the membrane fibres were potted in the module using the Epoxy resin Part A and Part B glue. The membrane gluing steps are presented in Figure 5.3. 30 fibres were glued together and potted in a plastic tube to be connected to the bottom of the MBR. After that the module was allowed to dry for 20 minutes before gluing the top. The potted membrane module was allowed to dry for at least 2 days to ensure the glue reached its maximum strength before wetting and testing.

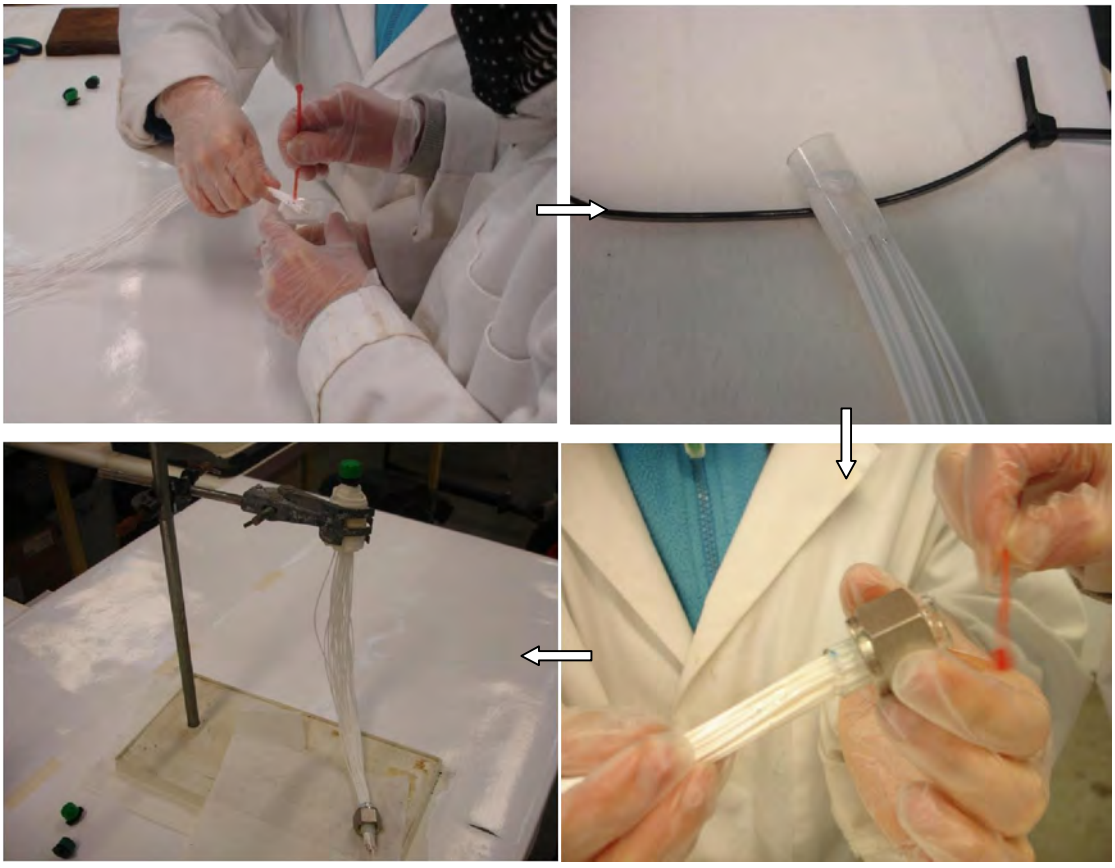


Figure 5.3 Membrane gluing

5.2.3. Wetting and testing of the membrane modules

In order to make sure the membrane module was not leaking and was working well, a clean water test was conducted to test TMP, membrane permeability and resistance for each module separately as shown in Figure 5.4.

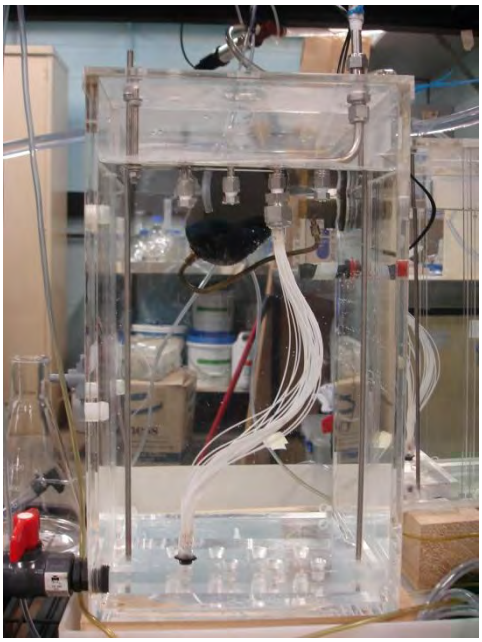


Figure 5.4 Membrane wetting and testing

The membrane module was first wetted with ultra pure water at 80 $L.m^{-2}.h^{-1}$ for 1 hour. After that, the pump was stopped. Once the pressure had stabilised, the initial pressure value (P_0) was recorded. The pump was then set to operate at nominal fluxes (J) of 10, 20, 40, 80 $L.m^{-2}.h^{-1}$. The pressure (P_t) was then recorded (after it had stabilised) at each flux value. TMP, permeability (K) and membrane resistance (R_m) were calculated as follows:

$TMP\ (kPa) = P_0 - P_t$

$K\ (L.m^{-2}.h^{-1}kPa^{-1}) = J/TMP$

$R_m\ (m^{-1}) = TMP/(viscosity \times J)$ (Judd and Judd, 2011)

The results of the clean water test for a typical good membrane module are shown in Table 5.1.

Table 5.1 Clean water test results for a typical membrane module

Nominal flux ($L.m^{-2}.h^{-1}$)	Measured flux ($L.m^{-2}.h^{-1}$)	P_0 (kPa)	P_t (kPa)	TMP (kPa)	K ($L.m^{-2}.h^{-1}.kPa^{-1}$)	R_m (m^{-1})
10	8.50	-3.47	-9.66	6.19	1.37	2.62×10^{12}
20	19.8	-3.47	-17.8	14.3	1.38	2.60×10^{12}
40	42.9	-3.47	-35.3	31.8	1.35	2.66×10^{12}
80	81.7	-3.47	-63.7	60.2	1.36	2.65×10^{12}
				Average ± stdev	1.37 ± 0.01	$2.63 \times 10^{12} \pm 0.03 \times 10^{12}$

The average K and R_m of all membrane modules were determined to be 1.4 ± 0.2 ($L.m^{-2}.h.kPa$) and $2.7 \times 10^{12} \pm 0.3 \times 10^{12}$ (m^{-1}), respectively.

After testing, each membrane module was immerged in ultra pure water in a plastic container (Figure 5.5) until use.

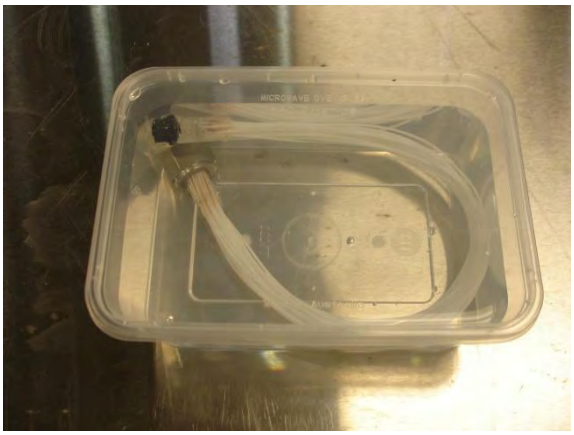


Figure 5.5 Membrane storage in ultrapure water

5.3. CLEAN WATER TESTS

A series of experiments were undertaken to assess whether any chemical contaminants or bulk water quality parameters may be introduced from the materials used to construct the experimental MBR systems themselves. This was considered to be important since some chemicals (eg, bisphenol A) are known to leach from some types of plastics into water (Sajiki and Yonekubo, 2004). Furthermore, it was necessary to assess whether substances such as the glues used to pot the membranes might

contribute such chemicals or lead to increases in COD in bioreactors and membrane permeates.

5.3.1. Experimental design and analysis

Clean water experiments were undertaken by operating the system with ultrapure water used as a feed and monitoring samples collected from various points within the system. The 200 L feed tank was filled with ultrapure water and the 4-MBR system was operated including aeration and membrane filtration processes. The system was operated with a HRT of 1 day and a flux 10 $L.m^{-2}.h^{-1}$. After operation for 1 day, samples were collected from:

- inside the influent tank,
- inside each of the four MBRs and
- inside each of the four effluent tanks.

These samples were analysed for chemical oxygen demand (COD). They were further analysed by Fluorescence Excitation-Emission Matrix (EEM) Spectroscopy as a means of identifying the presence of any (fluorescent) organic chemicals not directly targeted by the trace chemical analysis.

The MBR system was further flushed with ultra-pure water for 9 day and samples were also taken for trace chemical analysis using liquid chromatography tandem-mass spectrometry (LC-MS/MS) and gas chromatography tandem-mass spectrometry (GC-MS/MS) as described in Chapter 3.

5.3.2. COD results

After 1 day flushing the MBR system with ultra-pure water, COD concentration of 4-6 $mg.L^{-1}$ was detected in samples taken inside the MBRs. This compares with negligible COD measured from within the influent tank and effluent tanks (Table 5.2).

Table 5.2 COD concentrations in the system after flushing with ultra-pure water for 1 day

Sample location	COD ($mg.L^{-1}$)
Inside the influent tank	Under range (<1)
Inside each of the four MBRs	4-6
Inside each of the four effluent tanks	Under range (<1)

A suspected source of COD contamination was the Epoxy resin glue used for the membrane potting. This hypothesis was investigated by the use of fluoresence EEM spectroscopy as described in the following section.

5.3.3. Fluorescence excitation emission matrices

Samples were then analysed by EEM Spectroscopy to identify and characterise the source of COD contamination. An EEM spectrum collected after 1 day of flushing with ultra-pure water within an MBR tank is presented in Figure 5.6. Three distinct fluorescence peaks were observed with excitation wavelengths around 220, 275 and 395 nm and an emission wavelength centred around 300 nm.

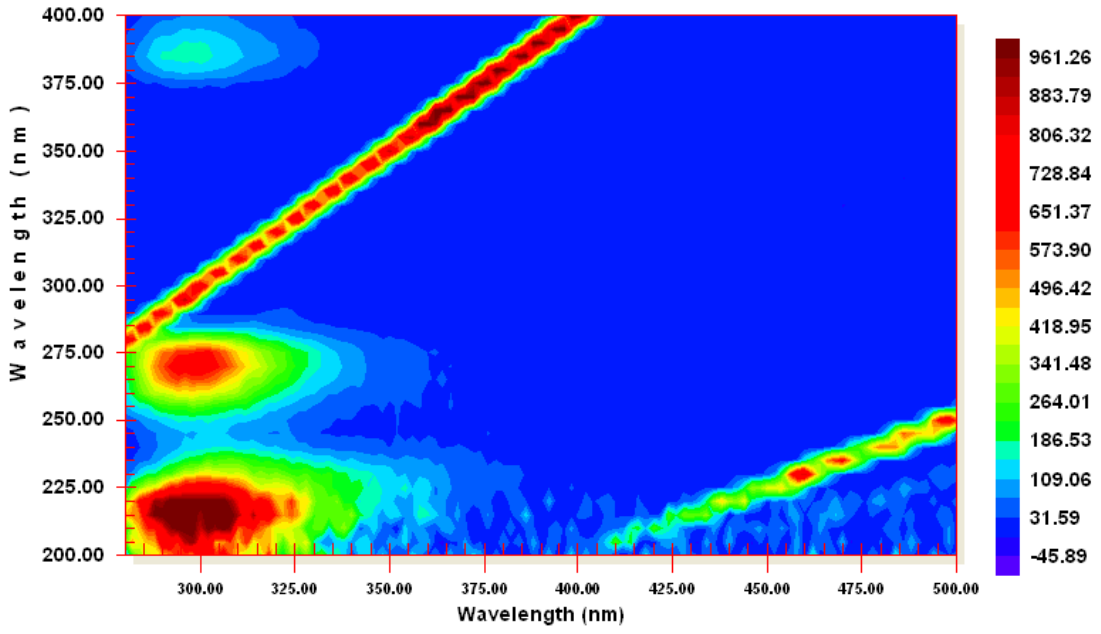


Figure 5.6 Fluorescence EEM of samples inside MBR after 1 day flushing with ultra-pure water

The fluorescence EEM of the epoxy resin (Selleys) glue was acquired by dissolving a small amount of the glue in ultra-pure water. This EEM is presented in Figure 5.7. Three distinct peaks were visible within this EEM, closely matching the peaks observed for the ultrapure water samples collected from the MBRs after 1 day. Therefore, it was

concluded that the potting glue was indeed the source of these peaks in the MBR tank water.

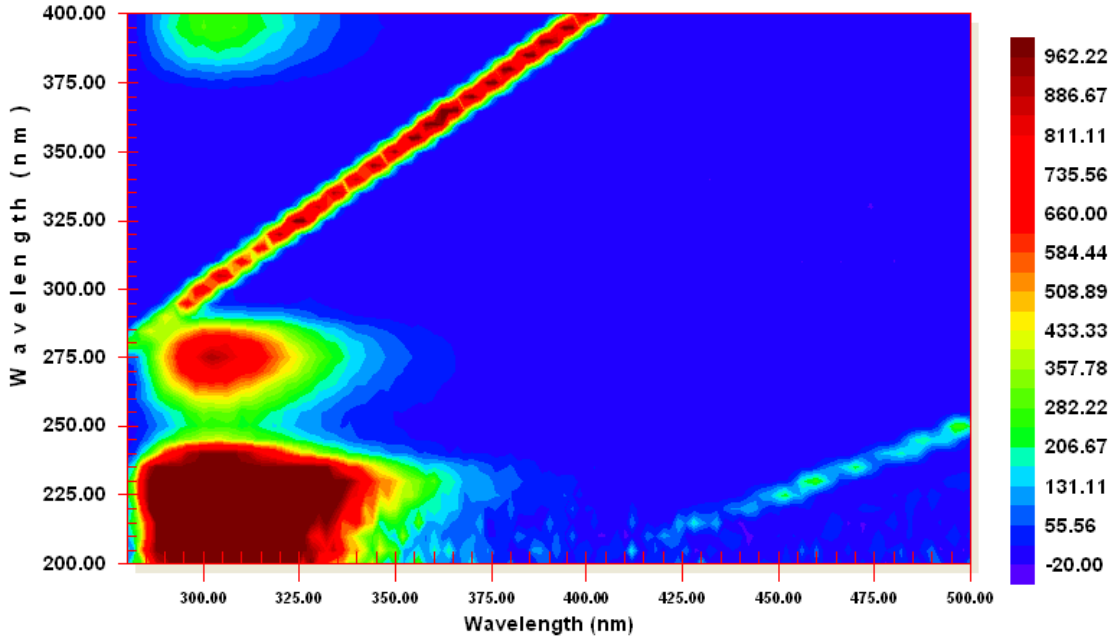


Figure 5.7 Fluorescence EEM of the Epoxy resin glue

The MBR system was then further flushed with ultra-pure water for 2 days and reanalysed for COD and fluorescence. The subsequent fluorescence EEM of the tank water is shown in Figure 5.8.

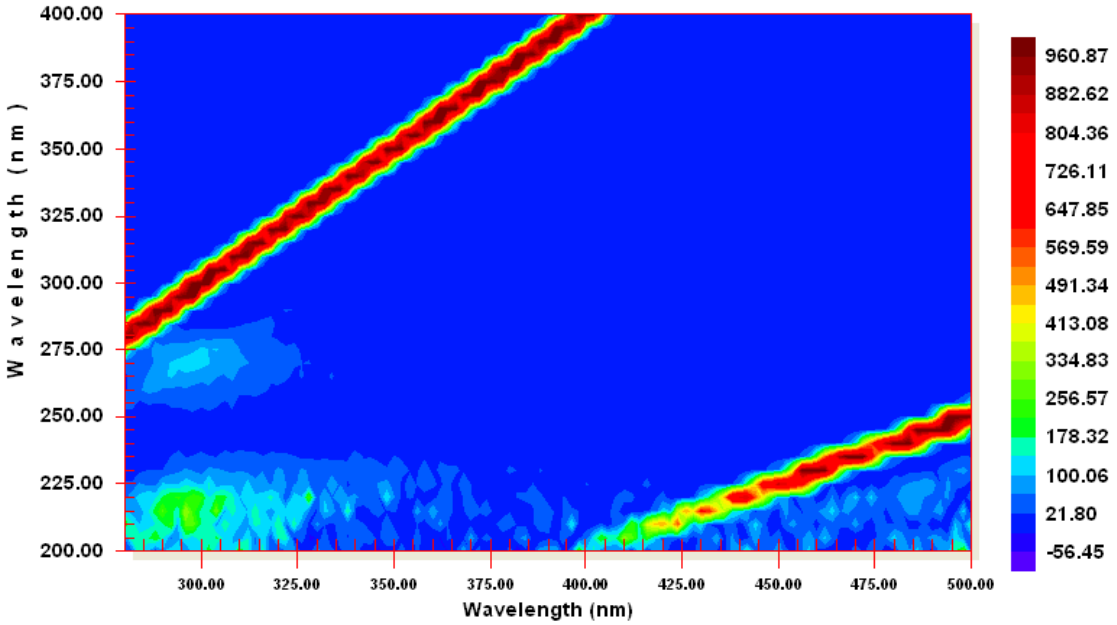


Figure 5.8 Fluorescence EEM of samples inside MBR after 3 days flushing with ultra-pure water

Comparing Figure 5.7 and Figure 5.8, it appeared that flushing was an effective mean of eliminating the contaminant as the characteristic 'glue signal' decreased significantly after 3 days. Thus, the MBRs were flushed 6 days more to ensure that no more contamination remained in the system. The final emission spectrum is presented in Figure 5.9.

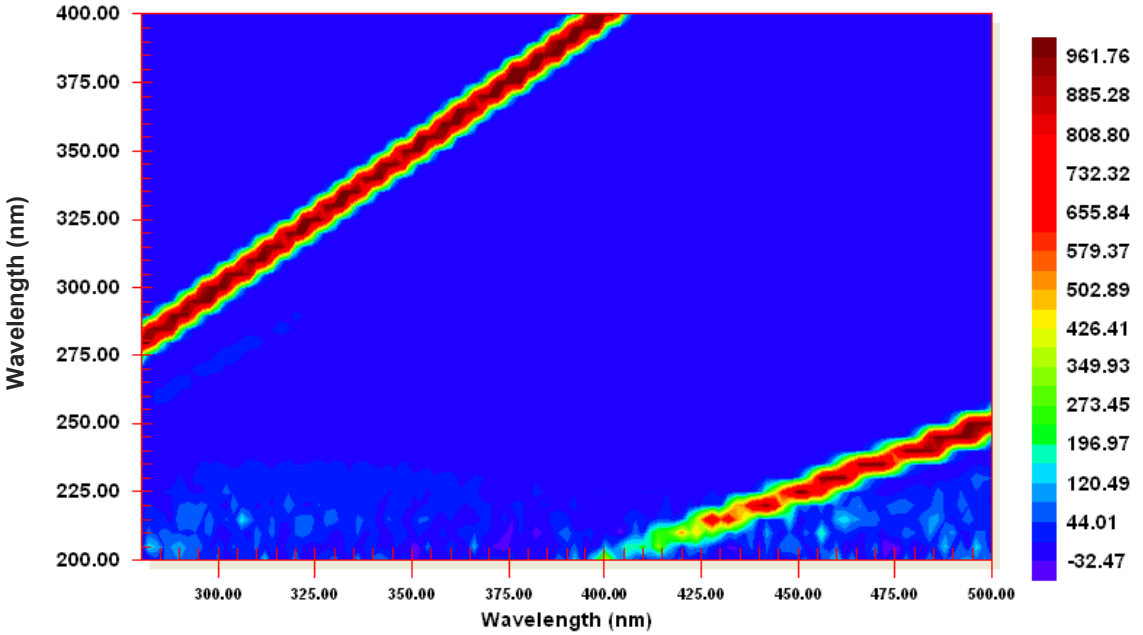


Figure 5.9 Fluorescence EEM of samples inside MBR after 9 days flushing with ultra pure water

COD was not detected in all samples taken after 9 days flushing the MBR system with ultra-pure water. As such, it was concluded that the identified fluoresence peaks were either the source of the COD contamination, or else a very useful surrogate measure of the COD contamination.

5.3.4. Trace chemical results

Results of trace chemical analysis show that except for bisphenol A, all other trace chemicals were not detected above the limit of quantification (LOQ) in the system. Bisphenol A was detected at high and variable concentrations in various samples from the system. The concentration of bisphenol A in the influent tank was 1615 $mg.L^{-1}$. The concentration of bisphenol A in the four MBRs was up to 1460 $mg.L^{-1}$ and the concentration of bisphenol A in the four effluent tanks was up 840 $mg.L^{-1}$. The results suggest that bisphenol A was leaching from the materials used to build the MBR system (i.e. the plastic influent tank, effluent tank, plastic pipes, or plastic valves etc.).

Bisphenol A was therefore removed from the list of analytes and not further considered for the MBR performance analysis described in this study.

5.4. OPERATION OF THE SYSTEM

Prior to experimental periods, the four bioreactor tanks were seeded with biomass from an existing pilot-scale MBR operating at the same municipal WWTP. This system had been operating and treating the same source water, with similar operational conditions for approximately 1 year. As such, the biomass was well acclimatised to the feed and operational conditions. Primary settled municipal effluent from the municipal WWTP was used to fill the influent tank daily. This filling process involved screening through the 1 mm fine screen mesh. The screened contents of the influent tank are subsequently referred to as the 'influent' to the MBR systems. The mixer in the influent tank was gently mixed continuously to assure a well-mixed environment in the tank. The influent from the influent tank flowed to the MBRs by gravity. A cistern valve was used to control influent flow for each reactor. Although this flow-control system generally worked well, a plastic tray was placed under each reactor to capture any overflow to floor. The MBRs were each operated at HRT of 1 day, SRT of 30 days and a flux of 10 $L.m^{-2}.h^{-1}$. The aerobic chamber of the MBR was intermittently aerated with 15 minutes on / 15 minutes off cycles to stimulate nitrification (aerobic) and denitrification (anaerobic) microbial process. The mixer in the aerobic chamber gently mixed the mixed liquor continuously. The membrane chamber of the MBR was aerated continuously in order to assist biofouling control. The peristaltic pump continuously sucked water through the hollow fibre membrane modules and the permeate (effluent) was stored in the effluent tank. TMP was monitored online by the computer.

The combination of low flux and continuous membrane aeration was effective for managing biofouling throughout the duration of the experiments. Since the TMP never exceeded the specified maximum value (50 kPa), no membrane backwashing was performed.

5.5. REPRODUCIBILITY EXPERIMENTS

Before starting the hazardous event simulation experiments, the four experimental MBRs were operated under the same designated conditions (HRT of 1 day, SRT of 30 days and a flux of 10 $L.m^{-2}.h^{-1}$) to assess the reproducibility of performance between the four parallel systems.

5.5.1. Experimental design and analysis

Primary settled effluent from the municipal WWTP was added to the influent tank of the MBR system daily after passage through the fine screen. The MBRs were also seeded with the biomass from the existing pilot MBR at the WWTP. The system was operated following the operating procedure as described in Section 5.4 above.

The system was operated for approximately 3 days (3 HRTs) before sampling was conducted. Screened primary settled effluent (from the MBR influent tank) and permeate (from each MBR effluent tank) were taken in triplicate every day for 5 days. Mixed liquor (from each MBR) was also taken in duplicate daily for 5 days. To maintain the mixed liquor suspended solid (MLSS) concentration in the MBRs above 5 $g.L^{-1}$, it was not possible to take larger volumes of mixed liquor so only duplicate samples of mixed liquor were taken. Influent and effluent samples were analysed for pH, COD and trace chemicals. Mixed liquor samples were analysed for MLSS, mixed liquor volatile suspended solid (MLVSS), capillary suction time (CST) and trace chemicals. In addition, TMP was also monitored during the experiment.

Removal efficiency was calculated from the influent samples of the previous day and effluent samples after 1 HRT. Each influent and effluent sample was collected in triplicate, enabling a mean and standard deviation to be estimated for the concentration values. However, in order to carry the measured variability over to the calculated removal efficiency, it was necessary to undertake a Monte Carlo simulation for Equation 5.1.

$$MBR\ Removal\ (\%) = \frac{Feed_{concentration} - Permeate_{concentration}}{Feed_{concentration}} \text{ x } 100 \quad \text{[Equation 5.1]}$$

This Monte-Carlo simulation was undertaken using @Risk 5.5 software. Normal distributions were defined using the calculated mean and standard deviation for each concentration value. The Monte Carlo simulations for Equation 5.1 were undertaken with 10,000 sampling iterations. The mean and standard deviations derived for MBR Removal (%) were then used to construct the following figures.

5.5.2. Key bulk water quality and operational parameter results

The results of key bulk water quality and operational parameters including pH, COD, MLSS, MLVSS, CST and TMP during the reproducibility experiment are presented in this section.

Table 5.3 summarise pH, COD, MLSS, MLVSS and CST results of the reproducibility experiments. During the experiment, influent pH ranged between 6.8 to 7.0 and MBR effluent pH from the 4 MBRs were similar, ranging between 6.2 to 7.4 during various operational dates.

Table 5.3 Permeate pH, COD removal, MLSS, MLVSS and CST results of the reproducibility experiments

Parameters	MBR1 (mean ± stdev)	MBR2 (mean ± stdev)	MBR3 (mean ± stdev)	MBR4 (mean ± stdev)
Permeate pH	6.7 ± 0.5	6.6 ± 0.4	6.6 ± 0.4	6.6 ± 0.3
COD removal	92.2 ± 2.96	93.5 ± 2.20	93.1 ± 2.73	93.3 ± 2.32
MLSS ($g.L^{-1}$)	5.11 ± 0.24	5.11 ± 0.10	5.08 ± 0.25	5.12 ± 0.28
MLVSS ($g.L^{-1}$)	4.65 ± 0.24	4.75 ± 0.14	4.71 ± 0.26	4.68 ± 0.27
CST ($s.gMLSS^{-1}.L^{-1}$)	3.28 ± 0.39	3.05 ± 0.33	2.95 ± 0.24	2.92 ± 0.29

The COD removal efficiency of the 4 MBRs during the reproducibility experiment is presented in Figure 5.10. High COD removal efficiency from 90 to 97% was achieved and the results were reproducible between the 4 MBRs.

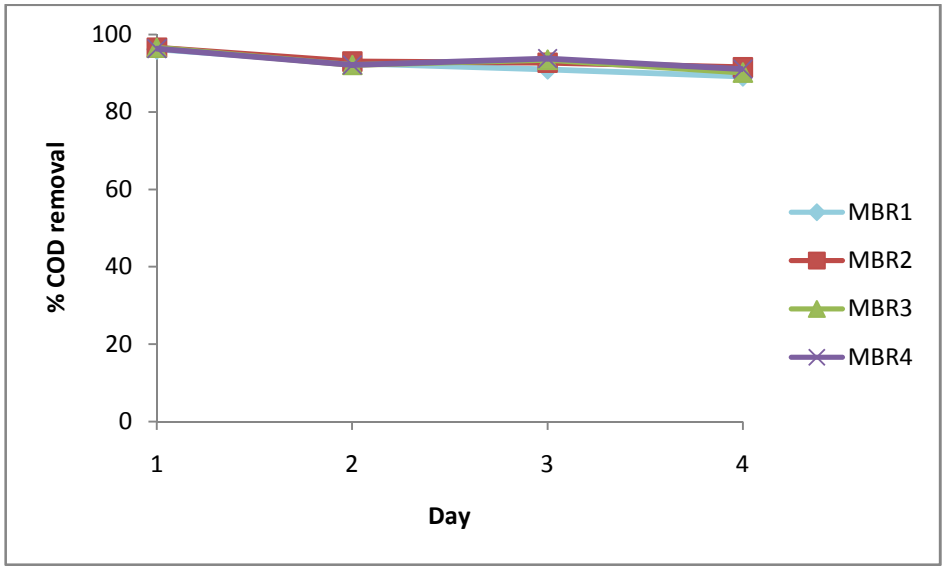


Figure 5.10 COD removal efficiency of the 4 MBRs

MLSS, MLVSS concentrations of the 4 MBRs during the reproducibility experiments is presented in Figure 5.11 and Figure 5.12. There were some small variations in MLSS and MLVSS concentrations between the 4 MBRs but in general, the results were reproducible between the 4 systems. The MLSS concentrations in the 4 MBRs varied

from 4.8 to 5.5 g.L^{-1}. The ratio between MLVSS concentration/MLSS concentration was from 89 to 95%, indicating high proportion of micro-organisms in the biomass.

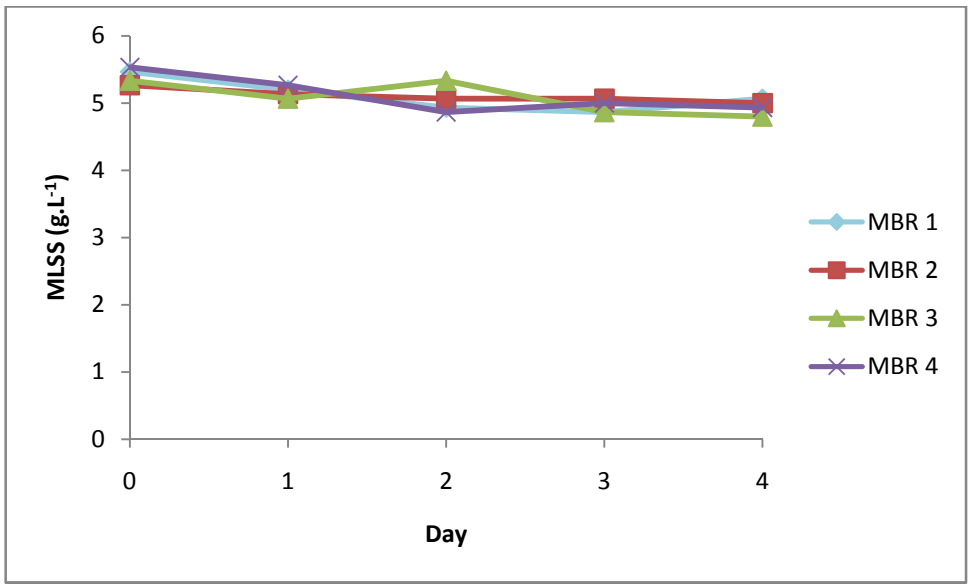


Figure 5.11 MLSS concentrations of the 4 MBRs

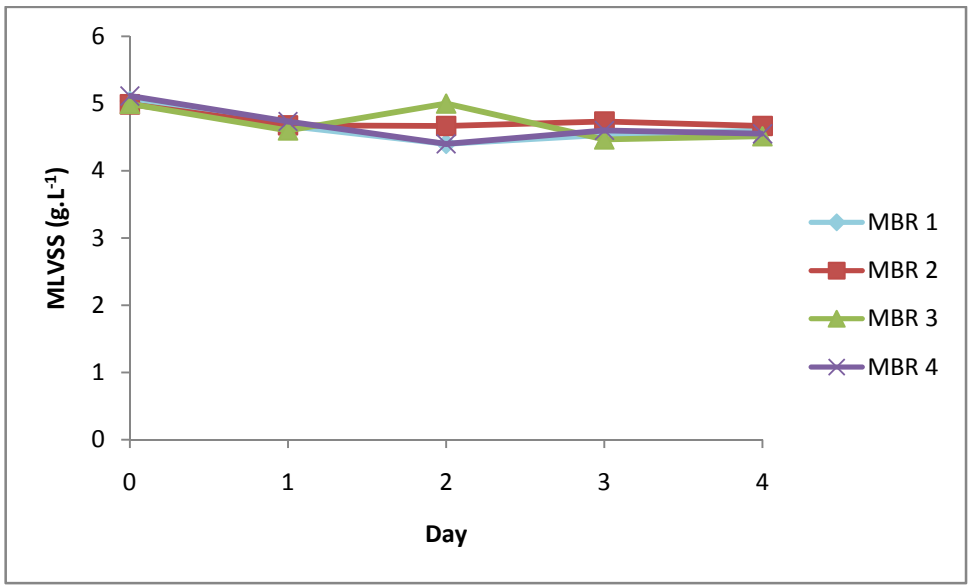


Figure 5.12 MLVSS concentrations of the 4 MBRs

The CST of mixed liquor from the 4 MBRs is presented in Figure 5.13. Generally, the lower CST value indicates the better sludge filterability. According to the CST manual (Triton Electronics, 2013), for activated sludge, the CST values ≤ 20 seconds, corresponding to 4 s.gMLSS^{-1}.L^{-1} in this case, are considered having sufficient filterability. CST results from the 4 MBRs were around 3 s.gMLSS^{-1}.L^{-1}. These results imply that filterability of the mixed liquor from the 4 MBRs was good. There were some

variations in CST observed on day 2 and day 3. However, these variations were within the error of the measurement itself (±10%). TMP of the MBRs gradually increased, reaching 34 kPa at the end of the experiment. However, since this was below the designated backwashing TMP setpoint, no backwashing was conducted during this experimental period. The TMP results were reproducible between the 4 MBRs.

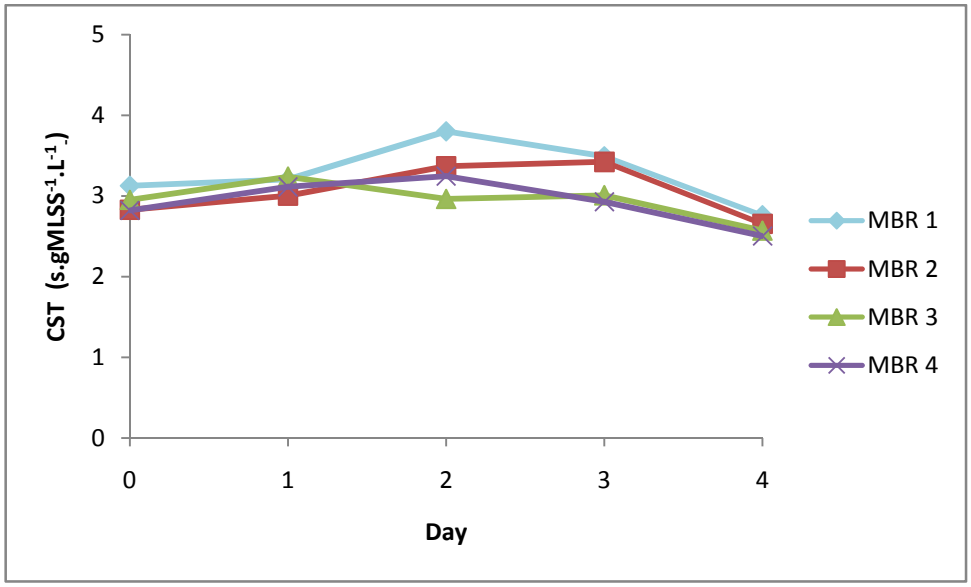


Figure 5.13 CST of mixed liquor of the 4 MBRs

5.5.3. Trace chemical results

The removals of trace chemicals that were consistently detected in the influent by the experimental MBRs are presented in Figure 5.14. Results from Figure 5.14 show that the overall removals of sulfamethoxazole, caffeine, ketoprofen, naproxen, ibuprofen, paracetamol, gemfibrozil, oestriol, propylparaben, testosterone, 2-phenylphenol, oestrone, etiocholanolone, androsterone, 17β-estradiol, triclosan were consistently above 90% and reproducible between the four MBRs. The percentage removals via adsorption to biomass were ≤ 7% for these compounds and were also reproducible between the four MBRs.

The overall removals of atenolol, trimethoprim, diclofenac and carbamazepine were reproducible with a maximum standard deviation of 8% between the four MBRs. However, the standard deviations of overall removals of atenolol, trimethoprim, diclofenac between different sampling dates within the same MBR was larger, varied from 12 to 13 % for atenolol, from 7% to 12% for trimethoprim and from 9 t0 14% for diclofenac.

The overall removal of triclocarban was consistently above 90% and reproducible between the four MBRs. However, the percentage removal via adsorption to biomass varied from 75% to 100% between four reactors for the same sampling date and it varied from 75% to 98% between different sampling dates within one reactor. This large variation was hypothesised to be due to the mass balance calculation and is discussed in further detail in Section 5.6.2 below.

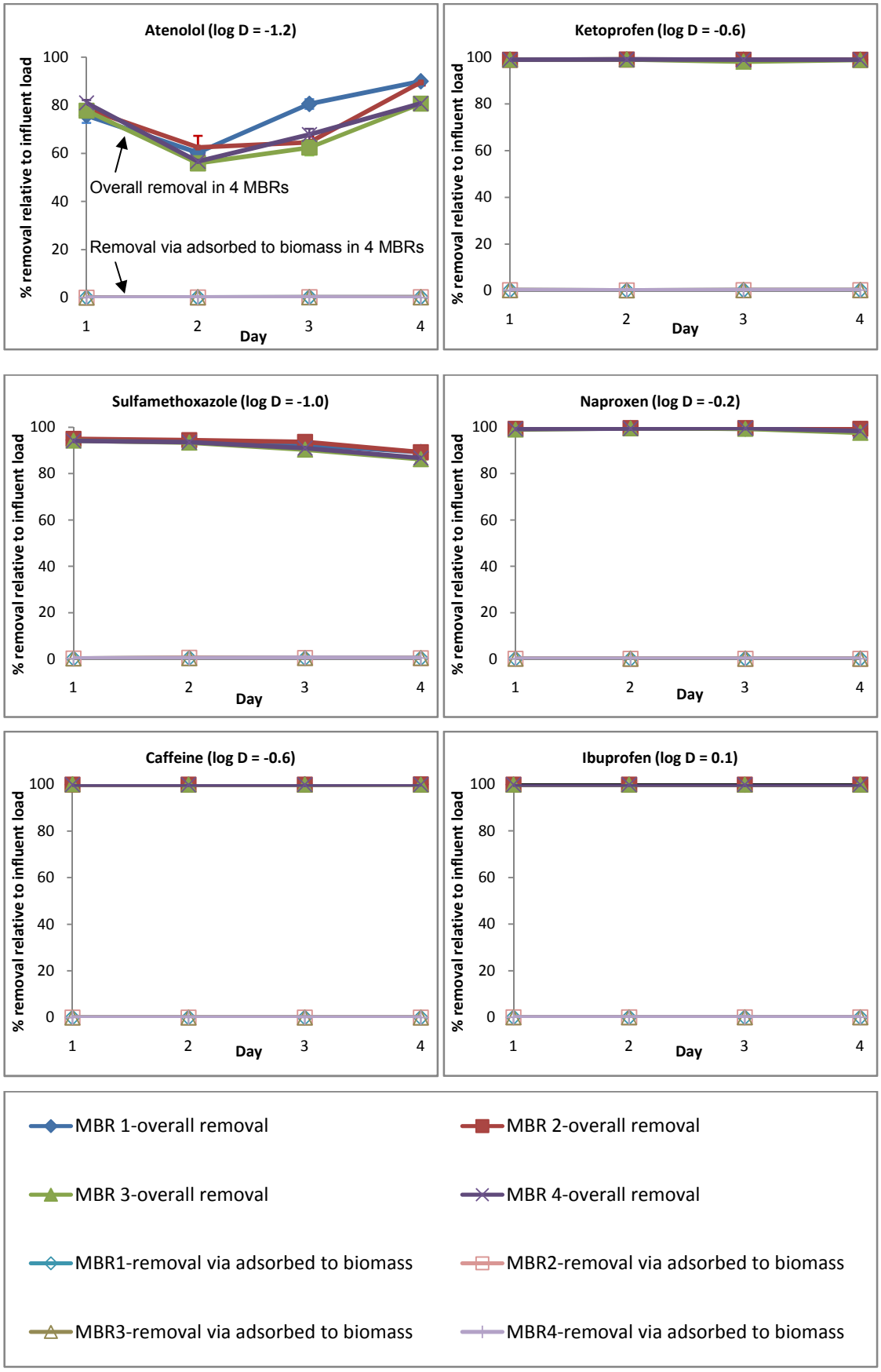


Figure 5.14 Removals of trace chemicals in reproducibility experiments

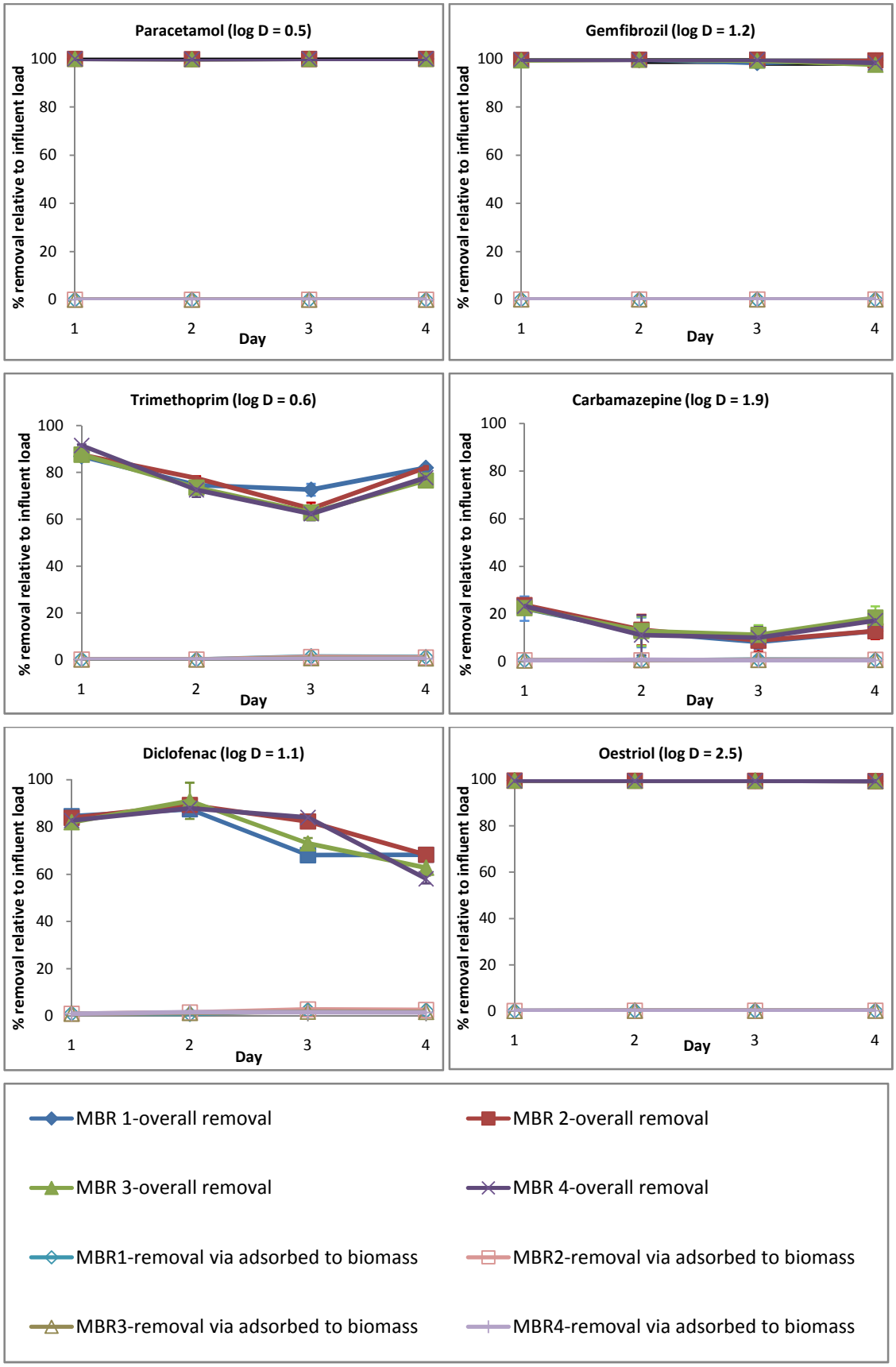


Figure 5.14 Removals of trace chemicals in reproducibility experiments (continue)

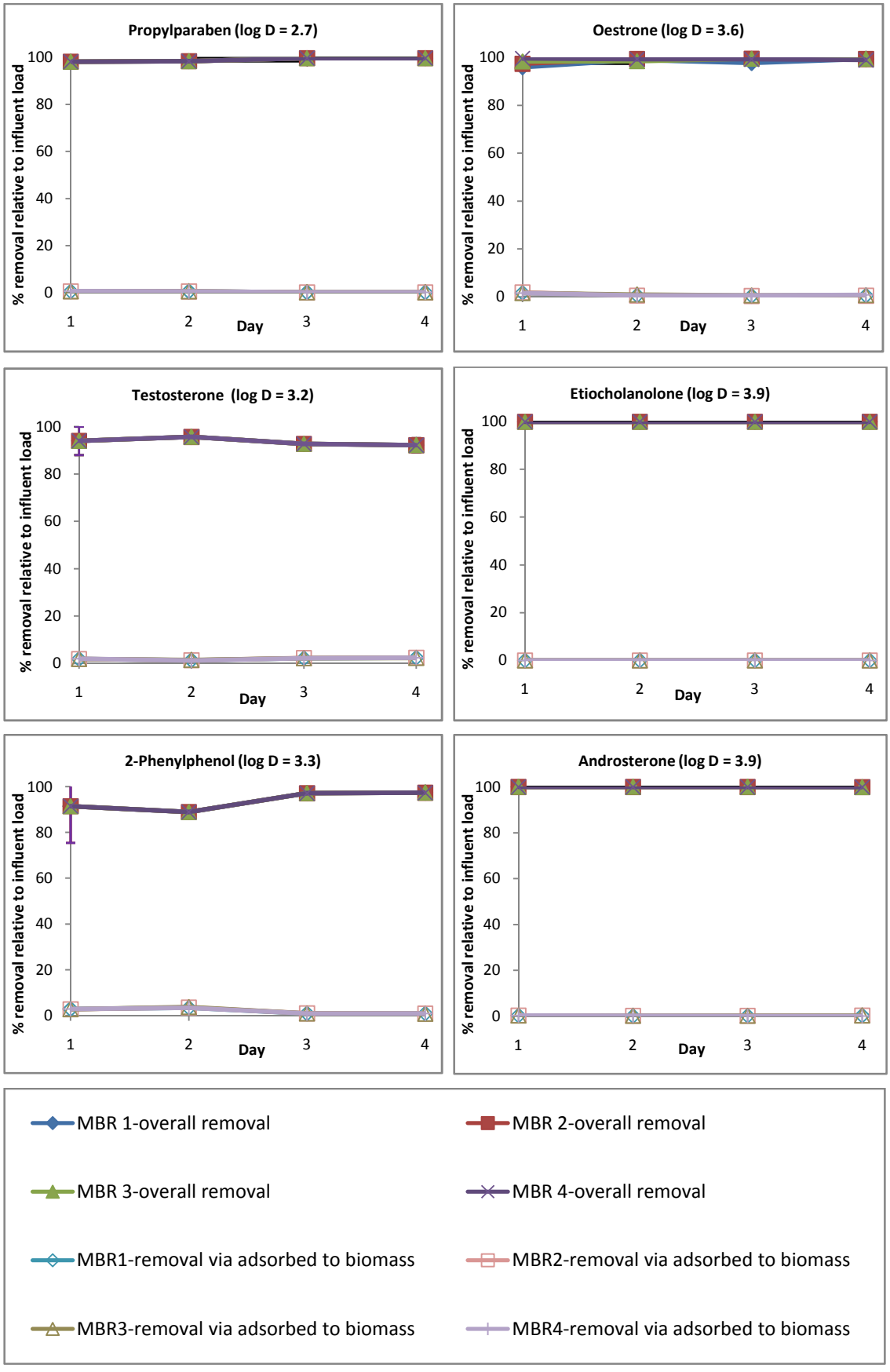


Figure 5.14 Removals of trace chemicals in reproducibility experiments (continue)

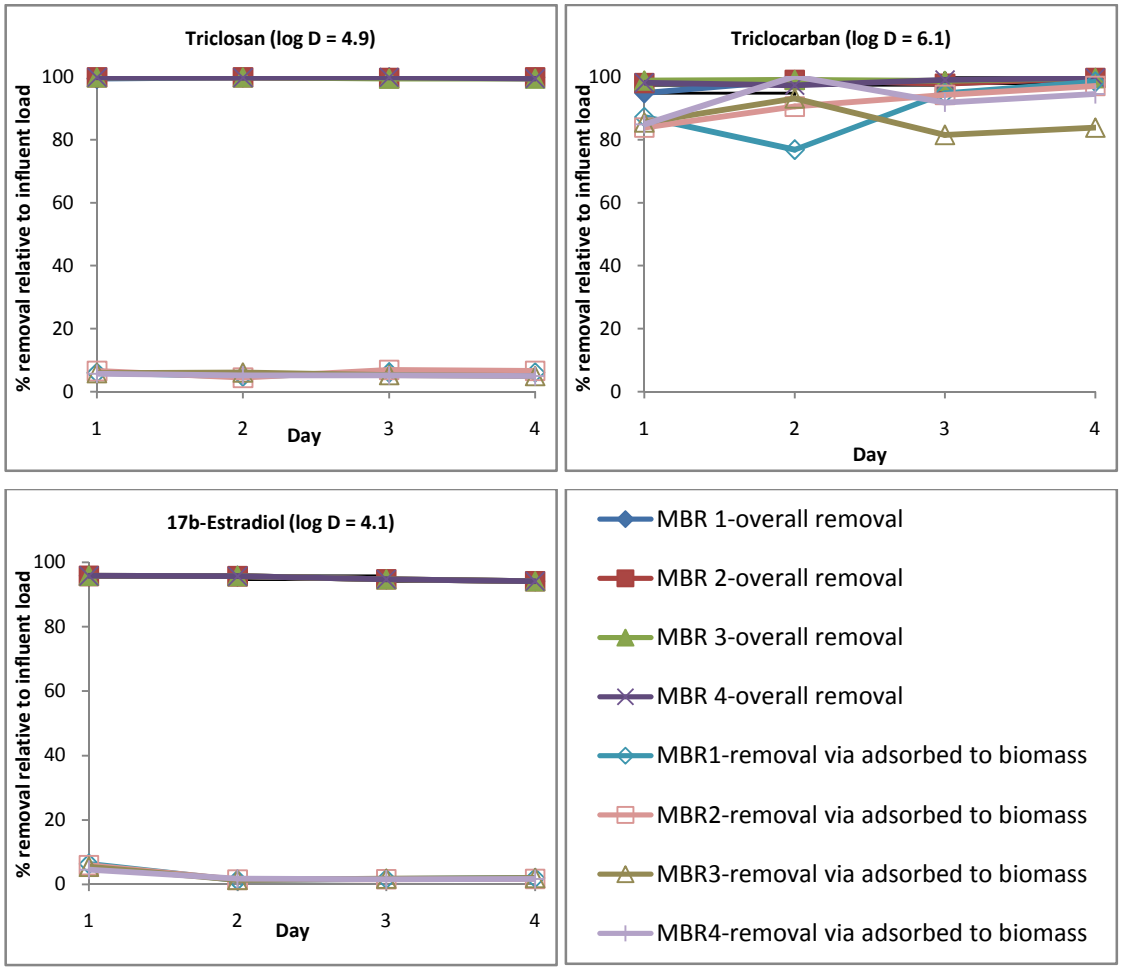


Figure 5.14 Removals of trace chemicals in reproducibility experiments (continue)

5.6. REMOVAL OF TRACE CHEMICALS BY THE EXPERIMENTAL MBRS UNDER NORMAL OPERATIONAL CONDITIONS

5.6.1. Concentrations of trace chemicals in the influent

Endocrine disrupting chemicals

Concentrations of endocrine disrupting chemicals (EDCs) in the influent are presented in Figure 5.15. These results are similar in terms of concentration and composition to the Wollumla package MBR, Bega Valley (presented in Chapter 4), with the main components being the contraceptives pills (17α-ethynylestradiol, mestranol and levonorgestrel), the breakdown product of the chemical used in detergents and personal care products (nonylphenol) were not detected above the limits of

quantification (LOQ) in the influent of the experimental MBRs. The natural estrogen 17α-estradiol was also not detected in the influent. The natural estrogens that were detected including 17β-estradiol and its metabolised products estrone and estriol. The androgenic hormones that were consistently detected including testosterone and its metabolised products, androsterone and etiocholanolone, while androstenedione and dihydrotestosterone were only detected in a few influent samples. Overall, the concentrations of steroidal hormones detected in the influent at the WWTP were in the same range with concentrations in raw sewage at the package MBR plant in Wolumla, Bega Valley. These results are consistent with previous Australian studies; with the exception of dihydrotestosterone that was one to two orders of magnitude higher than values reported in the literature (Coleman et al., 2010, Le-Minh et al., 2010). The detected estrogenic phenolic compounds include 2-phenylphenol and propylparaben. The concentration of 2-phenylphenol was an order of magnitude lower than values in the Wolumla package MBR, Bega Valley.

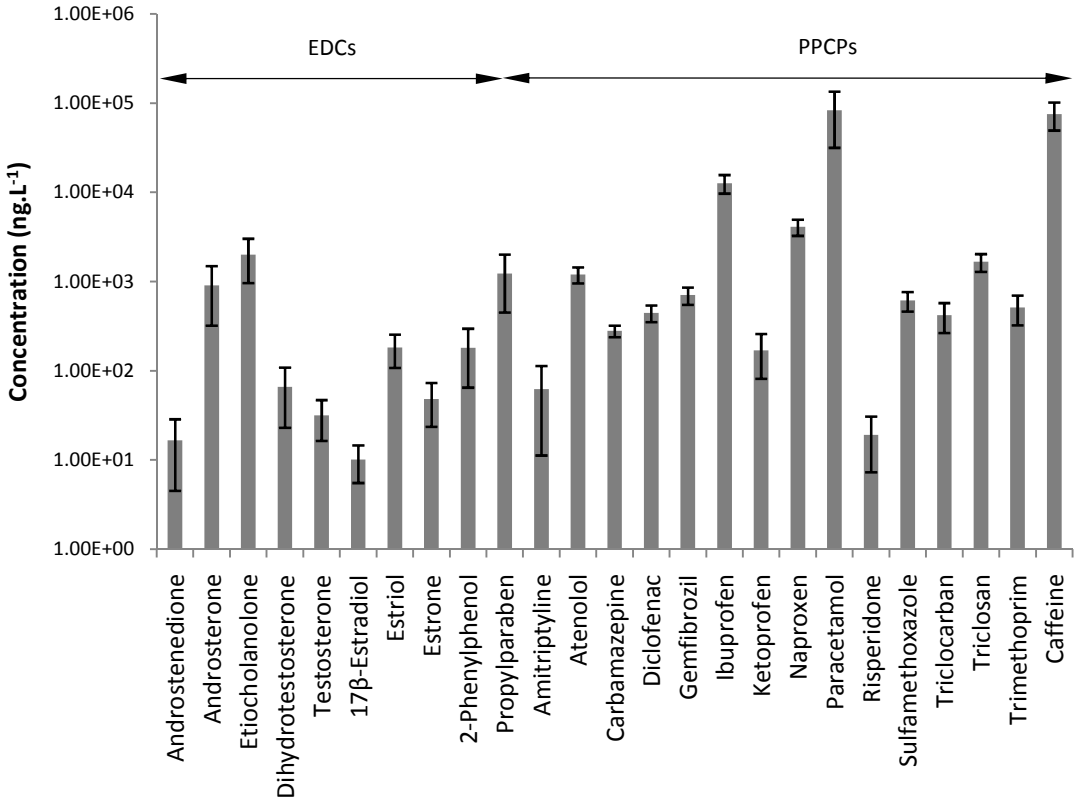


Figure 5.15 Concentrations of trace chemicals in the influent

Pesticides, pharmaceuticals and personal care products

The concentrations of pharmaceuticals and personal care products (PPCPs) that were detected in the influent are shown in Figure 5.15. Unfortunately, due to some technical problems during the LC-MS/MS run, atorvastatin, o-hydroxy atorvastatin, p-hydroxy atorvastatin and metformin were not able to be analysed, therefore, 32 (instead of 36) PPCPs were analysed in these reproducibility experiments and hazardous simulation experiments in the following chapters. Among the 32 analytes, 9 pharmaceuticals and 2 pesticides were not detected in the influent. These chemicals included diazepam, dilantin, enalapril, meprobamate, hydroxyzine, omeprazole, simvastatin, simvastatin hydroxy acid, triamterene, atrazine and linuron. Some chemicals e.g. amitriptyline, dihydrotestosterone, androstenedione and risperidone were only detected in a couple of influent samples. In general, the concentrations of PPCPs detected in the influent at this WWTP were in the same range of those at the Wolumla package MBR, Bega Valley. The day-to-day variability in concentrations of PPCPs at this WWTP was lower than in Wolumla, Bega Valley. This result was expected as the WWTP received wastewater from a much larger catchment (500,000 equivalent persons) compared to Wollumla, Bega Valley (800 equivalent persons). Previous studies have shown that larger catchments tend to lead to more homogenous concentrations of trace chemicals (Teerlink et al., 2012).

5.6.2. Removals of trace chemicals by the experimental MBRs

Androstenedione, amitriptyline, dihydrotestosterone and risperidone were only detected in a couple of influent samples. When these chemicals were detected, the removal of these chemicals by the MBRs was over 87% for androstenedione, over 70% for amitriptyline, over 95% for dihydrotestosterone and over 87% for risperidone.

The removals for other trace chemicals that were consistently detected in the influent during the experiment are presented in Figure 5.14 and also summarised in Table 5.4. A common measure of hydrophobicity of chemicals is log D (distribution coefficient). Log D is log K_{ow} (partition coefficient) corrected for ionisation at the ambient pH. The log D used in this thesis was log D at pH = 8. The chemicals were arranged from lower log D to higher log D. Results from Figure 5.13 show that despite log D varying from -1.0 to 4.9, the overall removal efficiency of sulfamethoxazole, caffeine, ketoprofen, naproxen, ibuprofen, paracetamol, gemfibrozil, oestriol, propylparaben, testosterone, 2-phenylphenol, oestrone, etiocholanolone, androsterone, 17β-estradiol and triclosan through the MBRs was always high, from above 90% to nearly 100%. This result was

consistent with results achieved from the package MBR at Wollumla, Bega Valley (presented in Chapter 4) as well as results from previous studies (Clara et al., 2005, Kim et al., 2007b, Radjenovic et al., 2007, Coleman et al., 2009, Radjenovic et al., 2009).

During the reproducibility experiment, some trace chemicals including atenolol, trimethoprim, diclofenac and carbamazepine were only partially removed by the MBRs. The overall removal of atenolol through the MBRs during reproducibility experiment varied from 60% to 89% between different sampling dates. Similarly, the overall removal was from 62% to 88% for trimethoprim and 63% to 89% for diclofenac during various sampling dates. The overall removal of carbamazepine was low, varying between 8% to 24%. These results were comparable with the results obtained from the package MBR plant in Wollumla, Bega Valley (Chapter 4). Previously reported studies have identified carbamazepine, diclofenac and trimethoprim as persistent compounds that are difficult to remove through MBRs. The reported removal efficiencies of these compounds were very variable, ranging from no removal or even negative removal for carbamazepine to 50% (Clara et al., 2004, Clara et al., 2005, Joss et al., 2005, de Wever et al., 2007, Kim et al., 2007b, Radjenovic et al., 2007, Vieno et al., 2007, Radjenovic et al., 2009). This most likely occur because these chemicals are not easily biodegradable and poorly adsorb to the biomass (Clara et al., 2005, de Wever et al., 2007, Kim et al., 2007b, Radjenovic et al., 2007, Radjenovic et al., 2009). The negative removal of carbamazepine was hypothesised due to the enzymatic cleavage of the glucuronic conjugate of carbamazepine and the re-release of the parent compound during the treatment process (Vieno et al., 2007).

Results from Figure 5.14 show that with the exception of triclocarban which had the percentage removal via adsorption to biomass varying from 75% to 100%, the percentage removal via adsorption to biomass for other reported chemicals was low. For sulfamethoxazole, caffeine, ketoprofen, naproxen, ibuprofen, paracetamol, gemfibrozil, oestriol, propylparaben, etiocholanolone, androsterone, atenolol and carbamazepine, the percentage removal via adsorption to biomass was less than 1.0% of the overall removal. The percentage removal via adsorption to biomass was from 0.4% to 1.8% for oestrone, from 1.3% to 2.5% for testosterone and from 0.8% to 3.5% for 2-phenylphenol. The percentage removal by adsorption to biomass was also insignificant for trimethoprim (from 0.1% to 1.3%) and diclofenac (from 0.9% to 2.9%). Removal via adsorption to biomass contributed 1.2%-6.3% to the overall removal of 17β-estradiol. The percentage removal via adsorption to biomass of triclosan in this

study was lower than that of Wollumla (Bega Valley) MBR study (7% compared to 52%). This may be due to higher SRT of the biomass used in this study compared to the Wollumla (Bega Valley) MBR study (30 days compared to 15 days). The results for overall removal efficiencies and removals via adsorption to biomass of these chemicals were reproducible between the 4 MBRs.

The removal mechanisms for trace organic chemical contaminants through MBRs are complex and include biotransformation, sorption to biomass, volatilisation and physical retention by the membrane (Stevens-Garmon et al., 2011). As the molecular weight cut off for ultra-filtration membranes is about 100-200 kDa, they are not expected to retain trace organic chemicals, unless the chemicals adsorb to larger particles (de Wever et al., 2007). In addition, previous studies state that adsorption of trace chemical contaminants on membrane itself is a temporary effect that occurs in the initial stages of filtration of clean membranes. When the membranes are saturated, this adsorption remains almost unchanged. In this study, the membranes in the reactors were operated and reached saturation before the experiments were conducted and the membranes were not backwashed or cleaned during the experiment. Thus, as discussed in Chapter 4, in this study, biotransformation and sorption to biomass are the two most important removal pathways for these trace chemicals. Results from this study show that the removals of most trace chemicals by adsorption to biomass were insignificant. This indicates that the main removal mechanism for these trace chemicals is biotransformation. The high biotransformability noted here can be attributed to the high SRT and MLSS concentration in the MBR (Clara et al., 2005, Chen et al., 2008b, Coleman et al., 2009).

A significant observation in mass balance calculation for triclocarban was identified here. As described in Chapter 4, the influent samples were filtered immediately after collection by Millipore glass fibre filtered paper with a pore size of 0.7 µm, after that a volume (500 mL in Wollumla MBR study in Chapter 4 and 250 mL in this experiment onwards) of filtered influent samples were measured by cylinders and transferred to amber glass bottles, then isotope-labelled internal standards of trace chemical contaminants were added to the sample bottles prior to solid phase extraction (SPE) and analysis by LC-MS/MS and GC-MS/MS. Therefore, the concentrations of trace chemicals in filtered influent were used to calculate the mass balance with an assumption that the concentrations of trace chemicals adsorbed to suspended solid in influent were negligible. This assumption was true for all other trace chemicals in this study as the fraction of these chemicals adsorbed to suspended solid in influent was

less than 1% of total load in the influent. However, a very hydrophobic chemical triclocarban with log D_{pH8} of 6.1 was identified as an exception. Triclocarban was found at a concentration up to 7.7 $\mu g.g^{-1}$ in the MBR biomass in this study. If the concentration of triclocarban in the MBR biomass was assumed to be the same as that in the suspended solids in the influent, then the load of triclocarban adsorbed to the suspended solids in the influent is significant, up to the same load of triclocarban in the filtered influent. Thus, the calculation of influent load of triclocarban is corrected to be included both the load of triclocarban in suspended solids and in filtered influent. Unfortunately, this problem was identified after all the experimental works of this PhD study were completed, therefore no modification of experimental procedure was possible. However, the concentration of triclocarban in the influent suspended solids was approximately accounted for by assuming it to be the same as the concentration of triclocarban in the MBR biomass. The data presented in Figure 5.14 and Table 5.4 have had this correction applied.

Table 5.4 Removals of consistently detected trace chemicals by the experimental MBRs

Chemicals	Overall removals (% relative to influent load)				Removals via adsorption to biomass (% relative to influent load)			
	MBR1 (mean ± stdev)	MBR2 (mean ± stdev)	MBR3 (mean ± stdev)	MBR4 (mean ± stdev)	MBR1 (mean ± stdev)	MBR2 (mean ± stdev)	MBR3 (mean ± stdev)	MBR4 (mean ± stdev)
Atenolol	76.5 ± 12.2	73.8 ± 12.7	69.2 ± 12.0	71.6 ± 11.7	0.11 ± 0.06	0.13 ± 0.08	0.06 ± 0.01	0.06 ± 0.01
Sulfamathoxazole	92.6 ± 2.41	93.1 ± 2.63	91.0 ± 3.65	91.4 ± 3.40	0.31 ± 0.09	0.35 ± 0.11	0.33 ± 0.10	0.35 ± 0.12
Caffeine	100 ± 0.02	100 ± 0.02	100 ± 0.01	99.9 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ketoprofen	98.9 ± 0.07	98.9 ± 0.06	98.7 ± 0.44	98.9 ± 0.06	0.28 ± 0.02	0.29 ± 0.02	0.28 ± 0.01	0.28 ± 0.03
Naproxen	99.4 ± 0.14	99.5 ± 0.21	98.9 ± 0.92	99.2 ± 0.54	0.03 ± 0.03	0.04 ± 0.04	0.04 ± 0.04	0.04 ± 0.02
Ibuprofen	99.9 ± 0.06	99.9 ± 0.03	99.9 ± 0.03	100 ± 0.02	0.02 ± 0.01	0.03 ± 0.03	0.03 ± 0.03	0.03 ± 0.01
Paracetamol	100 ± 0.06	100 ± 0.03	100 ± 0.05	100 ± 0.07	0.01 ± 0.01	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Trimethoprim	79.1 ± 6.61	77.9 ± 9.81	75.1 ± 10.2	76.0 ± 12.1	0.69 ± 0.68	0.58 ± 0.56	0.34 ± 0.28	0.25 ± 0.18
Diclofenac	77.1 ± 10.4	81.0 ± 8.99	77.2 ± 12.1	78.2 ± 13.6	1.66 ± 0.76	2.00 ± 0.86	1.49 ± 0.42	1.48 ± 0.30
Gemfibrozil	99.2 ± 0.63	99.5 ± 0.14	99.0 ± 0.93	99.3 ± 0.67	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Carbamazepine	14.0 ± 5.97	14.7± 6.34	16.3 ± 5.15	15.5 ± 6.08	0.76 ± 0.17	0.75 ± 0.14	0.72 ± 0.10	0.66 ± 0.10
Oestriol	99.5 ± 0.09	99.5 ± 0.09	99.5 ± 0.09	99.5 ± 0.09	0.16 ± 0.03	0.17 ± 0.03	0.16 ± 0.02	0.16 ± 0.02
Propylparaben	99.0 ± 0.83	99.0 ± 0.83	99.0 ± 0.83	99.0 ± 0.83	0.31 ± 0.27	0.31 ± 0.27	0.31 ± 0.27	0.31 ± 0.27
Testosterone	93.6 ± 1.54	93.6 ± 1.54	93.6 ± 1.54	93.6 ± 1.54	2.01 ± 0.49	2.03 ± 0.48	2.00 ± 0.40	2.01 ± 0.49
2-Phenylphenol	93.8 ± 4.17	93.8 ± 4.17	93.8 ± 4.17	93.8 ± 4.17	1.99 ± 1.33	2.01 ± 1.35	2.03 ± 1.43	1.99 ± 1.32
Oestrone	98.0 ± 1.56	98.9 ± 1.03	98.9 ± 0.60	99.3 ± 0.29	0.78 ± 0.59	0.78 ± 0.70	0.94 ± 0.42	0.86 ± 0.45
Etiocholanolol	99.9 ± 0.03	99.9 ± 0.03	99.9 ± 0.03	99.9 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01

Chemicals	**Overall removals (% relative to influent load)**				**Removals via adsorption to biomass (% relative to influent load)**			
	MBR1 (mean ± stdev)	**MBR2 (mean ± stdev)**	**MBR3 (mean ± stdev)**	**MBR4 (mean ± stdev)**	**MBR1 (mean ± stdev)**	**MBR2 (mean ± stdev)**	**MBR3 (mean ± stdev)**	**MBR4 (mean ± stdev)**
Androsterone	99.9 ± 0.02	99.9 ± 0.02	99.9 ± 0.02	99.9 ± 0.02	0.22 ± 0.08	0.26 ± 0.06	0.21 ± 0.06	0.15 ± 0.09
Triclosan	99.7 ± 0.22	99.8 ± 0.14	99.6 ± 0.25	99.7 ± 0.20	5.72 ± 0.61	6.13 ± 1.15	5.54 ± 0.54	5.24 ± 0.34
17β-Estradiol	95.1 ± 0.79	95.1 ± 0.79	95.1 ± 0.79	95.1 ± 0.79	2.80 ± 2.36	2.67 ± 2.21	2.64 ± 1.88	2.37 ± 1.42
Triclocarban	97.8 ± 2.13	98.6 ± 0.85	99.0 ± 0.42	98.5 ± 1.02	89.3 ± 9.53	91.4 ± 5.71	86.0 ± 5.04	92.8 ± 6.33

Trace chemicals excluded due to quality control

Bisphenol A was excluded due to quality control. DEET was also excluded as the ratio of quantification ion and qualification ion of the samples was outside the set ratio for valid quantification (±20%). It is possible that the DEET peak was contaminated from other chemical having one similar peak with the qualification or qualification peak of DEET.

5.7. CONCLUSIONS

The results from the clean water test revealed that the glue used to pot the membrane modules and the materials used to construct the MBRs may introduce chemical contaminants into the MBR systems. COD contaminant was detected in the MBR tanks and was characterised by EEM Spectroscopy to identify the source of COD contamination. It was concluded that the potting glue was indeed the source of the fluoresence peaks in the MBR tank water. It appeared that flushing was an effective method of eliminating the contaminant as the characteristic 'glue signal' decreased significantly after 3 d. COD was not detected in all samples taken after 9 d flushing the MBR system with ultra-pure water. As such, it was concluded that the identified fluoresence peaks were either the source of the COD contamination, or else a very useful surrogate measure of the COD contamination. Bisphenol A was detected at high and variable concentrations in various samples from the system. The results suggest that bisphenol A was leaching from the materials used to build the MBR system (i.e. the plastic influent tank, effluent tank, plastic pipes, or plastic valves etc.). Bisphenol A was therefore removed from the list of analytes and not further considered for the MBR performance analysis described in this study. DEET was also excluded from this study due to quality control.

The reproducibility experiments reveal that with the exception of triclocarban which had the percentage removal via adsorption to biomass varying from 75% to 100% between the four MBRs, results of key bulk water quality parameters, operational parameters and trace chemicals were reproducible between the four MBRs with a maximum standard deviation of 8% between the four MBRs. For atenolol, trimethoprim and diclofenac, although the overall removals of these chemicals were reproducible with a maximum standard deviation of 8% between the four MBRs, the standard deviations of overall removals of these chemicals between different sampling dates within the same MBR was larger, varied from 12 to 13 % for atenolol, from 7% to 12% for trimethoprim

and from 9 t0 14% for diclofenac. This needs to be taken into account when interpreting results from further experiments in coming chapters.

For a very hydrophobic chemical triclocarban, the load of this chemical adsorbed to the suspended solids in the influent was as significant as the load in the filtered influent so both loads need to be taken into account in the mass balance calculation. For other chemicals, the loads adsorbed to the suspended solids in the influent were negligible (< 1% of total load in the influent).

CHAPTER 6. IMPACTS OF TOXIC SHOCKS ON MBR PERFORMANCE

This chapter has been published in part in the following conference paper:

T. Trinh, H. Coleman, R. Stuetz, P. Le-Clech, J. Drewes and S. Khan, Impacts of 2,4 dinitrophenol shock on membrane bioreactor performance, **in proceeding of the Asian Pacific Water Recycling Conference**, 1-3 July 2013 in Brisbane.

6.1. INTRODUCTION

Toxic shock involves an influx of organic or inorganic elements, radicals or compounds, which wholly or partially inhibit, or damage the existing metabolic pathways or disrupt the established physiological condition of the microbial population (Gaudy and Engelbrecht, 1961). This chapter describes impacts of toxic shocks caused by various chemicals including an electron inhibitor 2,4-dinitrophenol (DNP), salinity and ammonia on MBR performance including impacts to pH, effluent chemical oxygen demand (COD), mixed liquor suspended solids (MLSS) concentration, mixed liquor volatile suspended solids (MLVSS) concentration, transmembrane pressure (TMP) and mixed liquor capillary suction time (CST). Removals of trace chemicals were also monitored to identify which trace chemicals provide useful roles as indicator chemicals to detect the impacts of the hazardous events on MBR performance. DNP shock was selected for these experiments as a representative shock caused by electron inhibitors. Salinity shock and ammonia shock were selected as these are common specific types of shock loads experienced by wastewater treatment plants (WWTPs).

6.2. MATERIALS AND METHODS

Experiments to assess impacts of toxic shocks were conducted in the experimental MBRs as described in Section 5.4 (Chapter 5). NH_4HCO_3, NaCl and DNP (all analytical standard) were purchased from Sigma Aldrich, Australia.

There have been a number of studies previously reporting the use of chemical shock experiments to assess the performance and/or resilience of activated sludge (AS) wastewater treatment processes (Ludzack and Noran, 1965, Kincannon and Gaudy, 1966, Kincannon and Gaudy, 1968, Li and Zhao, 1999, Kelly et al., 2004, Ng et al., 2005, Henriques et al., 2007). Based on these studies, 200 $mg.L^{-1}$ DNP was selected as a shock dose for the DNP shock experiments, 20 $g.L^{-1}$ NaCl was chosen as a shock dose for the salinity shock experiments and 700 $mg.L^{-1}$ ammonia was selected as a shock dose for the ammonia shock experiments. These shock doses were selected with the anticipation that there would be some visible impacts on the MBR performance.

In these experiments, ammonia was introduced in the form of ammonium bicarbonate (NH_4HCO_3). As previously reported by Henriques et al. (2007), this salt was selected in order to buffer against marked pH decreases due to nitrification increases, which would have otherwise complicated the assessment of shock from ammonia toxicity. Salinity

was introduced as sodium chloride (NaCl), as previously reported by Kincannon and Gaudy (1968), NaCl is one of the more common salts found in large amounts in some carriage waters and is a major inorganic constituent of wastes from several industrial processes. DNP was introduced as an uncoupling chemical to inhibitor energy (ATP) production as it is know to cause a reduction biochemical oxygen demand (BOD) removal efficiency during biological treatment processes (Love and Bott, 2002).

The four experimental MBRs were operated simultaneously. One of the MBRs was operated under steady-state conditions as a control and the other three MBRs were subjected to DNP shock, salinity shock and ammonia shock, respectively.

DNP, NaCl and NH_4HCO_3 were each introduced as a single shock-dose to the mixed liquor (bioreactor tank) of the specified MBR. Initial mixed liquor concentrations were as indicated above. Influent samples (0.25 L) were taken in triplicate every day after filling the influent tank. Effluent samples (1 L) from the control and the shock reactors were taken before introducing the shocks (t = 0 h) and after introducing the shocks at 1 h, 2 h, 3 h, 24 h, 48 h and 72 h. Mixed liquor samples (0.5 L) were also taken before introducing the shocks (t = 0 h) and after introducing the shocks at 3 h, 24 h, 48 h and 72 h (1 h and 2 h samples were not collected in order to avoid significantly impacting the MLSS concentration due to sample collection). Influent and effluent samples were analysed for pH, chemical oxygen demand (COD) and trace organic chemicals. Mixed liquor samples were analysed for mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), capillary suction time (CST) and trace organic chemicals. Transmembrane pressure (TMP) was also continuously monitored throughout these experiments. The methods for these analyses were described in Chapter 3.

6.3. RESULTS AND DISCUSSION

Results of key bulk water quality and operational parameters including pH, COD, MLSS, MLVSS, CST and TMP during toxic shock experiments are presented in this section.

6.3.1. pH

pH of the MBR permeate from the DNP shock reactor was around 6.3 to 6.8, which is similar to that of the control experiment. During the first 24 h, the pH of the MBR permeate from the salinity shock reactor varied between 6.7 to 7.4, which is within the

range observed during the reproducibility experiments (Chapter 5). During this time, the pH in the ammonium shock reactor was observed to increase to between 6.7 and 8.2. All pH values were observed to revert to within the control range by the end of the experiment trials.

6.3.2. COD

The variability in COD removal efficiency for the control and the toxic shock studies is presented in Figure 6.1. Results show that after introducing DNP, COD removal efficiency in the shocked reactor, immediately decreased from 92% to 69% after 1 h and was reduced further to 64% after 2 h and 59% after 3 h. The COD removal efficiency was then stable for the remaining of the first 48 h after the shock load. Only partial recovery (to 74%) was observed within the 72 h experiment period.

DNP is a well-known inhibitor of efficient energy (ATP) production in cells with mitochondria. It uncouples oxidative phosphorylation by carrying protons across the mitochondrial membrane, leading to a rapid consumption of energy without generation of ATP. As such, at high concentrations, DNP can disrupt a variety of important bacterial metabolic processes (Brummett and Ordal, 1977, Decker and Lang, 1977, Nicholas and Ordal, 1978, Bakker and Randalll, 1984, Henriques et al., 2005).

Bacteria have been shown to exhibit biochemical indicators of stress in response to DNP shock (Bott et al., 2001). Furthermore, under DNP shock conditions, significant potassium (K+) efflux has been reported, resulting in deflocculation of biomass (Bott and Love, 2002, Love and Bott, 2002).

A wide variety of DNP concentrations have been reported for the inhibition of COD removal efficiency for AS systems. One study using a batch AS reactor fed with synthetic wastewater found that at 20 $mg.L^{-1}$ DNP, COD removal was reduced from 90% to 53% (Chen et al., 2006a). In contrast, a study using a sequencing batch reactor (SBR) fed with municipal wastewater reported no effect on COD removal efficiency at DNP concentration up to 107 $mg.L^{-1}$ (Henriques et al., 2007). It has been hypothesised that some variation may be explained by variable endogenous concentrations of DNP (or other chemicals with similar properties) in municipal wastewaters, and hence, variable populations of DNP-degrading bacteria in wastewater treatment plants (Jo and Silverstein, 1998).

The experiment reported here was conducted in an MBR fed with real municipal wastewater and real biomass from an existing MBR. Hence, the presence of DNP-degrading bacteria is likely. However, the shock dose of 200 mg.L^{-1} was selected such that it would exceed the likely toleration of the biomass in the MBR, leading observable inhibition of biodegradation processes and a significant reduction in COD removal efficiency.

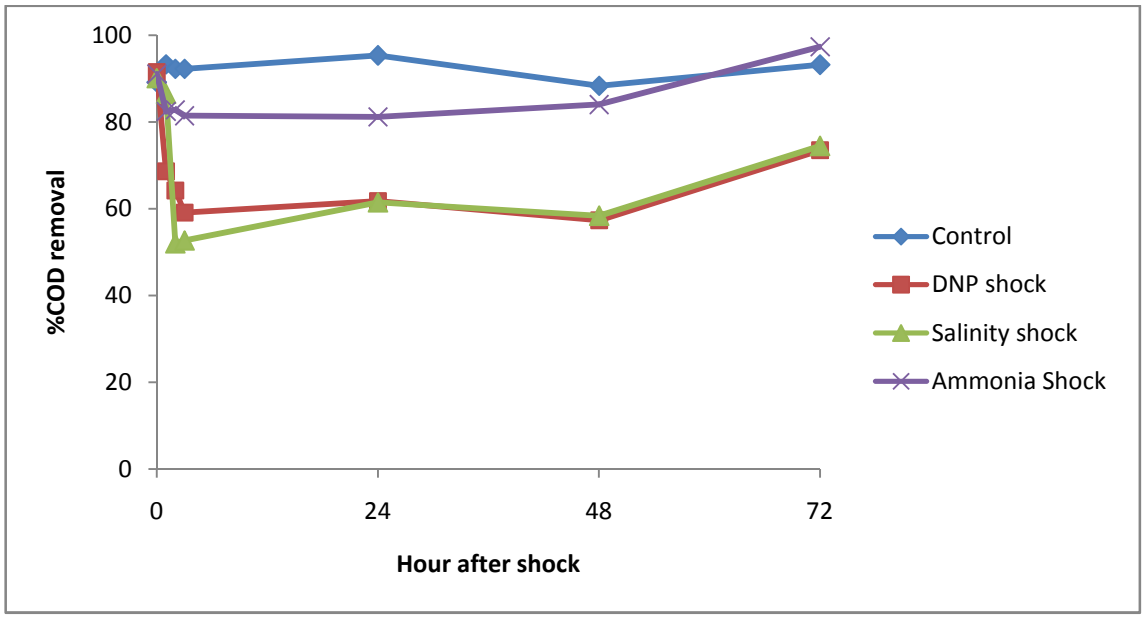


Figure 6.1 COD removal efficiency of the control and the DNP, salinity and ammonia shock reactors

The impact of salinity shock on COD removal efficiency by the MBR was similar to that of the DNP shock, after introducing NaCl, COD removal efficiency in the salinity shock reactor immediately decreased from 92% to 86% after 1 h and reduced further to 53% after 2 h and 52% after 3 h. This result was consistent with a number of previous studies on MBRs (Reid et al., 2006, Yogalakshmi and Joseph, 2010) and AS reactors (Ludzack and Noran, 1965, Kincannon and Gaudy, 1966, Kincannon and Gaudy, 1968, Ng et al., 2005). These studies found that COD removal was severely reduced when influent NaCl concentrations reached 20 g.L^{-1}. This is the result of the salty conditions producing a higher osmotic pressure on bacterial cells, which then inhibits bacterial growth and floc formation (Dan et al., 2003). A decrease in COD removal efficiency was found to correlate almost linearly with increasing influent NaCl concentrations between 20 to 60 g.L^{-1} (Ng et al., 2005). The COD removal efficiency in the salinity shock reactor recovered slightly to around 60% after 24 h and remained around this level until 48 h. It then continued to recover to 74% after 72 h from the shock exposure.

The ammonia shock produced less significant impacts on COD removal efficiency compared to the DNP and salinity shocks. After introducing NH_4HCO_3, COD removal efficiency in the shock reactor immediately decreased from 90% to 82% after 1 h and remained at this level until 24 h. The COD removal efficiency then improved slightly to around 84% after 48 h and fully recovered to 97% after 72 h. This result is consistent with a previous study, which found that COD removal efficiency of an AS reduced from 98% to 78% when the ammonia-N concentration increased from 50 $mg.L^{-1}$ to 800 $mg.L^{-1}$ (Li and Zhao, 1999). High ammonia concentration has shown to inhibit microbial activity by reducing the specific oxygen uptake rate (SOUR) and dehydrogenase enzyme activity (Li and Zhao, 1999, Henriques et al., 2007).

6.3.3. MLSS and MLVSS

MLSS and MLVSS concentrations in mixed liquor of the control and the toxic shock reactors are presented in Figure 6.2 and Figure 6.3. Results show that the MLSS and MLVSS concentrations in the DNP shock and the ammonia shock reactors were lower than that of the control. This result was expected since DNP and ammonia have been shown to inhibit the growth of activated sludge (Li and Zhao, 1999, Chen et al., 2006a, Henriques et al., 2007). The MLSS and MLVSS results of the DNP shock reactor show the same qualitative trends with previous investigations of AS systems (Kelly et al., 2004, Chen et al., 2006a, Henriques et al., 2007). However, the magnitude of the biomass reduction in the DNP shock reactor was smaller than those of these previous studies. This may be due to the much higher MLSS concentrations in MBRs in the current study compared to the MLSS concentrations in AS. There is evidence that reactors with higher MLSS and MLVSS concentrations provide better tolerance to DNP shock (Hess et al., 1993, Jo and Silverstein, 1998).

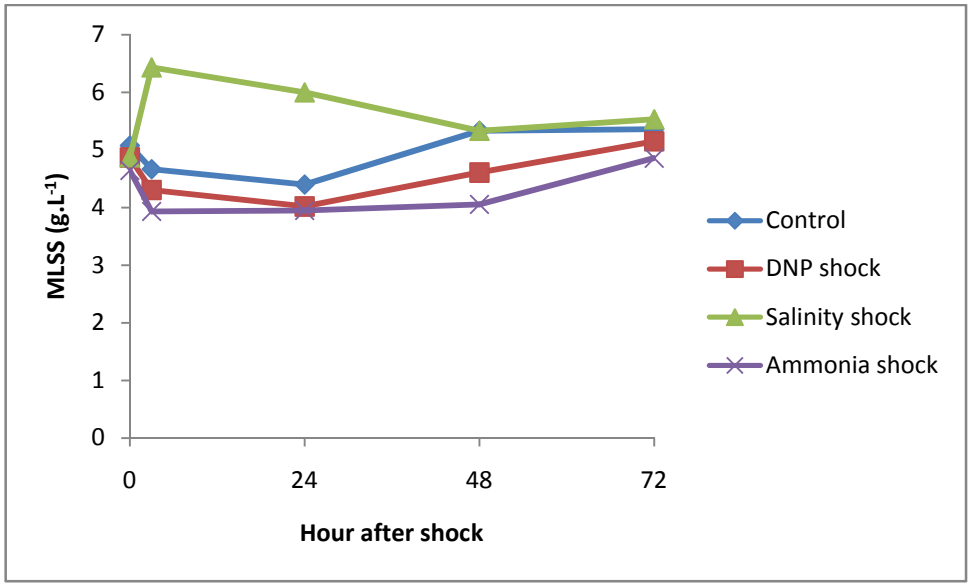


Figure 6.2 MLSS concentrations of the control and the DNP, salinity and ammonia shock reactors

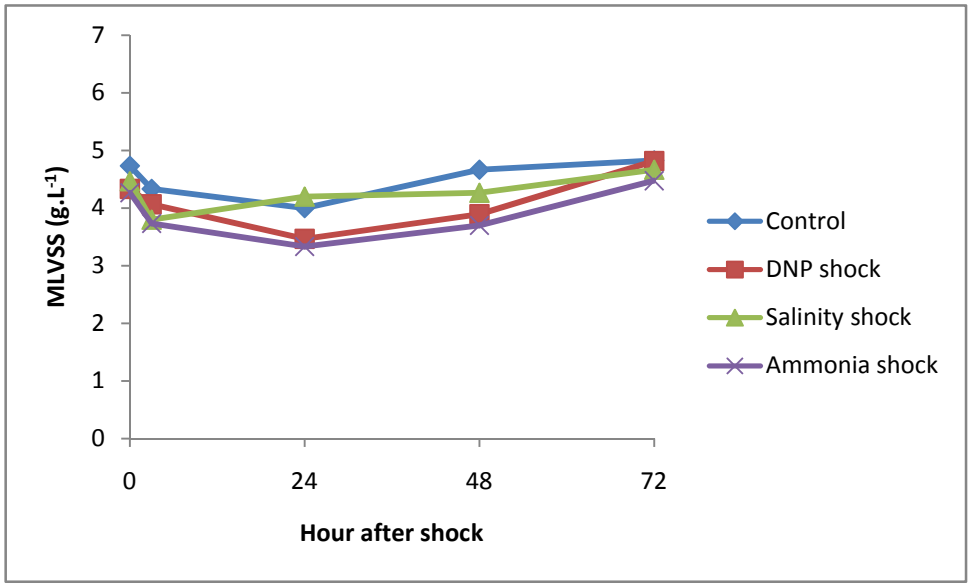


Figure 6.3 MLVSS concentrations of the control and the DNP, salinity and ammonia shock reactors

In contrast to the DNP and ammonia shock experiments, MLSS concentrations in the salinity shock reactor were observed to increase from 4.9 $g.L^{-1}$ to 6.4 $g.L^{-1}$ within 3 h after introducing NaCl. It then decreased to 6 $g.L^{-1}$ after 24 h. MLSS concentrations returned to the same value as the control (5.3 $g.L^{-1}$) after 48 h and remained at the same level until the end of the experiment. The apparent increase in MLSS concentrations after introducing NaCl may be due to the incorporation of Na^+ and Cl^- ions onto biomass floc, thus increasing its mass. This explanation is supported by the observation of white salt crystals on the dried MLSS filter papers from the samples

collected after 3 h and 24 h. Although the MLSS concentrations increased after introducing the salinity shock, the MLVSS concentrations of the reactor remained at similar level as the control across the experiment. Again, this provides further support for the hypothesis that the apparent MLSS concentration increase after introducing the salinity shock was due to additional mass from NaCl. It is known that an NaCl shock load can cause changes in population variety and activity of activated sludge (Stewart et al., 1962, Ludzack and Noran, 1965, Ng et al., 2005). Sludges grown in high salt concentrations have low carbohydrate and protein contents and abnormally high lipid and ribonucleic acid (RNA) contents (Kincannon and Gaudy, 1966). Sustained high chloride concentrations generally depress respiration (Ludzack and Noran, 1965). High salt concentrations also reduce gravity separation due to lower density difference between water and biomass (Ng et al., 2005) and thus decrease biomass settleability (Ludzack and Noran, 1965).

6.3.4. CST and TMP

CST of mixed liquor from the control and the toxic shock reactors are presented in Figure 6.4. Results show that after introducing the shocks, the CST from the DNP and ammonia shock reactors, increased quickly and was significantly higher than that of the control after 24 h. This implies that filterability of the mixed liquor from the DNP shock reactor and ammonia shock reactors was notably reduced. This result is in agreement with previous AS studies which found that activated-sludge settling and dewatering properties could be deteriorated in the presence of ammonia concentrations higher than 20 $mg.L^{-1}$ or DNP concentrations higher than 5 $mg.L^{-1}$ (Novak, 2001, Chen et al., 2006a, Henriques et al., 2007). High concentration of monovalent cations like ammonium could replace divalent cations in the floc, weakening the binding biopolymers and causing weaker and less-dense flocs (Higgins and Novak, 1997, Novak, 2001). Following this, the settling properties of the sludge improved and recovery seemed to have been due to replacement with new flocs rather than alteration of the existing biomass (Novak, 2001). DNP shock causes change in sludge hydrophobicity and thus affects its settling and dewatering ability (Chen et al., 2006a). CST of the DNP shock reactor was remained higher than that of the control while CST of the ammonia shock reactor was fully recovered 72 h after introducing the shocks. CST of the NaCl shock experiment was slightly increased 24 h after introducing NaCl and continued rising slowly after 48 h. It was then recovered and nearly reached the control value after 72 h.

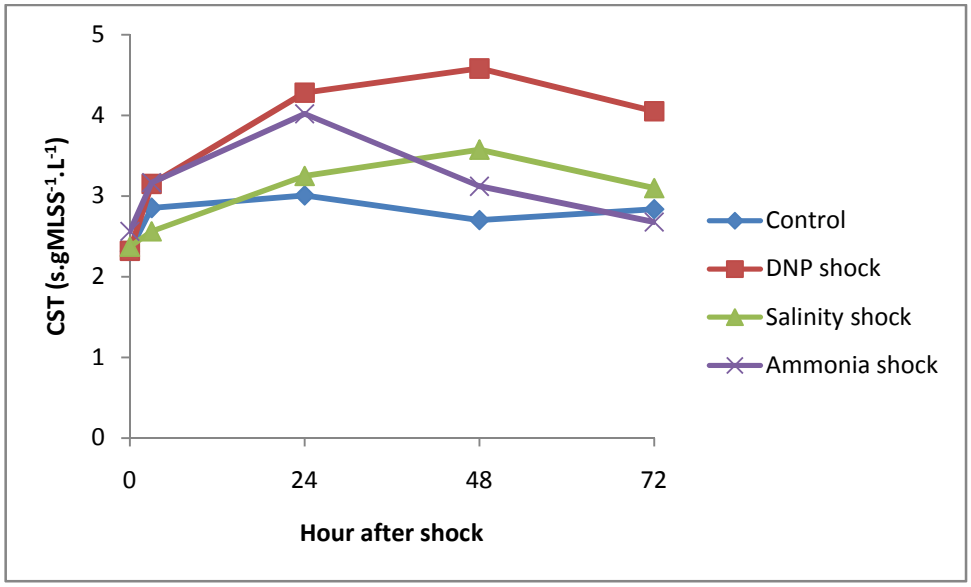


Figure 6.4 CST of the control and the DNP, salinity and ammonia shock reactors

After introducing the shocks, the TMP of the DNP shock and salinity shock reactors rose rapidly from 3-5 kPa and reached 25 kPa after 24 h, while the TMP of the control slowly rose from 3 kPa and reached 11 kPa after 24 h. The TMP of the DNP shock and salinity shock reactors continued rise quickly and reached 34 kPa after 72 h whereas the TMP of the control increased more slowly, reaching 22 kPa after 72 h. TMP of the ammonia shock reactor increased more slowly than that of the DNP and salinity shock reactors, reaching 28 kPa after 72 h. Previous studies have reported that a natural response of bacteria upon exposure to a toxic shock can be to increase the release of soluble microbial product (SMP) and extracellular polymeric substance (EPS) into the mixed liquor (Aquino and Stuckey, 2004, Chen et al., 2006a, Reid et al., 2006). This is generally believed to be the cause of increases in CST and fouling leading to rising in TMP in MBRs under toxic shock conditions (Reid et al., 2006, Judd and Judd, 2011).

6.3.5. Trace chemicals

Removals of trace organic chemicals by the MBRs following DNP, salinity and ammonia shock conditions are compared with removals under control conditions in this section. Among the analysed chemicals, 17α-estradiol, 17α-ethynylestradiol, mestranol, levonorgestrel, nonylphenol, 4-tert-octylphenol, diazepam, dilatin, meprobamate, enalapril, hydroxyzine, omeprazole, simvastatin, simvastatin hydroxy acid, atrazine and linuron were not detected in influent samples. Some trace chemicals (dihydrotestosterone, androstenedione, amitriptyline, risperidone, triamterene) were only detected in a few influent samples.

The results of trace chemicals that were consistently detected in the influent during the experimental period were divided in 3 groups including hydrophilic chemicals (log D_{pH8} < 2), moderately hydrophobic chemicals (2 ≤ log D_{pH8} ≤ 3. 2), and very hydrophobic chemicals (log D_{pH8} > 3.2), as previously described by (Tadkaew et al., 2011).

Figure 6.5 presents the removal efficiencies of hydrophilic trace chemicals by the DNP, salinity and ammonia shock reactors in comparison with the control reactor. Similar to COD results, DNP and salinity shocks were observed to impact the trace chemical removal efficiencies to a greater extent than the ammonia shock did.

After introducing the DNP shock, the overall removals of sulfamethoxazole, ibuprofen, ketoprofen, gemfibrozil and naproxen were significantly reduced from above 80% to below 40% and remained at this level or just slightly improved at 72 h after the shock. However, the removals of caffeine and paracetamol in the shock reactor were only reduced slightly by the DNP shock. This may be because paracetamol and caffeine are very easily biotransformable compounds and their biotransformation is thus maintained by the subset of organisms or metabolic processes not impacted by the shock. The easily biotransformable characteristic of paracetamol in an MBR has previously been demonstrated (Joss et al., 2006). As shown in Table 6.1, paracetamol has a relatively high biotransformation rate constant (K_{biol} = 106 - 240 $L.gMLSS^{-1}.d^{-1}$) compared to other hydrophilic chemicals including sulfamathoxazole, ketoprofen, naproxen, ibuprofen and gemfibrozil (K_{biol} from 0.2 to 38 $L.gMLSS^{-1}.d^{-1}$). A reported K_{biol} for caffeine could not be identified, but a study on the biotransformability of this compound confirmed that caffeine is a very easily biotransformable substance (Lin et al., 2010).

Table 6.1 Published biotransformation rate constants (K_{biol}) for hydrophilic chemicals

Chemicals	Process conditions: SRT (d)/MLSS(g.L^{-1})/t (◦C)	K_{biol} (L.gMLSS^{-1}.d^{-1})	References
Sulfamethoxazole	MBR: >100/7.2/25	0.13-0.39	(Fernandez-Fontaina et al., 2013)
	MBR: >100/6.2/16	0.18-0.22	(Abegglen et al., 2009)
	MBR: >150/3.8/23	0.16-0.22	(Abegglen et al., 2009)
Caffeine	Lab-scale aqueous biodegradation experiment	n.a but study concluded that caffeine is very easy biotransformable.	(Lin et al., 2010)
Ketoprofen	AS: n.a/2.5/20	0.03	(Urase and Kikuta, 2005)
Naproxen	MBR: >100/7.2/25	0.65-5.45	(Fernandez-Fontaina et al., 2013)
	MBR: >100/6.2/16	0.06-0.96	(Abegglen et al., 2009)
	MBR:30-40/3.2/17	0.4-0.8	(Joss et al., 2006)
Ibuprofen	MBR: >100/7.2/25	7.8-49.3	(Fernandez-Fontaina et al., 2013)
	MBR: >100/6.2/16	>3	(Abegglen et al., 2009)
	MBR: >150/3.8/23	1.31-1.35	(Abegglen et al., 2009)
	MBR:30-40/3.2/17	9-22	(Joss et al., 2006)
Paracetamol	MBR:30-40/3.2/17	106-240	(Joss et al., 2006)
Gemfibrozil	MBR:30-40/3.2/17	0.06	(Urase and Kikuta, 2005)

In the salinity shock reactor, the overall removals of sulfamethoxazole and ibuprofen reduced from above 90% to below 70% in the first 24 h after introducing the shock, but they recovered fully after 72 h. The overall removals of gemfibrozil, naproxen and ketoprofen reduced from above 99% to 21%-66% in the first 24 h after introducing the shock. They then decreased further to 9%- 46% after 48 h and slightly recovered after 72 h. In contrast to other hydrophilic chemicals, removal of caffeine and paracetamol through the MBR was not affected by the salinity shock. This again may be explained by the more readily biotransformability of caffeine and paracetamol compared to those of other hydrophilic chemicals.

After introducing the ammonia shock, the overall removal efficiencies of ketoprofen and gemfibrozil were significantly reduced while the removals of other hydrophilic chemicals including sulfamethoxazole, ibuprofen, caffeine, paracetamol and naproxen were only slightly reduced or not affected. Among these hydrophilic chemicals, ketoprofen and gemfibrozil have the lowest reported biotransformation rate constants K_{biol} as shown in Table 6.1. Hence the relatively high susceptibility of their biotransformation to ammonia

shock suggests that the least readily biotransformable compounds are the least resistant to loss of biotransformability from toxic shocks. This is logically consistent with the previous observation (above) that the most biotransformable compounds are the most resistant to impacts from toxic shock. The susceptibility of ketoprofen and gemfibrozil to toxic shock may be explained by the reliance upon specific organisms or metabolic pathways for their biotransformation. As these organisms or metabolic pathways are impeded, so too is the biotransformation of these chemicals. Removal of ketoprofen and gemfibrozil was fully recovered within 72 h after shock.

Results from Figure 6.5 confirm that removals via adsorption to biomass were an insignificant removal mechanism (< 2%) for these hydrophilic chemicals and this was unchanged under DNP, salinity and ammonia shock conditions. Biotransformation was the main removal mechanism for these hydrophilic chemicals and this removal mechanism was generally inhibited under DNP, salinity and ammonia shock conditions since the some biological transformation processes of the reactors were inhibited.

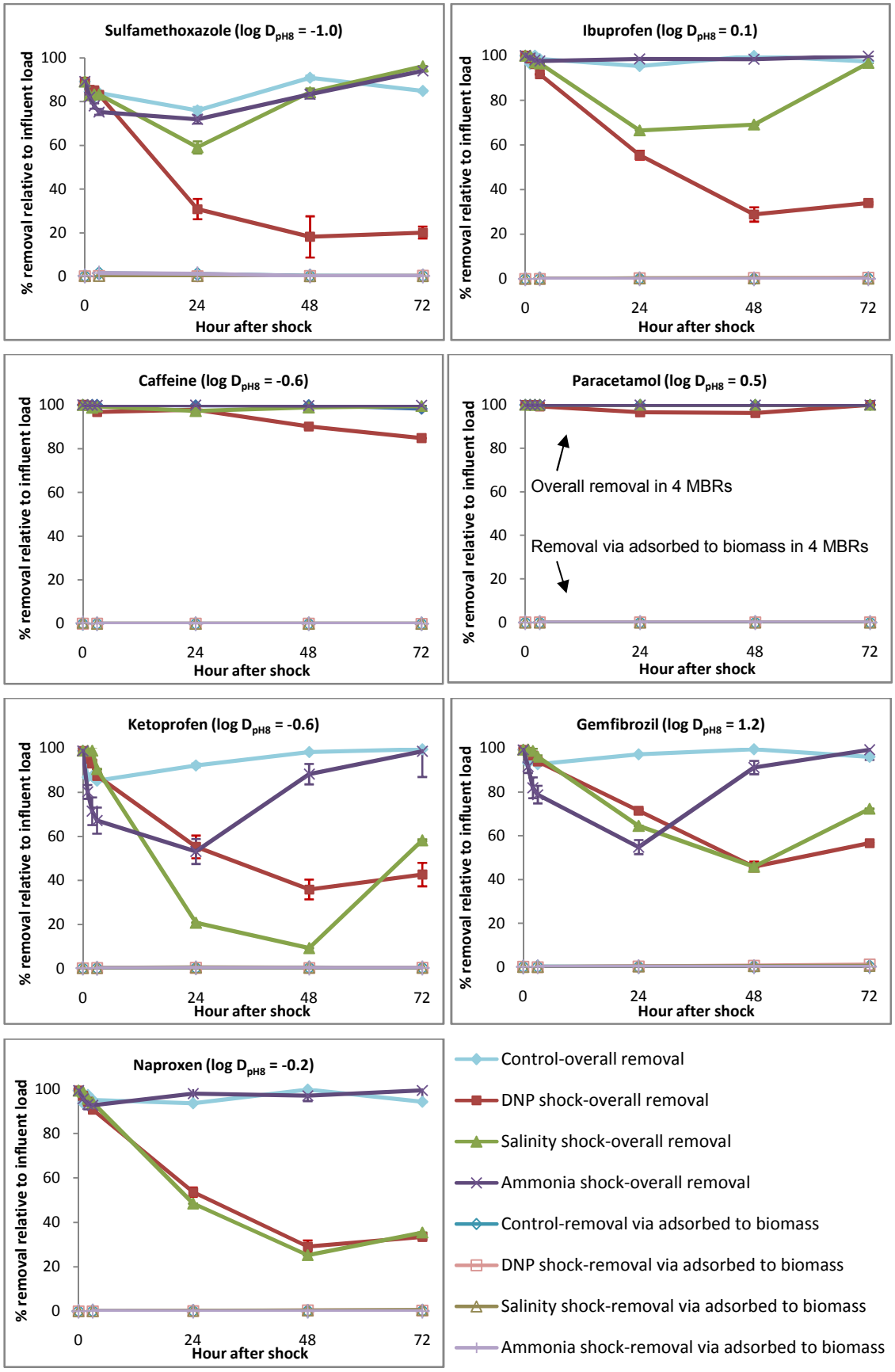


Figure 6.5 Removals of hydrophilic chemicals (log D_{pH8} < 2) by the control and the DNP, salinity and ammonia shock reactors

Figure 6.6 presents the removal efficiencies of moderately hydrophobic chemicals by the control and the toxic shock reactors. Results show that the overall removals of the moderately hydrophobic chemicals oestriol, propylparaben and testosterone were high (above 90%) and not affected by DNP, salinity and ammonia shocks. Removals via adsorption to biomass were an insignificant removal mechanism for these chemicals. It is noted that the concentration of testosterone in the biomass was less than the limit of quantification (LOQ), so the LOQ value was used to calculate the mass balance, so the percentage of testosterone removed via adsorption to biomass was < 5%.

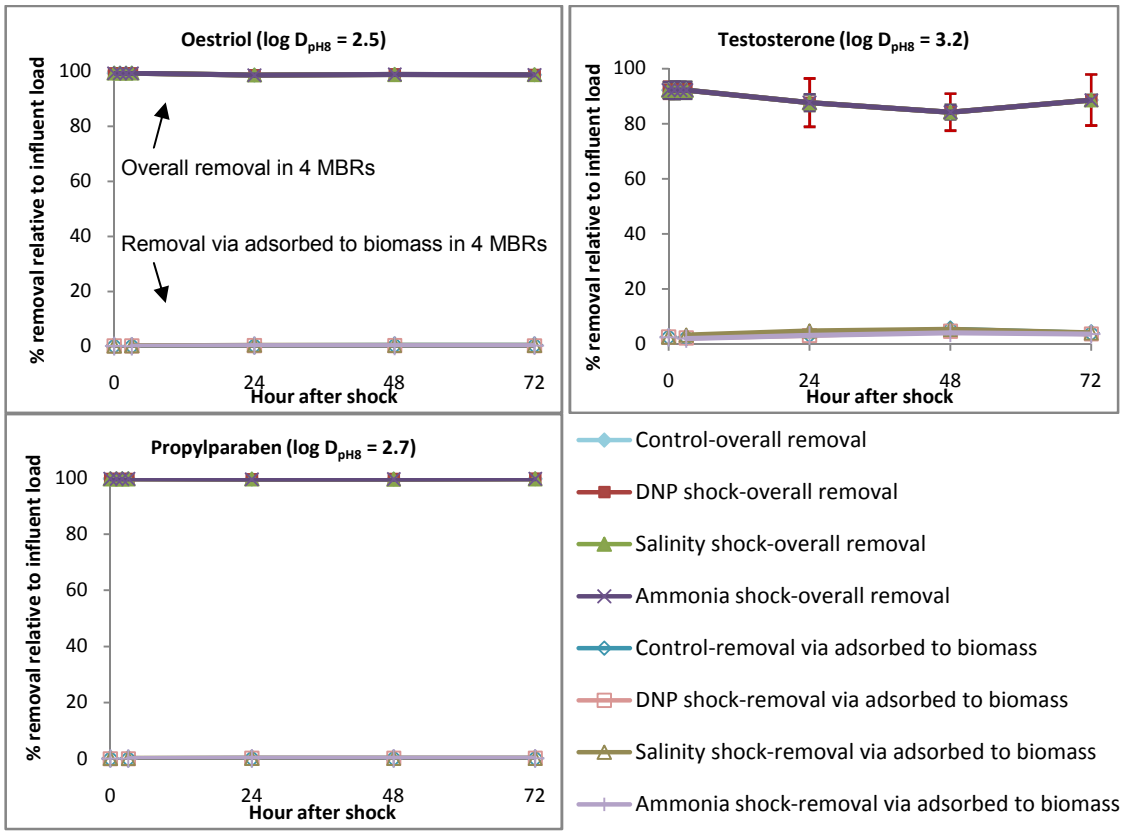


Figure 6.6 Removals of moderately hydrophobic chemicals (2 ≤ log D_{pH8} ≤ 3. 2) by the control and the DNP, salinity and ammonia shock reactors

Figure 6.7 presents the removal efficiencies of very hydrophobic chemicals by the control and the toxic shock reactors. Similar to moderately hydrophobic chemicals, results show that the removal efficiencies of very hydrophobic chemicals were not affected by the DNP, salinity or ammonia shock conditions. The overall removals of 2-phenylphenol, oestrone, etiocholanolone, androsterone, 17β-estradiol, triclosan and triclocarban were always high, above 90% during the experiment. Removal via adsorption to biomass contributed up to 14% to the overall removal of triclosan. For triclocarban, the percentage removal via adsorption to biomass was 90%-100% in the control reactor and 80%-100% in the shock reactors. This variation was within the range observed during reproducibility experiment in Chapter 5. The removals by adsorption to biomass were less than 5% for the other hydrophobic chemicals.

In general, the results show that under DNP, salinity and ammonia shock conditions, the removals of moderately and very hydrophobic chemicals were not affected. It is possible that the shocks inhibited biotransformation processes in the aqueous phase of the reactors but, within the biomass structures, some biotransformation processes continued largely unaffected. The moderately hydrophobic and very hydrophobic chemicals can adsorb to the biomass and thus these chemicals were still biotransformed. For hydrophilic chemicals, the removals of chemicals with moderate or less readily biotransformability were significantly reduced while the removals of easily biotransformable chemicals were just slightly affected.

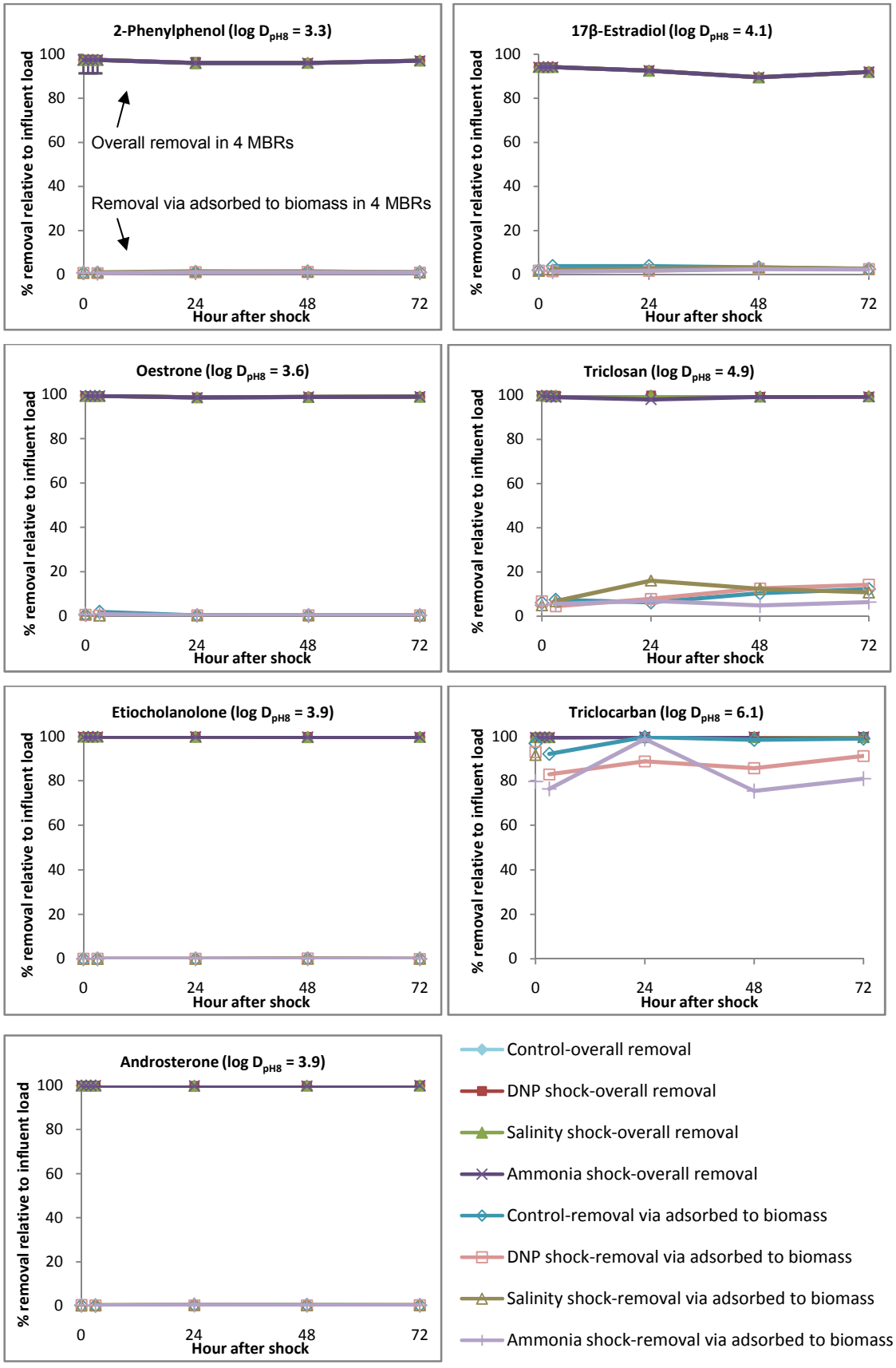


Figure 6.7 Removals of very hydrophobic chemicals (log D_{pH8} > 3.2) by the control and the DNP, salinity and ammonia shock reactors

6.4. CONCLUSIONS

DNP and salinity shock conditions were shown to cause a significant reduction in COD removal and a considerable increase in CST and TMP. CST in the ammonia shock reactor also increased significantly after introducing the shock. TMP in the ammonia shock reactor increased faster than that of the control but slower than that of DNP and salinity shock reactors. These results reveal that COD removal, CST and TMP are effective MBR operational parameters for monitoring impacts of toxic shocks such as DNP, salinity and ammonia shocks on MBR performance.

Under DNP, salinity and ammonia shock conditions, the removal of moderately and very hydrophobic chemicals was not affected. It is possible that the shock inhibited biotransformation processes in the aqueous phase of the reactors but, within the biomass structures, some biotransformation processes continued largely unaffected. The moderately hydrophobic and very hydrophobic chemicals can adsorb to the biomass and thus these chemicals were still being biotransformed. For hydrophilic chemicals, the most biotransformable compounds (caffeine and paracetamol) were found to be the most resistant to impacts from toxic shocks while the least biotransformable compounds (ketoprofen and gemfibrozil) are found to be the least resistant to loss of biotransformability from the shocks. The toxic shocks appear to impact some specific organisms or metabolic processes, thus most affecting the less readily biotransformable chemicals (ketoprofen and gemfibrozil), which rely upon specific organisms or metabolic pathways for their biotransformation. In contrast, readily biotransformable compounds such as caffeine and paracetamol may be more easily transformed by a wider range of organisms or metabolic pathways so they are still widely biotransformed under the shock conditions. Therefore, hydrophilic chemicals with low biotransformability (e.g ketoprofen and gemfibrozil) may be sensitive indicators for monitoring impacts of toxic shocks on removal of trace chemicals by MBR.

CHAPTER 7. IMPACTS OF ORGANIC SHOCK AND FEED STARVATION CONDITIONS ON MBR PERFORMANCE

7.1. INTRODUCTION

Exposure to sudden organic shock loads has been an important area of research aimed at understanding the impacts of hazardous events to biological systems for more than 50 years (Gaudy and Engelbrecht, 1961). This is because of the common occurrence of challenging conditions with organic composition in influent wastewater often varying substantially over a single diurnal period as well as from day to day (Selna and Schroeder, 1979). However, the majority of the previously reported research on the impacts of organic shock loads has been undertaken on activated sludge (AS) systems (Saleh and Gaudy, 1978, Normand and Perdrieux, 1981, Manickam and Gaudy, 1985, Mora et al., 2003, Thanh et al., 2009, Seetha et al., 2010). Comparatively very little research has been conducted using MBR systems (Al-Malack, 2007). Most of these studies on both AS and MBR were conducted using lab-scale reactors treating synthetic wastewaters (Saleh and Gaudy, 1978, Normand and Perdrieux, 1981, Manickam and Gaudy, 1985, Mora et al., 2003, Thanh et al., 2009, Seetha et al., 2010). The reported impacts have been related to the impairment of the ability of the systems to remove measurable bulk parameters and nutrients. Similar to organic shock loads, much of the previous studies on the impacts of feed starvation conditions have been carried out on AS systems (Kjelleberg et al., 1987, Urbain et al., 1993, Coello Oviedo et al., 2003, Li et al., 2006). Little starvation research has been conducted on MBRs (Yogalakshmi et al., 2007, Le-Minh, 2011). As such, there has been an important knowledge gap regarding the impacts of organic shock and feed starvation conditions on MBR performance, especially their impacts on the removal of trace chemical contaminants.

This chapter reports the impacts of organic shock and feed starvation conditions on MBR performance including impacts to pH, effluent chemical oxygen demand (COD), mixed liquor suspended solids (MLSS) concentration, mixed liquor volatile suspended solids (MLVSS) concentration, transmembrane pressure (TMP) and mixed liquor capillary suction time (CST). Removals of trace chemicals were also monitored to identify which trace chemicals provide useful roles as indicator chemicals to detect the impacts of the hazardous events on MBR performance

7.2. MATERIALS AND METHODS

Experiments to assess impacts of organic shock and feed starvation conditions were conducted in the experimental MBRs as described in Section 5.4 (Chapter 5). Glucose

and glutamic acid (analytical standard grade) was purchased from Sigma Aldrich, Australia.

A COD concentration of 5 $g.L^{-1}$ was selected as a shock dose for the organic shock experiment based on previously reported investigations using AS systems (Gaudy and Engelbrecht, 1961, Saleh and Gaudy, 1978, Normand and Perdrieux, 1981, Manickam and Gaudy, 1985, Urbain et al., 1993, Coello Oviedo et al., 2003). A 6 day feed starvation period was selected for the starvation experiment based on previous starvation studies using MBR systems (Yogalakshmi et al., 2007, Le-Minh, 2011). These shock conditions were selected with the anticipation that there would be some visible impacts on the MBR performance.

A control MBR was run in parallel to a MBR subjected to organic shock conditions. Organic shock was introduced in the form of a mixture of glucose and glutamic acid (1:1) to represent a range of assimilable organic compounds. The glucose/glutamic acid mixture was introduced as a single dose to the mixed liquor (bioreactor tank) of the shock MBR. Initial mixed liquor concentration was 5 $g.L^{-1}$ COD. Influent samples (0.25 L) were taken in triplicate every day after filling the influent tank. Effluent samples (1 L) from the control and the shock reactor were taken before introducing the shock (t = 0 h) and after introducing shock at 1 h, 2 h, 3 h, 24 h, 48 h and 72 h. Mixed liquor samples were taken before introducing the shock (t = 0 h) and after introducing the shock at 3 h, 24 h, 48 h and 72 h (1 h and 2 h samples were not collected in order to avoid significantly impacting the MLSS concentrations due to sample collection). Influent and effluent samples were analysed for pH, COD and trace chemicals. Mixed liquor samples were analysed for MLSS, MLVSS, CST and trace chemicals. TMP was also continuously monitored throughout these experiments. The methods for these analyses were described in Chapter 3.

Similar to the organic shock experiment, a control MBR was run in parallel to a MBR subjected to feed starvation conditions. The starvation conditions were simulated by stopping the feed to the MBR. During the starvation period, the permeate pump remained on and the permeate was recycled to the MBR to prevent effects on membrane performance. Influent samples (0.25 L) were taken in triplicate every day after filling the influent tank. Effluent samples (1 L) from the control and the shock reactor were taken before starvation (t = 0 h). During the starvation period, no effluent sample from either the control or the starvation MBRs was taken. After 6 days under starvation, MBR feeding was reinitiated and effluent samples were taken from both the control and the starvation MBRs every day for 4 days. Influent and effluent samples

were analysed for pH, COD and trace chemicals. Mixed liquor samples from both the control and the starvation MBR reactors were taken daily during the experiment including the starvation period when possible (excluding weekends and public holidays as access to the plant was not permitted). However, during the starvation period, only 15 mL of mixed liquor samples were taken for MLSS and MLVSS analysis while before and after starvation period, 500 mL samples were taken for MLSS, MLVSS and trace chemical analysis. TMP was also continuously monitored throughout these experiments. The methods for these analyses were described in Chapter 3.

7.3. RESULTS AND DISCUSSION

7.3.1. Key bulk water quality and MBR operational parameters

Organic shock experiment

Results of key bulk water quality and MBR operational parameters including pH, COD, MLSS, MLVSS, CST and TMP during the organic shock experiment are presented in this section. pH of the MBR effluent from the shock reactor decreased from 7.0 to 3.8 in the first 3 h after introducing the shock. This was because the large amount of glutamic acid added in the reactor exceeded the metabolic rate of the existing microorganisms. Thus, this organic acid was accumulated in the reactor leading to a decrease in pH of the mixed liquor and MBR effluent". The pH slightly increased to 4.9 after 24 h and fully recovered back to 7.9 after 48 h indicating that the microorganisms had metabolised the accumulated glutamic acid in the reactor. pH of the MBR effluent from the control remained at around 7.0 throughout the experiment.

As COD was introduced directly into the MBR in the organic shock experiment, the COD data was not presented as removal efficiency but presented as effluent COD in the control and the organic shock reactors (Figure 7.1).

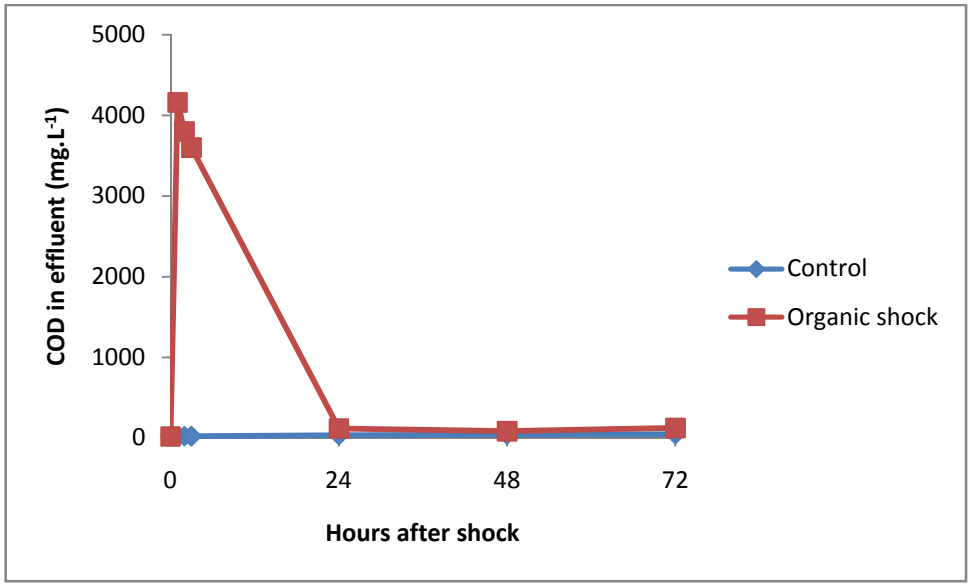


Figure 7.1 Effluent COD of the control and the organic shock reactors

Results show that after introducing the organic shock, effluent COD of the shock reactor immediately increased sharply from 20 $mg.L^{-1}$ to 4160 $mg.L^{-1}$ after 1 h after which it decreased slightly to 3800 $mg.L^{-1}$ and 3600 $mg.L^{-1}$ after 2 h and 3 h respectively. If the mixed liquor COD concentration was used to calculate COD removal efficiencies, then the COD removal decreased significantly from 95 % to 17 % after 1 h introducing the shock. So it is clear that the COD removal has, in fact, been impacted. That is, the increased COD concentration observed in the effluent is not simply proportional to the increased COD in the mixed liquor.

Effluent COD of the shock reactor reduced to 117 $mg.L^{-1}$ after 24 h and remained around this level until the end of the experiment. Effluent COD from the control remained stable at around 20 $mg.L^{-1}$ throughout the experiment. This result is consistent with previous studies on AS, which reported that the influent shock concentrations around 3000 $mg.L^{-1}$ COD caused significant increases in effluent COD concentrations, resulting in a reduction in COD removal efficiency from 98 % to 86 % (Saleh and Gaudy, 1978, Manickam and Gaudy, 1985). Four to six days after a 3000 $mg.L^{-1}$ COD shock was applied, the COD in an AS effluent was observed to return to a low level (Saleh and Gaudy, 1978, Manickam and Gaudy, 1985). In contrast, a previous study found that organic shock loads with influent COD concentration from 5 to 16 $g.L^{-1}$ COD provided no significant impact on the effluent COD of an immersed MBR system (Al-Malack, 2007). This inconsistency may be because the MBR in the previous research was operated with synthetic wastewater that may contain only very easily biodegradable organic compounds. In addition, the MBR in the previous

research was operated at much higher MLSS concentration (15 $g.L^{-1}$) than MLSS concentration in this study (around 5 $g.L^{-1}$) (Al-Malack, 2007). This higher MLSS may provide some resilience against sudden COD increases in the influent. The organic shock also caused reactor foaming and overflow of biomass as shown in Figure 7.2.

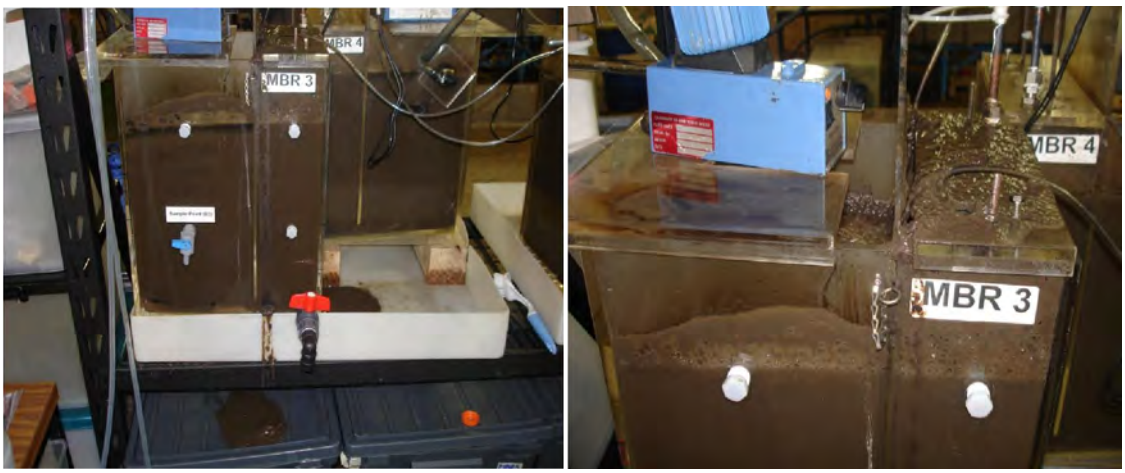


Figure 7.2 Foaming and overflow of biomass in the organic shock reactor

MLSS and MLVSS concentrations of mixed liquor of the control and the organic shock reactors are presented in Figure 7.3 and Figure 7.4. A rapid growth in biomass concentrations after the shock was expected but the results indicate that the MLSS concentrations in the organic shock reactor were only slightly increased after 24 h and returned to the same level as the control after 48 h. This may be, in part, due to the biomass loss during foaming and overflow in the first 48 h after the shock as shown in Figure 7.2. In a previous study, a 3000 $mg.L^{-1}$ COD shock load to an AS system was reported to cause a rapid growth in biomass, a noticeable change in colour of the mixed liquor, a decrease in floc size, an increase in filamentous forms and a reduction in the number of protozoa (Saleh and Gaudy, 1978). Disruption in COD removal capacity and the change in colour of an AS system were observed to be correlated with changes in the biochemical composition of the sludge (Manickam and Gaudy, 1985). It was also reported that the dynamics of the biological treatment depended less on the growth rate of microorganisms than on other mechanisms, notably the storage of the carbon substrate in the cells (Normand and Perdrieux, 1981). This study also suggested that the impacts depended essentially on the magnitude of the perturbation imposed and not on the initial biomass concentration (Normand and Perdrieux, 1981). This suggestion is inconsistent with the results in the study above where higher MLSS concentrations are suggested to provide some resilience against sudden COD increases in the influent. As there is conflicting evidence available, more research is

required to fully understand the relationships between some of the operational parameters and resilience of the systems to hazardous events. Sludge age was reported to have a very distinct effect with lower response in time with older sludges (Normand and Perdrieux, 1981).

Organic shock loads have been found to change the dominant bacterial type in the reactor from gram-positive rods to gram-negative oval shaped bacteria (Seetha et al., 2010). In this case, it was assumed to be likely that autotrophs were outcompeted by heterotrophs and washed out of the system. In general, high organic concentration in influent wastewater is known to inhibit nitrification as it supports the growth of heterotrophic bacteria, which compete with autotrophic nitrifying bacteria for oxygen, nutrients and space (Sharma and Ahlert, 1977, Hanaki et al., 1990, Ohashi et al., 1995, van Benthum et al., 1997, Zhu and Chen, 2001, Chen et al., 2006b).

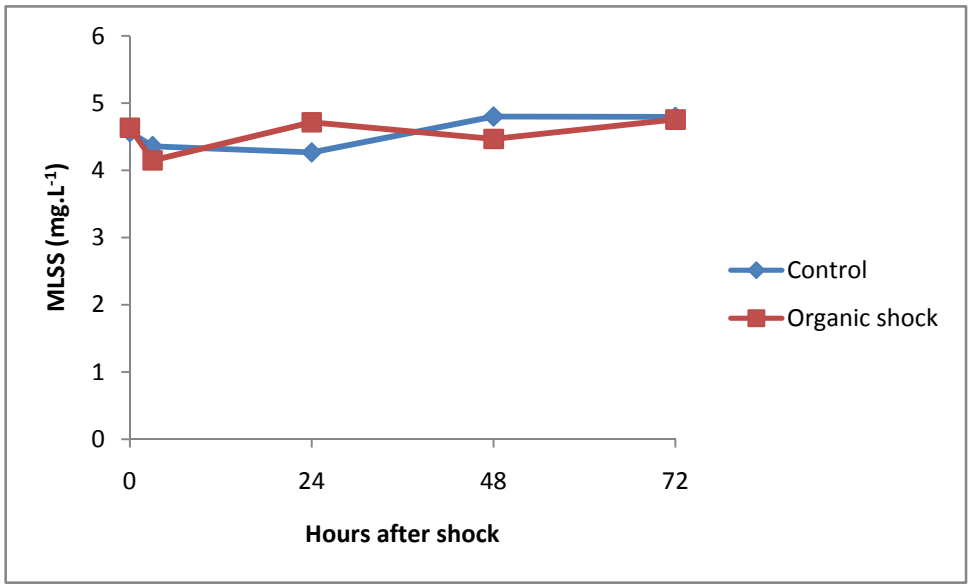


Figure 7.3 MLSS concentrations of the control and the organic shock reactors

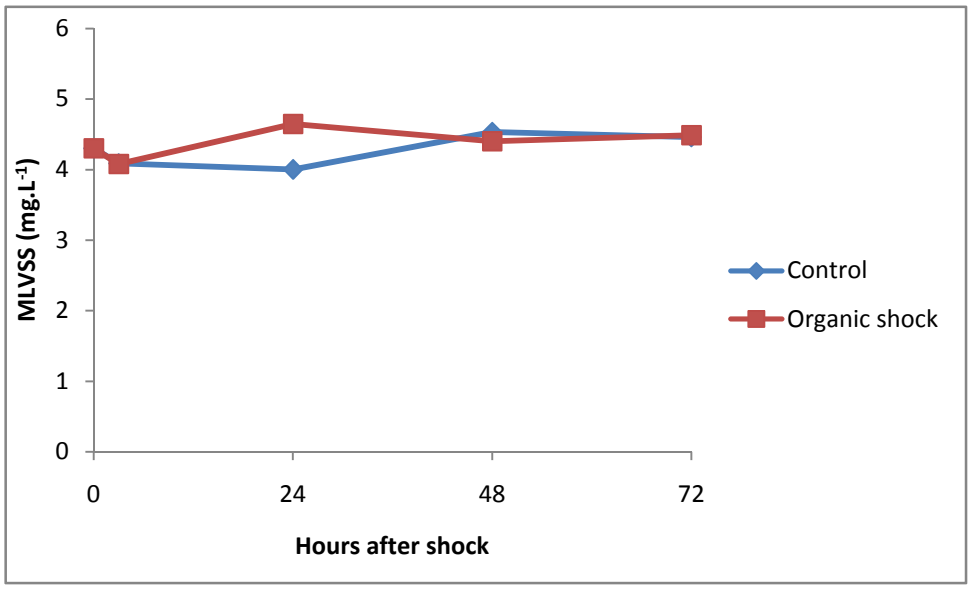


Figure 7.4 MLVSS concentrations of the control and the organic shock reactors

The mixed liquor CST from the control and the organic shock reactors is presented in Figure 7.5. Results show that CST of the organic shock reactors increased sharply after introducing the shock and remained significantly higher than that of the control until the end of the experiment although it was slightly improved after 72 h. This implies that filterability of the mixed liquor from the organic shock reactor was notably reduced after introducing organic shock and still not fully recovered 72 h after the shock. In previous studies, organic shock loads have been found to cause a decrease in floc-size, changes in dominant micro-organism types and changes in the biochemical composition of the sludge (Saleh and Gaudy, 1978, Manickam and Gaudy, 1985,

Seetha et al., 2010). These changes may be the causes for an observed reduction in filterability of the mixed liquor after being subjected to the organic shock. The TMP of the organic shock reactor rapidly rose from 10 kPa to 43 kPa while TMP of the control gradually rose from 10 kPa to 23 kPa until the end of the experiment.

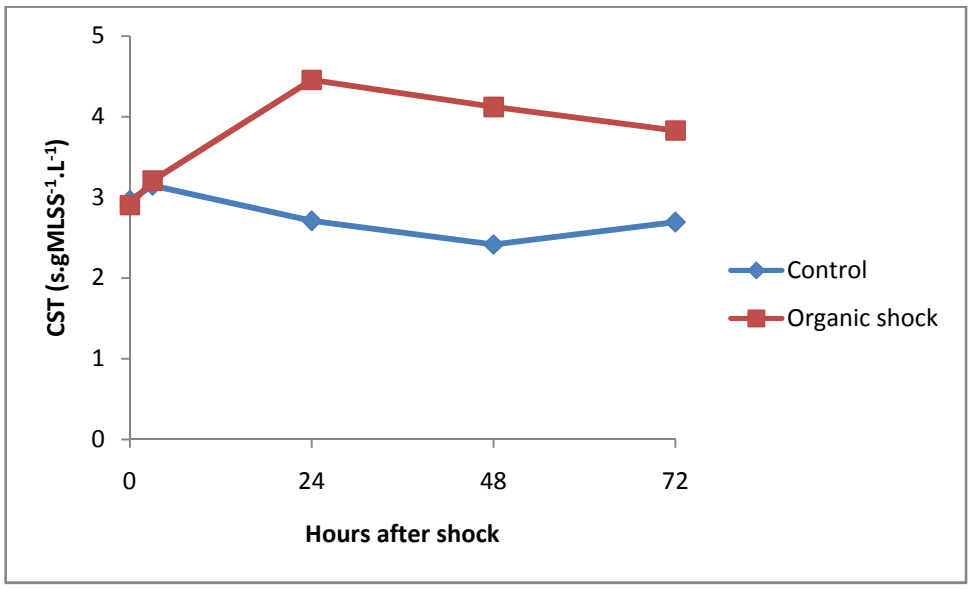


Figure 7.5 CST of the control and the organic shock reactors

Feed starvation experiment

Results of key bulk water quality and MBR operational parameters including pH, COD, MLSS, MLVSS and TMP during the starvation experiment are presented in this section. Unfortunately, due to some technical problems, the CST data is not available for the starvation experiment. Results show that pH of the MBR permeate from the control varied from 6.5 to 7.0 and pH of the permeate from the starvation reactor varied from 6.4 to 6.8. This variation was within the range observed during the reproducibility experiments (Chapter 5).

The COD removal efficiency of the control and the starvation reactors are presented in Figure 7.6. Results show that after re-feeding the starvation MBR, COD removal efficiency in the reactor immediately reached 97%, which suggests the rapid microbial utilisation of the available carbon sources after the long starvation period (Li et al., 2006). This high COD removal efficiency was stable and the same as the control for the next 4 days. A previous study found that DOC and nitrogen removal by lab-scale MBRs was not affected under a feed starvation shock of 2 days (Le-Minh, 2011). In this study, the starvation period was extended to 6 days and the COD removal efficiency reached the same value of the control immediately after re-feeding. The result of this

study suggests that the MBR system can withstand starvation conditions well and can recover back to pre-shock steady-state conditions quickly after re-feeding. Another lab-scale MBR study for a starvation period of 5 days reported that, 3 days after re-feeding, the COD removal efficiency reached 90% and fully recovered back to steady state conditions after 6 days of normal operation (Yogalakshmi et al., 2007). The MBR in this previous study had MLSS concentration of 15 $g.L^{-1}$, which was high compared to typical MLSS concentrations in MBRs in practice and three times higher than MLSS concentration in the current study (5 $g.L^{-1}$). This may be the reason for longer recovery time as the proportion of the dead biomass may be larger. The concentration of organic matter released from inside dead cells to the outside liquid medium thus may be higher (Coello Oviedo et al., 2003) and such systems require longer recovery times.

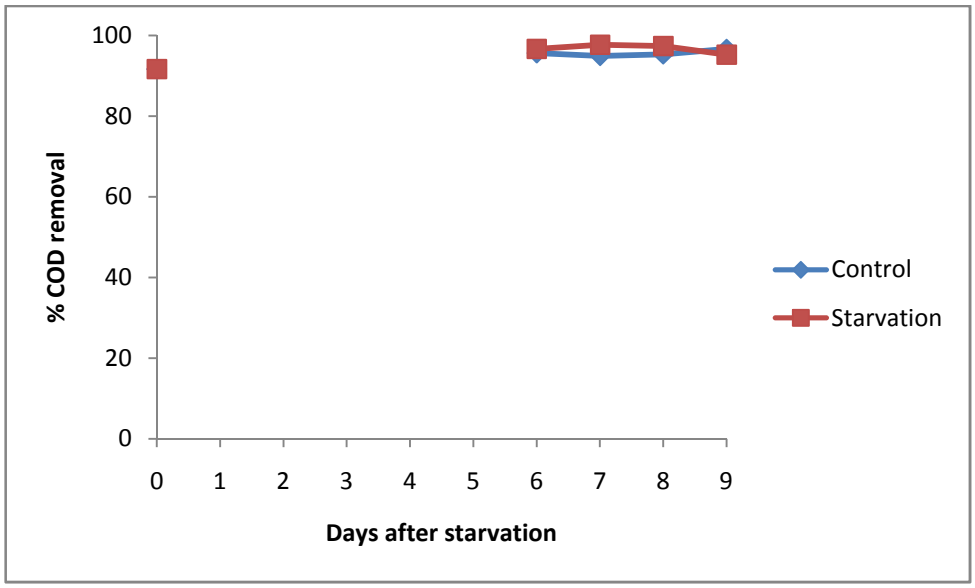


Figure 7.6 COD removal efficiency of the control and the starvation reactors

The MLSS and MLVSS concentrations of mixed liquor of the control and the starvation reactors are presented in Figure 7.7 and Figure 7.8. Results show that MLSS concentration in the starvation reactor was reduced from 4.6 $g.L^{-1}$ to 4.1 $g.L^{-1}$ in 1 day after starvation and 3.4 $g.L^{-1}$ after 2 days. After 6 day starvation, MLSS concentration in the starvation reactor was reduced to 3 $g.L^{-1}$. Although feeding of the MBR was re-established at the 6th day, MLSS concentration still remained around 3 $g.L^{-1}$ until the end of the experiment (the 9th day). The MLVSS data showed a similar trend as the MLSS data. This result is consistent with previous studies on AS, which found that biomass concentrations in AS decreased sharply during the first 4 days of the starvation period and then reduced more slowly after that (Urbain et al., 1993, Coello Oviedo et al., 2003). A previous study on MBR also found MLSS concentration reduced

significantly after a starvation period of 5 days and it took nearly a month of continuous operation to regain the amount of biomass lost during feed starvation (Yogalakshmi et al., 2007). These responses were reported to be related to the degradation of both proteins and polysaccharides contents of the sludge (Urbain et al., 1993). Starvation shocks also resulted in disappearance of some of the typical microbial groups usually found in an AS, and the appearance of other opportunistic microorganisms (Coello Oviedo et al., 2003).

At t = 0 h, TMP of the control and the starvation reactor was around 4.3-5.3 kPa. During the starvation period, TMP of the control increased quicker and was about 3 kPa higher than that of the starvation reactor. After re-feeding, TMP of the starvation reactor rose up quickly and reached the same value of that of the control (28 kPa) after a few hours. TMP of the control and the starvation reactors increased gradually and reached 32 kPa until the end of the experiment.

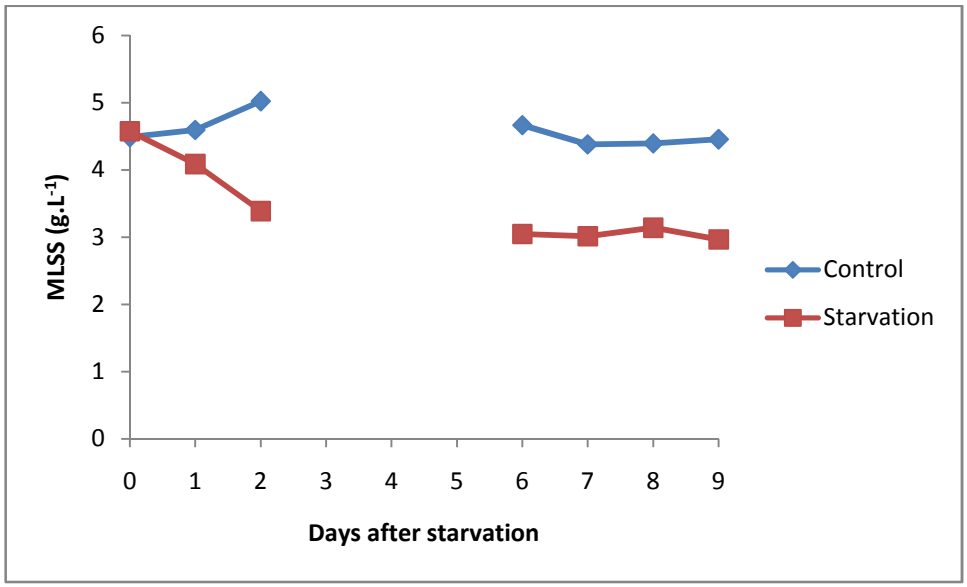


Figure 7.7 MLSS concentrations of the control and the starvation reactors

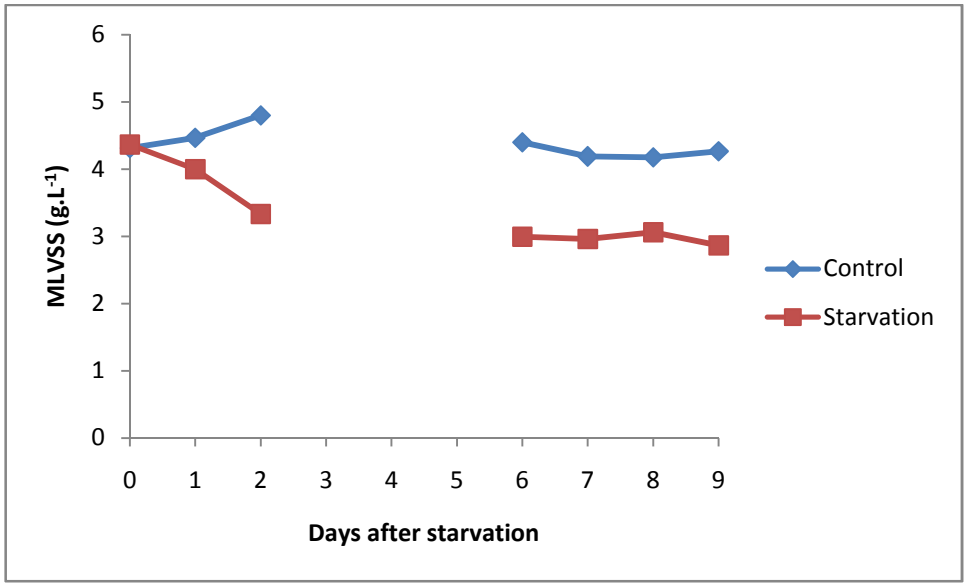


Figure 7.8 MLVSS concentrations of the control and the starvation reactors

7.3.2. Trace chemicals

Removals of trace chemicals by the MBRs under organic shock and feed starvation conditions are presented in this section. Among the analysed chemicals, 17α-estradiol, 17α-ethynylestradiol, mestranol, levonorgestrel, nonylphenol, 4-tert-octylphenol, diazepam, dilatin, meprobamate, enalapril, hydroxyzine, omeprazole, simvastatin, simvastatin hydroxy acid, atrazine and linuron were not detected in influent samples. Some trace chemicals (dihydrotestosterone, androstenedione, amitriptyline, risperidone, triamterene) were only detected in a few influent samples.

Similar to Chapter 6, the results of trace chemicals that were consistently detected in the raw sewage during the experimental period were divided in 3 groups including hydrophilic chemicals (log $D_{pH8} < 2$), moderately hydrophobic chemicals ($2 \leq \log D_{pH8} \leq 3.2$), very hydrophobic chemicals ($\log D_{pH8} > 3.2$) as previously described by Tadkaew et al. (2011).

Organic shock experiment

Figure 7.9 presents the removal efficiencies of the hydrophilic trace chemicals by the control and the organic shock reactors. At 2 h after introducing the organic shock, the overall removals of ketoprofen, gemfibrozil and naproxen were significantly reduced from above 98% to 73% for naproxen, and 51% for ketoprofen and gemfibrozil. The removal of naproxen reduced further to 39% after 24 h and the removal of gemfibrozil decreased further to 29% after 48 h. The removals of ketoprofen, gemfibrozil and

naproxen were not fully recovered 72 h after the shock. The impacts of organic shock on removals of other hydrophilic chemicals including sulfamethoxazole, ibuprofen, caffeine and paracetamol were less than those of ketoprofen, gemfibrozil and naproxen. The removal of paracetamol steadily decreased from over 99% to 80% within the first 3 h after introducing the shock, it then fully recovered after 48 h. The removals of ibuprofen and caffeine slightly reduced from over 99% to 92%-95% in the first 3 h after introducing the shock. The removal of caffeine fully recovered after 48 h while it took 72 h for the removal of ibuprofen to recover completely.

Similar to observations in Chapter 6, the different degrees of impacts of organic shock on various hydrophilic chemicals again may be explained by their different biotransformability. The less readily biotransformable compounds (ketoprofen with K_{biol} = 0.03 L.gMLSS^{-1}.d^{-1} and gemfibrozil with K_{biol} = 0.06 L.gMLSS^{-1}.d^{-1}) (Urase and Kikuta, 2005, Joss et al., 2006) were shown to suffer a significant reduction in removal efficiency by the organic shock. Naproxen having published K_{biol} varying from 0.06 – 5.45 L.gMLSS^{-1}.d^{-1} (Joss et al., 2006, Abegglen et al., 2009, Fernandez-Fontaina et al., 2013) also experienced a considerable decrease in removal efficiency after introducing the organic shock. In contrast, the most biotransformable compounds (caffeine and paracetamol with K_{biol} = 106-240 L.gMLSS^{-1}.d^{-1}) (Joss et al., 2006, Lin et al., 2010) were less affected by the organic shock. The removal of ibuprofen that has a K_{biol} varying from 1.3 to 49.3 L.gMLSS^{-1}.d^{-1} (Joss et al., 2006, Fernandez-Fontaina et al., 2013) was also just slightly reduced by the organic shock. These results are consistent with previous findings from toxic shock experiments (Chapter 6). The susceptibility of ketoprofen, gemfibrozil and naproxen to organic shock may again be explained by the reliance upon specific organisms or metabolic pathways for their biotransformation. As these organisms or metabolic pathways are inhibited, the biotransformation of these chemicals was also reduced.

Results from Figure 7.9 confirm that removal via adsorption to biomass was an insignificant removal mechanism (< 2%) for these hydrophilic chemicals and this was unchanged under organic shock conditions. Biotransformation was the main removal mechanism for these hydrophilic chemicals and this removal mechanism was affected since some of the biological transformation processes of the reactors were inhibited.

Figure 7.10 presents the removal efficiencies of moderately hydrophobic chemicals by the control and the organic shock reactors. Results show that the overall removals of the moderately hydrophobic chemicals oestriol, propylparaben and testosterone were high (above 90%) and not affected by the organic shock. Removal via adsorption to

biomass was an insignificant removal mechanism for these chemicals. It is noted that the concentration of testosterone in biomass was below the limit of quantification (LOQ), so the LOQ value was used to calculate the mass balance, revealing that the percentage of testosterone removed via adsorption to biomass was < 4%.

Figure 7.11 presents the removal efficiencies of very hydrophobic chemicals by the control and the organic shock reactors. Similar to moderately hydrophobic chemicals, results show that the removal efficiencies of very hydrophobic chemicals were not affected by the organic shock conditions. The overall removals of 2-phenylphenol, oestrone, etiocholanolone, androsterone, 17β-estradiol, triclosan and triclocarban were always high above 90% during the experiment. Removal via adsorption to biomass contributed up to 14% to the overall removal of triclosan. For triclocarban, the percentage removal via adsorption to biomass was 71%-81% in the control reactor and 74%-93% in the shock reactors. This variation was within the variation of percentage removal via adsorption to biomass of triclocarban between the MBRs during the reproducibility experiment (25%). Removal via adsorption to biomass was less than 5% for other very hydrophobic chemicals. These results show that adsorption to biomass was the main removal mechanism for triclocarban, both adsorption to biomass and biotransformation are important removal pathways for triclosan and biotransformation was the dominant removal mechanism for other very hydrophobic chemicals.

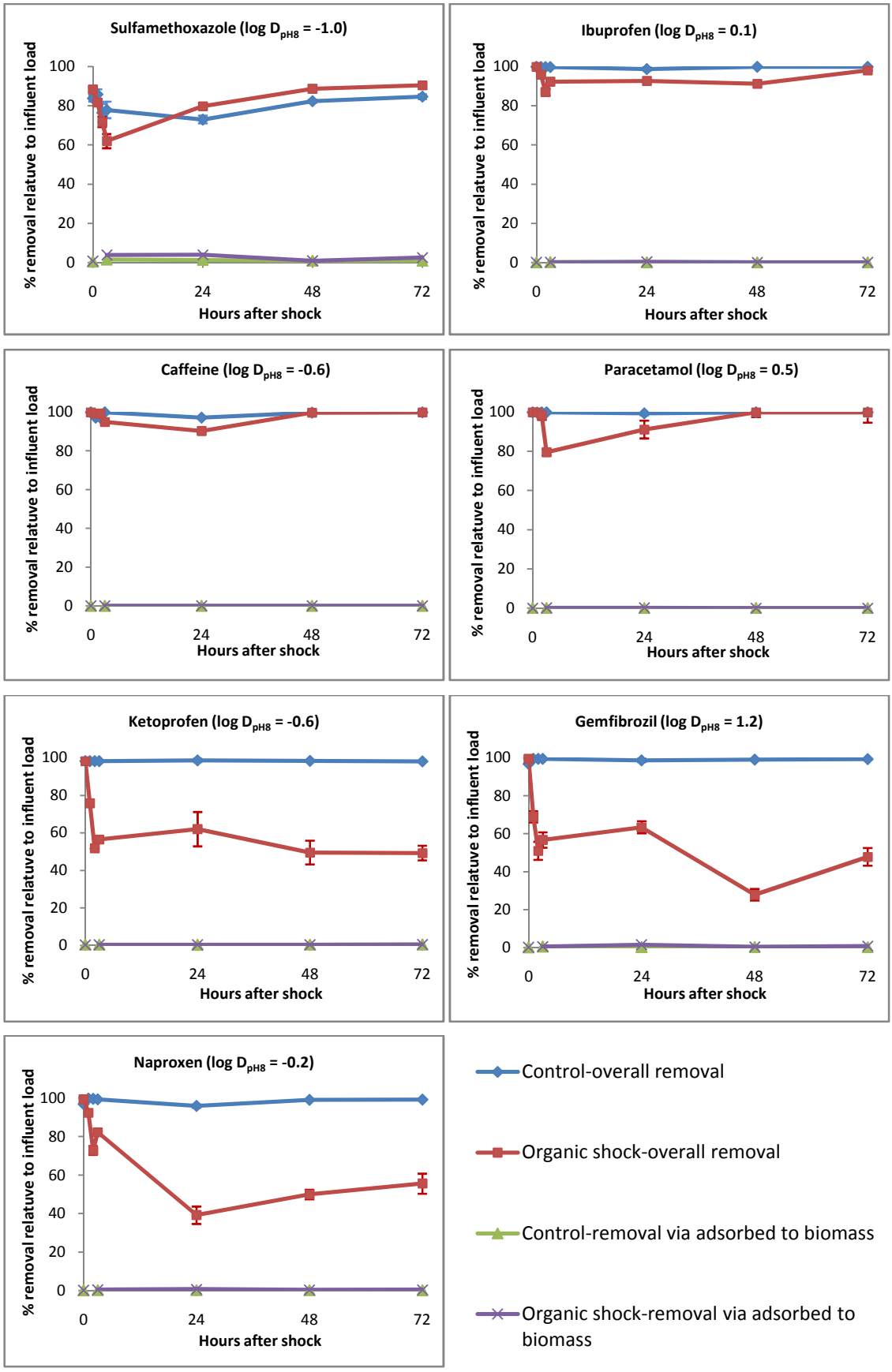


Figure 7.9 Removals of hydrophilic chemicals (log D_{pH8} < 2) by the control and the organic shock reactors

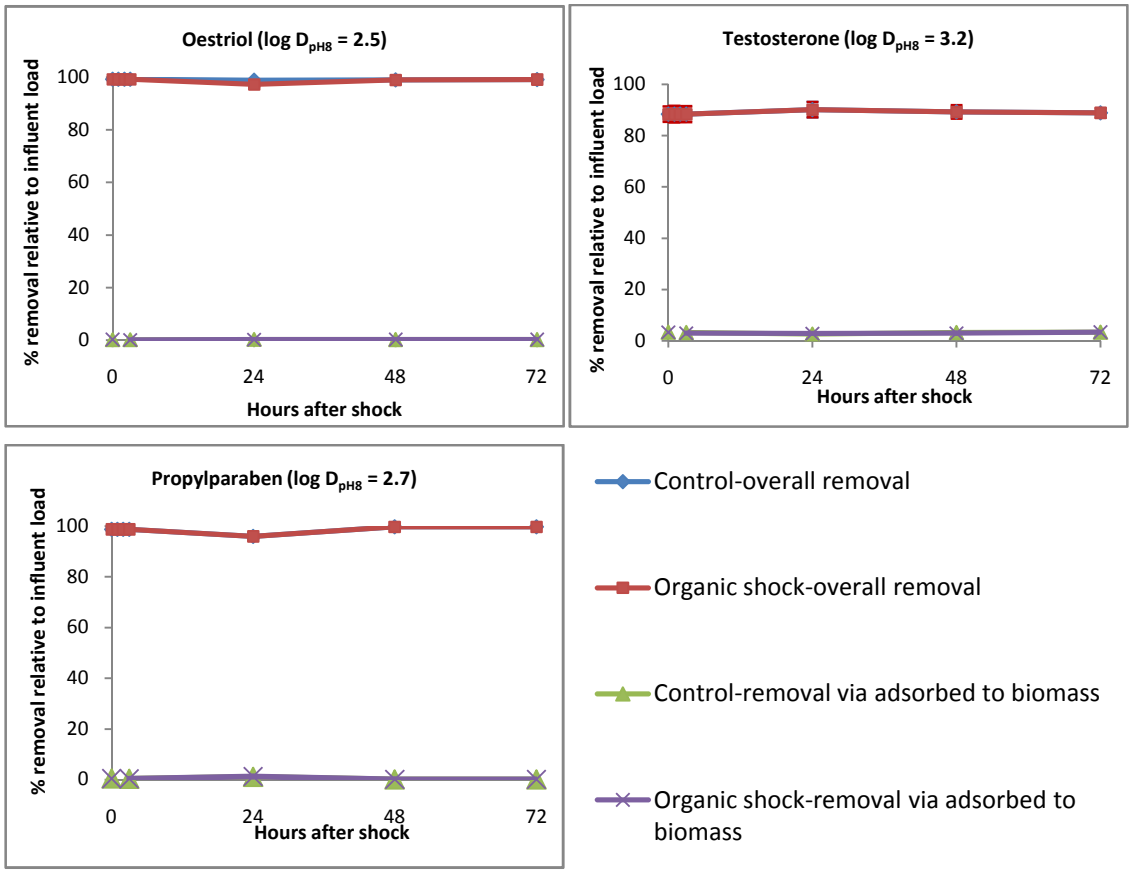


Figure 7.10 Removals of moderately hydrophobic chemicals (2 ≤ log D_{pH8} ≤ 3. 2) by the control and the organic shock reactors

In general, the results show that under organic shock conditions, the removals of moderately and very hydrophobic chemicals were not affected while the removals of hydrophilic chemicals were affected to various degrees. A previous study found that bacteria cells that are close to the interface with the liquid medium are more susceptible to toxins than those deeply embedded in extracellular polymeric substance (EPS) of the activated sludge flocs (Henriques, 2006). That is because the EPS acts as a barrier between toxins and bacteria cells. EPS can react with toxins or impede the penetration of toxins to bacteria cells to give the cells a better chance to express specific stress responses such as expressing a stress regulator functioning as a protection mechanism of biofilm against exogenous insults (Henriques, 2006, Poole, 2012). In addition, some cells induce a specific protection phenotype functioning different from the other bacteria in the biofilm, such as increasing expression of multiple chemical efflux pumps or altering the composition of outer membrane proteins (Henriques, 2006). Thus, it is possible that the organic shock in this study inhibited some biotransformation processes in the aqueous phase of the reactor but, within the biomass structures due to these protective responses, some biotransformation processes continued largely unaffected. The moderately hydrophobic and very

hydrophobic chemicals can adsorb to the biomass and thus these chemicals were still biotransformed. For hydrophilic chemicals, the removal of chemicals with moderate or less readily biotransformability was significantly reduced while the removal of easily biotransformable chemicals was just slightly affected. These results are consistent with previous findings from toxic shock experiments (Chapter 6).

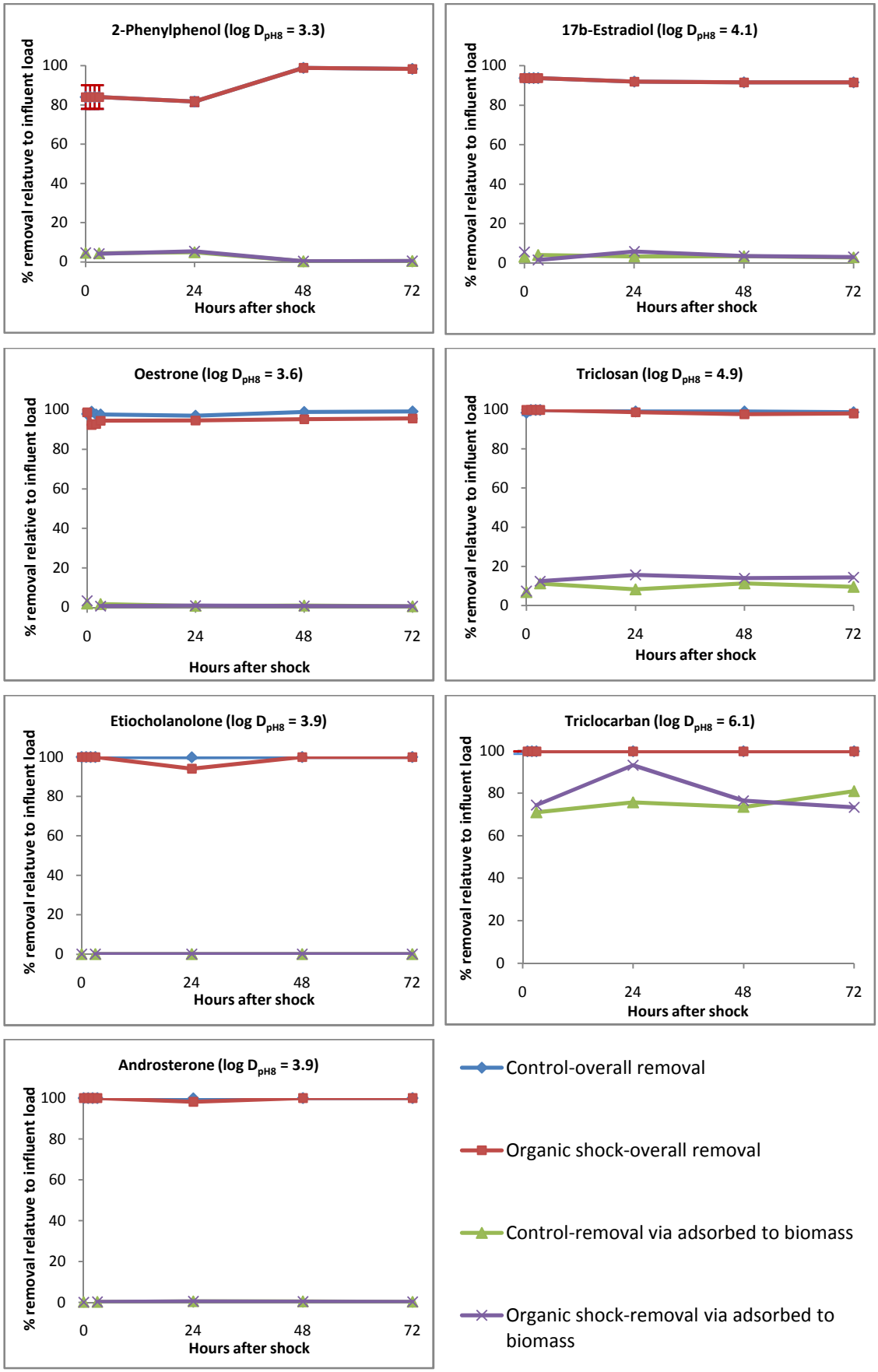


Figure 7.11 Removals of very hydrophobic chemicals (log D_{pH8} > 3.2) by the control and the organic shock reactors

Starvation experiment

The removals of hydrophilic, moderately hydrophobic and hydrophobic chemicals during the starvation experiment are presented in Figure 7.12, Figure 7.13 and Figure 7.14, respectively. Results show that the removals of these chemicals were not affected by the starvation conditions. This result is consistent with a previous study that found that the removals of sulfonamide and trimethoprim antibiotics by MBRs were not affected by a feed starvation shock load of 2 days (Le-Minh, 2011). The overall removals of these chemicals were high (above 80% during the experiment), with the exception of one sampling date where the removal of sulfamethoxazole was 70%. Removal via adsorption to biomass contributed up to 8% to the overall removal of triclosan. For triclocarban, the percentage removal via adsorption to biomass was 92%-105% in the control reactor and 70%-95% in the shock reactors. This variation was within the variation of percentage removal via adsorption to biomass of triclocarban between the MBRs during reproducibility experiment (25%). Removal via adsorption to biomass contributed less than 5% to the overall removals of other chemicals including sulfamethoxazole, caffeine, ketoprofen, naproxen, ibuprofen, paracetamol, gemfibrozil, oestriol, testosterone, propylparaben, 2-phenylphenol, oestrone, etiocholanolone, androsterone and 17β-estradiol.

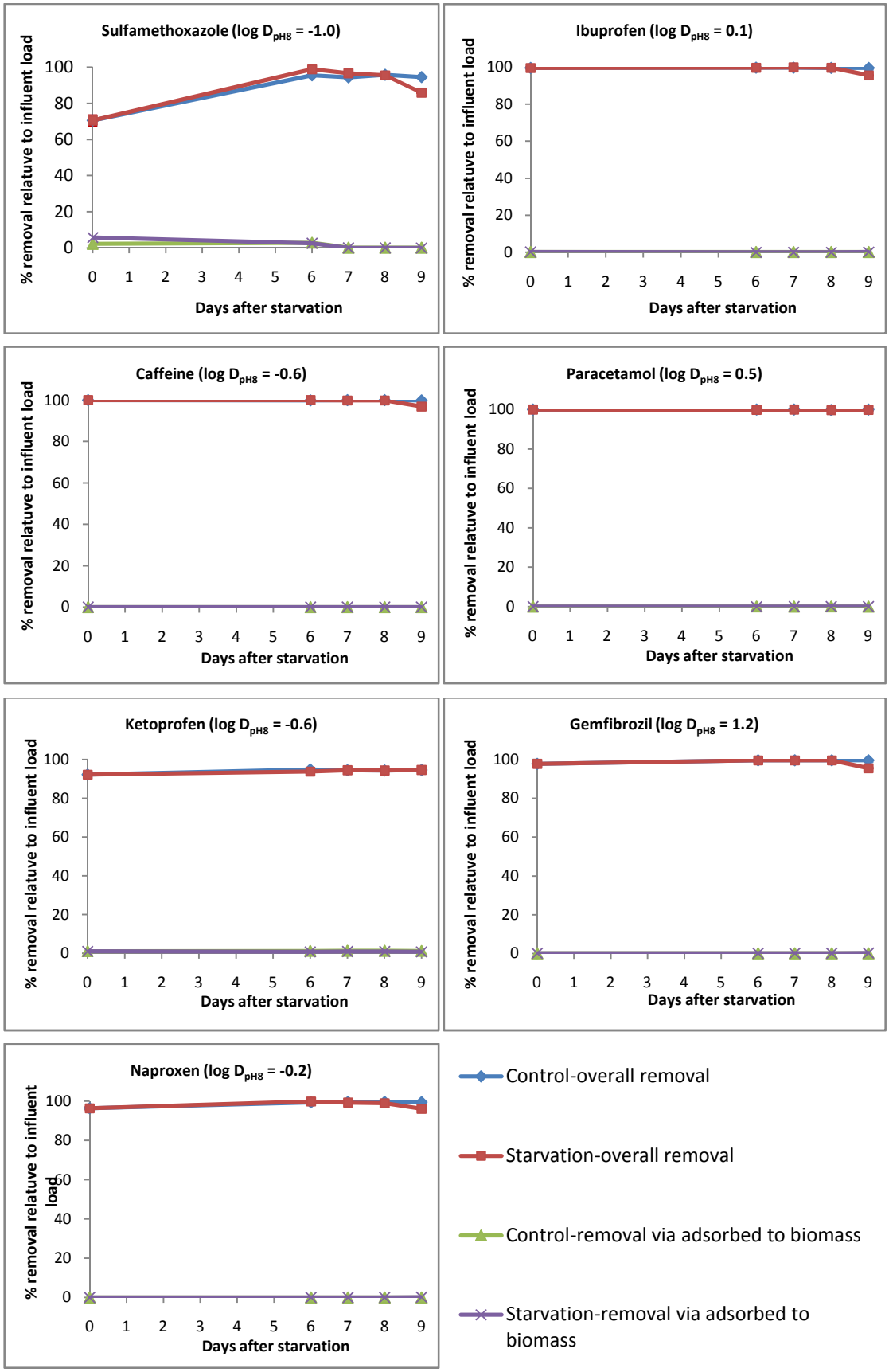


Figure 7.12 Removals of hydrophilic chemicals (log D_{pH8} < 2) by the control and the starvation reactors

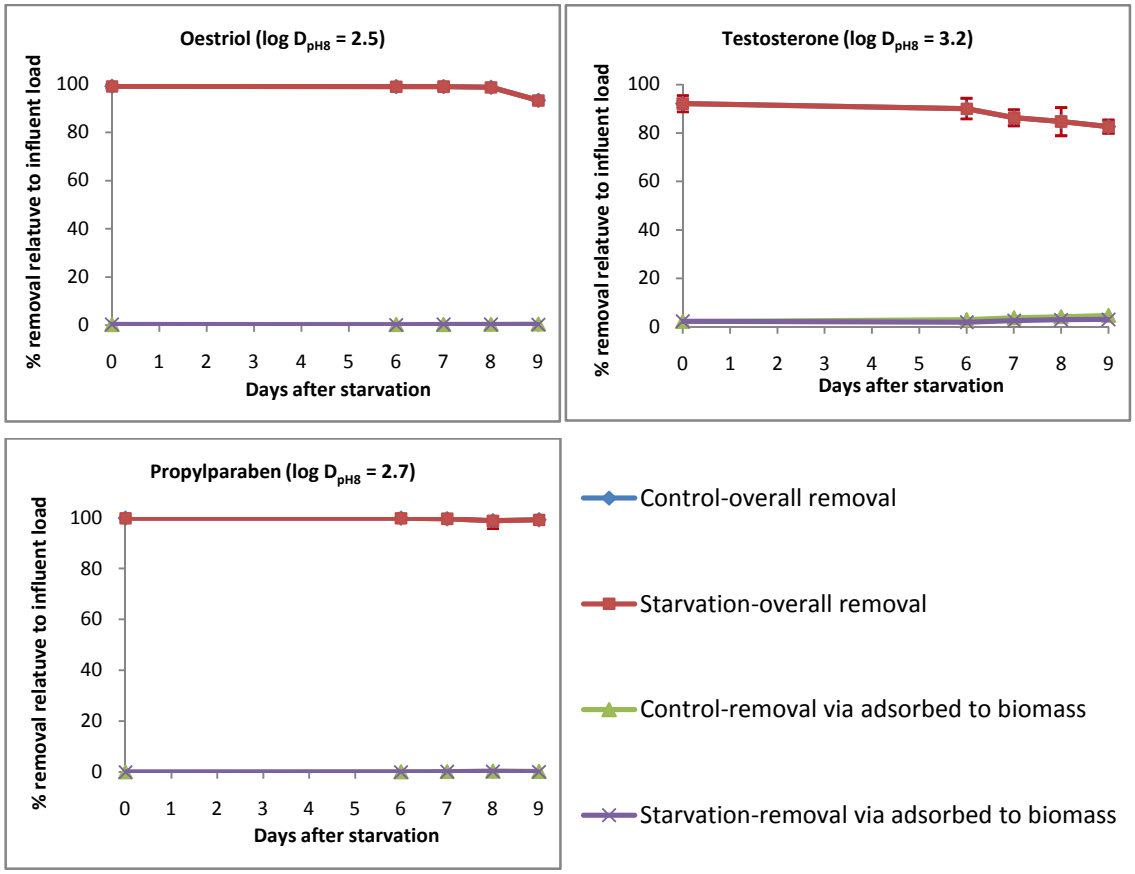


Figure 7.13 Removals of moderately hydrophobic chemicals (2 ≤ log D_{pH8} ≤ 3. 2) by the control and the starvation reactors

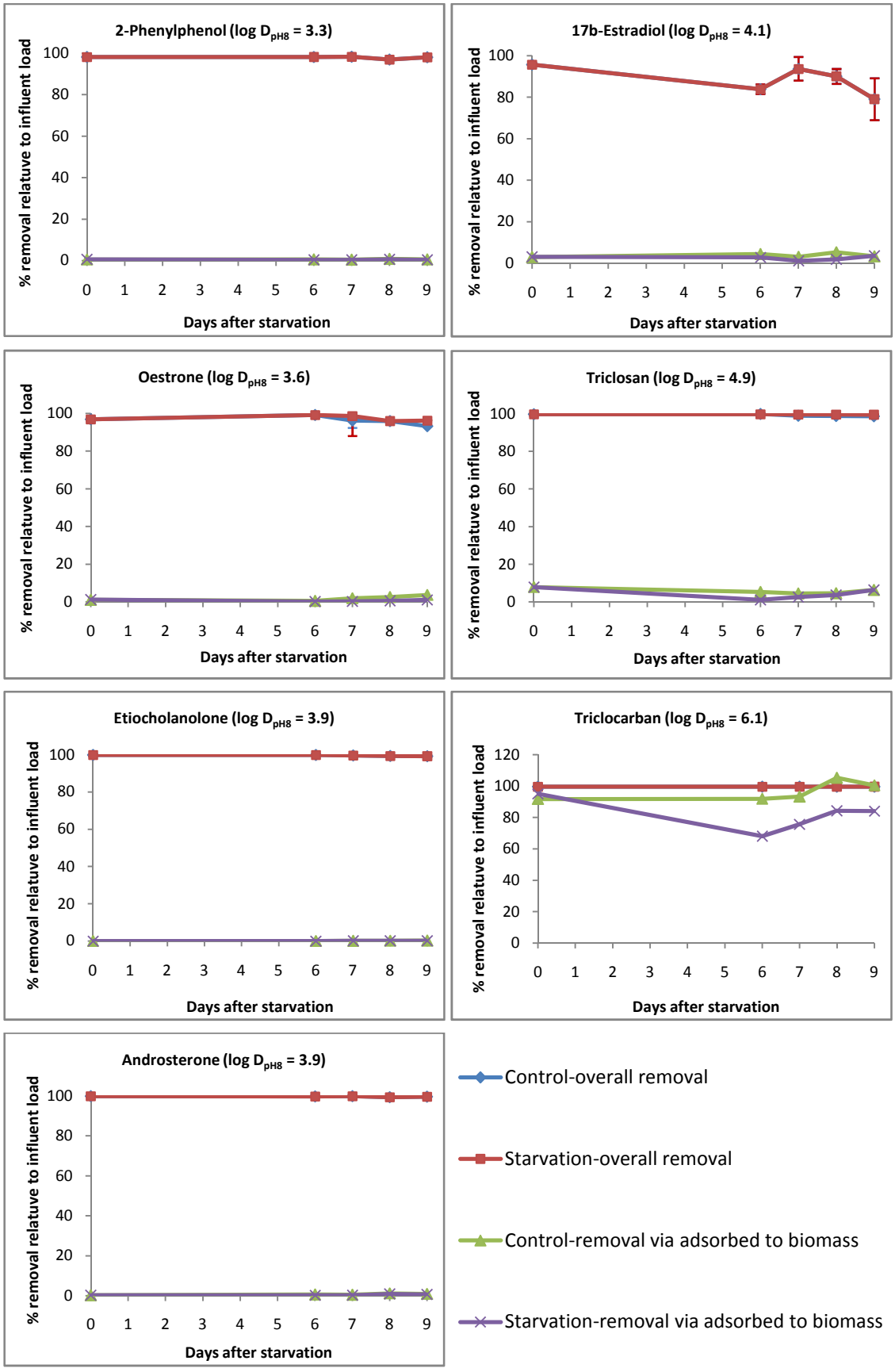


Figure 7.14 Removals of very hydrophobic chemicals (log D_{pH8} > 3.2) by the control and the starvation reactors

7.4. CONCLUSIONS

The organic shock was shown to cause a significant increase in effluent COD, CST and TMP. These results reveal that effluent COD, CST and TMP are effective parameters for monitoring impacts of organic shock on MBR performance.

The impacts of organic shock on trace chemicals removals observed in this chapter are consistent with those observed for toxic shocks (Chapter 6). The same groups of chemicals (moderately and very hydrophobic compounds) seem to be more resistant and the same groups (hydrophilic chemicals with less readily biotransformability) seem to be highly impacted. The organic shock possibly inhibited some biotransformation processes in the aqueous phase of the reactor but, within the biomass structures, some biotransformation processes continued largely unaffected as a result of biofilm protection. The moderately hydrophobic and very hydrophobic chemicals can adsorb to the biomass and thus these chemicals were still biotransformed. Similar to Chapter 6, the different degrees of impacts of the organic shock on various hydrophilic chemicals may be explained by their different biotransformability., the less readily biotransformable compounds (ketoprofen and gemfibrozil) are the least resistant to loss of biotransformability from the organic shock, whereas the most biotransformable compounds (caffeine and paracetamol) are the most resistant to impacts from the organic shock. The susceptibility of ketoprofen and gemfibrozil to organic shock may be explained by the need for specific organisms or metabolic pathways for their biotransformation. As these organisms or metabolic pathways are impeded, so too is the biotransformation of these chemicals. In contrast, readily biotransformable compounds such as caffeine and paracetamol may be more easily transformed by a wider range of organisms or metabolic pathways so they are still widely biotransformed under the shock conditions. The results reveal that hydrophilic chemicals with low biotransformability (e.g. ketoprofen and gemfibrozil) may be sensitive indicators for monitoring impacts of organic shock on removals of trace chemicals by MBR.

Starvation has a significant and noticeable effect on MLSS concentrations, but the systems nonetheless appear to be resilient in terms of COD and trace chemicals removals. MLSS concentrations may be a sensitive indicator of fluctuations in feed compositions. However, this indication may not necessarily translate into immediate performance problems.

There is conflicting evidence available about the role of operational parameters (e.g MLSS concentrations) in response to hazardous events, hence more research is

required to fully understand the relationships between some of the operational parameters and resilience of the systems to hazardous events.

CHAPTER 8. IMPACTS OF PHYSICAL MEMBRANE DAMAGE AND LOSS OF POWER SUPPLY ON MBR PERFORMANCE

8.1. INTRODUCTION

Beside the sudden changes in influent flow and concentration, other potentially hazardous events including physical membrane damage and loss of aeration are also expected to affect the MBR treatment process performance. Available literature on impacts of physical membrane damage and loss of power supply on MBR performance is very limited, revealing a lack of attention on this topic. This knowledge gap requires further investigation to fully understand and characterise the operational robustness of MBR systems.

This chapter reports the impacts of physical membrane damage and loss of power conditions on MBR performance including impacts to pH, effluent chemical oxygen demand (COD), mixed liquor suspended solids (MLSS) concentration, mixed liquor volatile suspended solids (MLVSS) concentration, transmembrane pressure (TMP) and mixed liquor capillary suction time (CST). Removals of trace chemicals were also monitored to identify, which trace chemicals provide useful roles as indicator chemicals to detect the impacts of the hazardous events on MBR performance.

8.2. MATERIALS AND METHODS

Experiments to assess impacts of physical membrane damage and loss of power supply conditions were conducted in the experimental MBRs as described in Section 5.4 (Chapter 5).

8.2.1. Physical membrane damage

A control MBR was run in parallel to a MBR subjected to physical membrane damage. The total numbers of membrane fibres in each MBR were 120 fibres. A preliminary test was conducted in the laboratory to determine how to damage the membrane and how many membrane fibres should be damaged in the bioreactor experiments. This involved cutting submerged hollow fibre membranes into two parts at various depths and monitoring the impact to permeate turbidity. During these tests, it was observed that if the membrane was cut at insufficient depth (less than 3 cm), the top of the capillary fibre would float in the surface and no water would be drawn through it. Cutting a single membrane (at lower depth) led to negligible impact to turbidity, however cutting two (or more) membranes led to a drastic increase in permeate turbidity (>300 NTU). The starkly different impacts between cutting the first and second fibre, reveal that there is a significant element of ‘chance’ regarding whether a specific

fibre breakage will be quickly blocked (leading to negligible turbidity rise) or remain open (leading to drastic turbidity rise). Accordingly, the final experimental protocol for this hazardous event simulation involved sequential cutting of membranes until a major turbidity breach was observed.

The final physical membrane simulation was undertaken by initially cutting one membrane fibre by a sharp knife at a depth about 10 cm. After cutting the membrane fibre, effluent samples were immediately taken directly from the tube of the permeate pump every min for 10 min, these samples were analysed for turbidity onsite (and COD was also analysed after bringing samples back to the lab). Based on the turbidity results, a second fibre of the same module was cut at a similar position (depth 10 cm), and effluent samples were continuously taken from the tube of the permeate pump, every min for 20 min. Additional effluent samples were taken at 60 min, 90 min, and 120 min. Again, these were analysed for turbidity on site (and COD after bringing samples back to the lab).

Effluent samples (1 L) were also collected for the analysis of trace chemical contaminants and COD. These were collected before cutting the first fibre (at t = 0 h) and after cutting the second fibre at 0.5 h, 2 h, 3 h, 24 h and 48 h. Influent samples (0.25 L) were taken in triplicate every day after filling the influent tank. Mixed liquor samples (0.5 L) were taken before cutting the first fibre (at t = 0 h) and after cutting the second fibre at 3 h, 24 h and 48 h (0.5 h and 2 h samples were not collected in order to avoid significantly impacting the MLSS concentrations due to sample collection). Influent samples were analysed for COD and trace chemicals. Mixed liquor samples were analysed for MLSS, MLVSS and trace chemicals. TMP was also continuously monitored throughout these experiments. The methods for these analyses were previously described in Chapter 3.

8.2.2. Loss of power supply

The loss of power conditions were simulated by stopping the power supply to the system for a duration of 2 h. This included stopping the feed to the MBR, the aeration inside the bioreactor and the membrane chamber, and the permeate pump. A control MBR was run in parallel to the MBR subjected to the loss of power supply.

Influent samples (0.25 L) were taken in triplicate every day after filling the influent tank. Effluent samples (1 L) from the control and the shock reactor were taken before stopping the power supply (at t = 0 h). During the loss of power duration, no effluent

samples from either the experimental or control MBRs were taken. After 2 h under the loss of power conditions, the power supply was returned and the MBR was operated as normal. Effluent samples were taken from both the control and the shock MBR at 1 h. 2 h, 3 h, 24 h, 48 h and 72 h after turning the power supply on. Mixed liquor samples were taken before the loss of power duration (at t = 0 h) and after the loss of power duration at 3 h, 24 h, 48 h and 72 h. Influent and effluent samples were analysed for pH, COD and trace chemicals. Mixed liquor samples were analysed for MLSS, MLVSS, CST and trace chemicals. TMP was also continuously monitored throughout these experiments. The methods for these analyses were described in Chapter 3.

8.3. RESULTS AND DISCUSSION

8.3.1. Key bulk water quality and MBR operational parameters

Physical membrane damage experiment

Results of key bulk water quality and MBR operational parameters including COD, MLSS, MLVSS and TMP during the physical membrane damage experiment are presented in this section. Turbidity and COD in the MBR permeate of the control and the physical membrane damage reactors in the first 20 min after cutting the fibre are presented in Figure 8.1 and Figure 8.2.

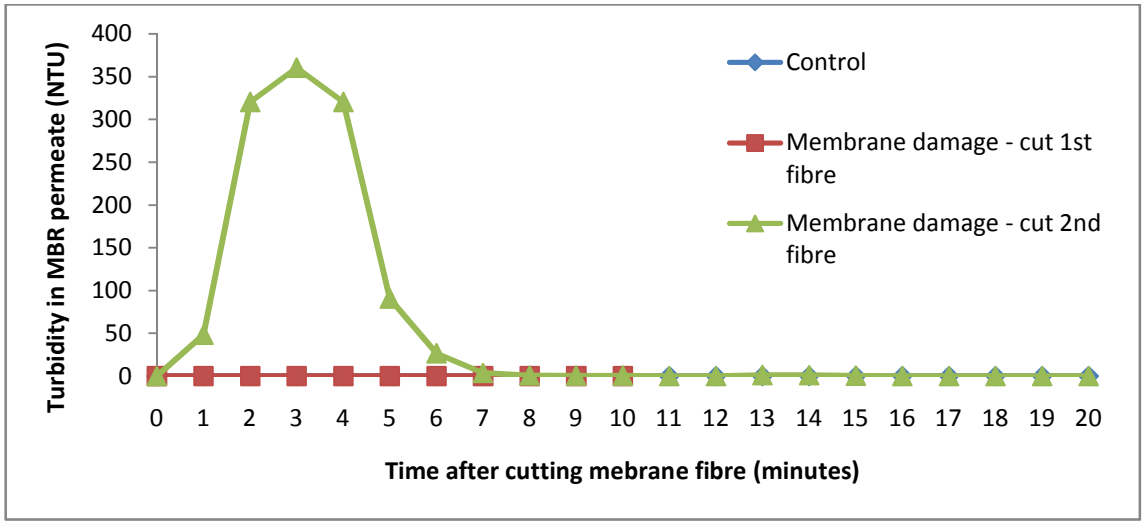


Figure 8.1 Turbidity in the MBR permeate of the control and the physical membrane damage reactors in the first 20 min after cutting the fibre

Results show that after cutting the first fibre, turbidity in the MBR permeate was not changed. The turbidity continued to closely match that of the control (0.2 NTU) for 10 min, however after cutting the second fibre, the turbidity in the MBR permeate

immediately increased to 49 NTU after 1 min, 320 NTU after 2 min and 360 NTU after 3 min. It then reduced to 320 NTU after 4 min and quickly decreased to 91 NTU after 5 min, 27 NTU after 6 min and 4 NTU after 7 min. This indicates that biomass had clogged and sealed the breakage. The turbidity reduced to 0.3 NTU after 9 min, it was then slightly increased to 1.1 and 1.2 NTU at 13 min and 14 min indicating some loss of clogging effect. The turbidity was reduced back to 0.4 NTU again after 15 min and then gradually reduced to 0.2 after 18 min and remained stable at this level until the end of the experiment. Results confirm that turbidity is a good indicator for online monitoring and provides rapid detection of physical membrane damage. A picture of the permeate samples from the physical membrane damage reactors taken every min after cutting the second fibre is presented in Figure 8.3.

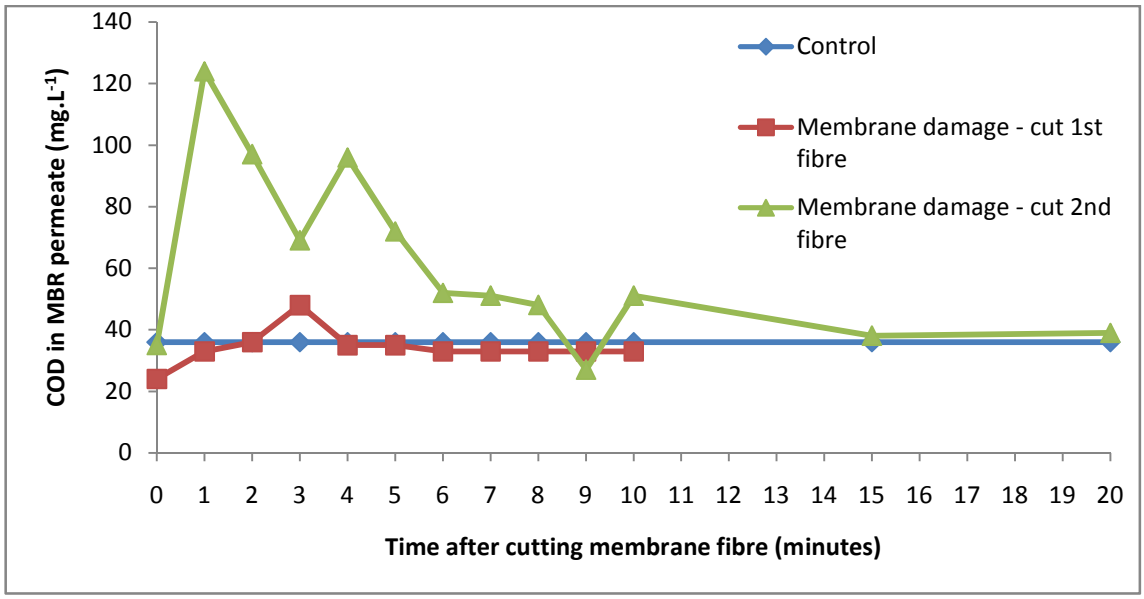


Figure 8.2 COD in the MBR permeate of the control and the physical membrane damage reactors in the first 20 min after cutting the fibre

COD data in the MBR permeate of the control and the physical membrane damage reactors are presented in Figure 8.2. After cutting the first fibre, COD concentration in the MBR permeate gradually increased from 24 to 48 mg.L^{-1} after 3 min and then reduced to 33 mg.L^{-1} after 4 min and remain stable at this level. After cutting the second fibre, COD in the permeate immediately increased to 124 mg.L^{-1} after 1 min, it then reduced to 100 mg.L^{-1} after 2 min, 72 mg.L^{-1} after 5 min and 51 mg.L^{-1} after 10 min. This result indicates leaking of organic matter through the membrane breakage. The result is anticipated as membrane integrity failure was expected to impair permeate water quality (Judd and Judd, 2011). The COD slowly reduced to 38 mg.L^{-1}

after 15 min and then 35 $mg.L^{-1}$ after 60 min and remained stable at this level until the end of the experiment.

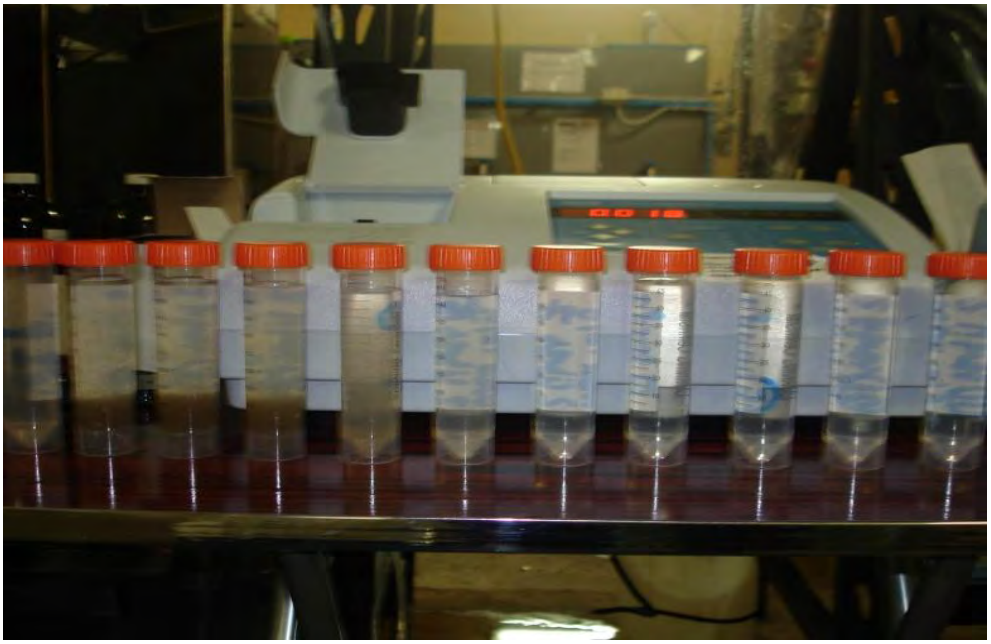


Figure 8.3 Permeate samples from the physical membrane damage experiment taken every min after cutting the second fibre

MLSS and MLVSS concentrations of mixed liquor of the control and the physical membrane damage reactors are presented in Figure 8.4 and Figure 8.5. Results show that the difference of MLSS and MLVSS concentrations between the control and the membrane damage reactor was within the variation range between the MBRs observed during the reproducibility experiments. As physical membrane damage does not affect the characteristics of the biomass, it would not be expected to cause any significant variation in MLSS and MLVSS concentrations in the MBR.

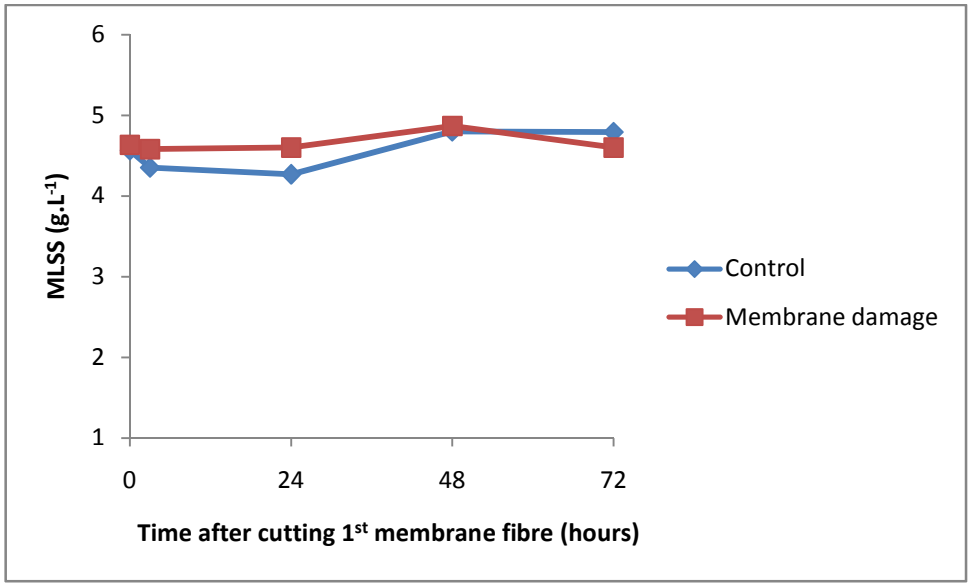


Figure 8.4 MLSS concentrations of the control and the physical membrane damage reactors

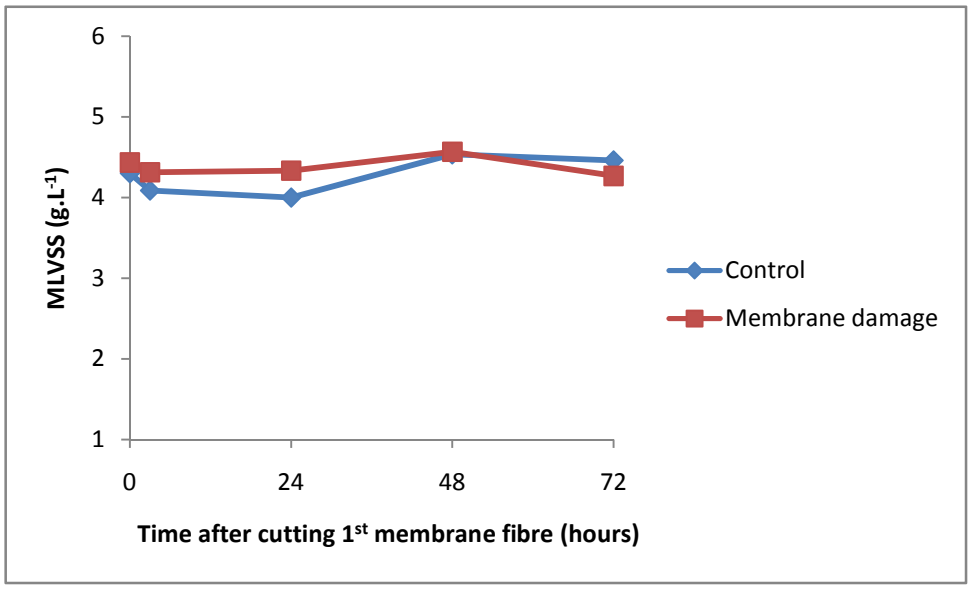


Figure 8.5 MLVSS concentrations of the control and the physical membrane damage reactors

CST of mixed liquor from the control and the physical membrane damage reactors is presented in Figure 8.6. Results show that the variation of CST of membrane damage reactor was within the variation range of that of the control. Again this was expected since physical membrane damage does not affect the characteristics of the biomass.

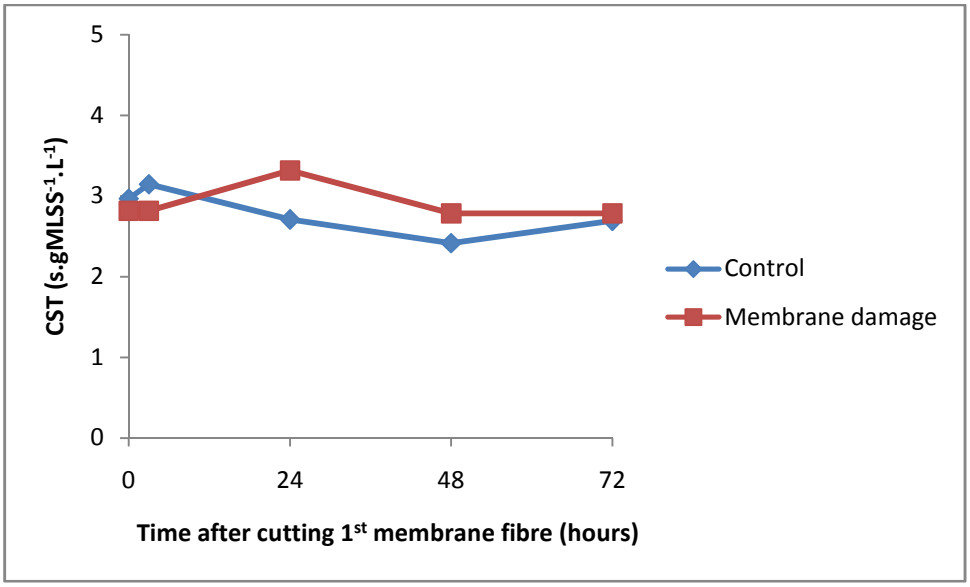


Figure 8.6 CST of the control and the physical membrane damage reactors

After cutting the membrane fibres, TMP of the physical membrane damage reactor rose very slowly from 8 kPa to 10 kPa until the end of the experiment while TMP of the control increased more quickly from 10 kPa and reached 23 kPa at the end of the experiment. Furthermore, the permeate flow rate of the experimental MBR was observed to increase (by about 20%) relative to the control MBR. This increased permeate flow continued for at least the first 24 h after the membrane breakage (i.e. significantly longer than the turbidity and COD increases). These observations suggest that while biomass (and turbidity and COD) breakthrough from the breached fibre was minimised by the clogging effect, some increased hydraulic flow through the breached membrane was maintained.

Loss of power experiment

Results of key bulk water quality and MBR operational parameters including pH, COD, MLSS, MLVSS, CST and TMP during the loss of power experiment are presented in this section. The pH was not affected by the loss of power conditions, with the pH of MBR effluent of the shock reactor remained around 6.6 during the experiment. After turning the power supply off, DO in the reactor rapidly reduced and reached 0 $mg.L^{-1}$ within 30 minutes. When the power supply was returned, DO in the reactor immediately increased and reached 4 $mg.L^{-1}$ within 15 minutes.

COD removal efficiency for the control and the loss of power reactors is presented in Figure 8.7. Results show that COD removal efficiency of the loss of power reactor was slightly reduced (by about 3%) when compared to the control in the first 3 h after

returning the power supply. After that, the COD removal efficiency for the loss of power reactor recovered to a similar value to that of the control and remained the same until the end of the experiment. These results suggest that the MBR performance was sufficiently robust to be unaffected by this particular hazardous event scenario. No previous literature reports on the impacts of loss of power on AS or MBR performance were able to be identified.

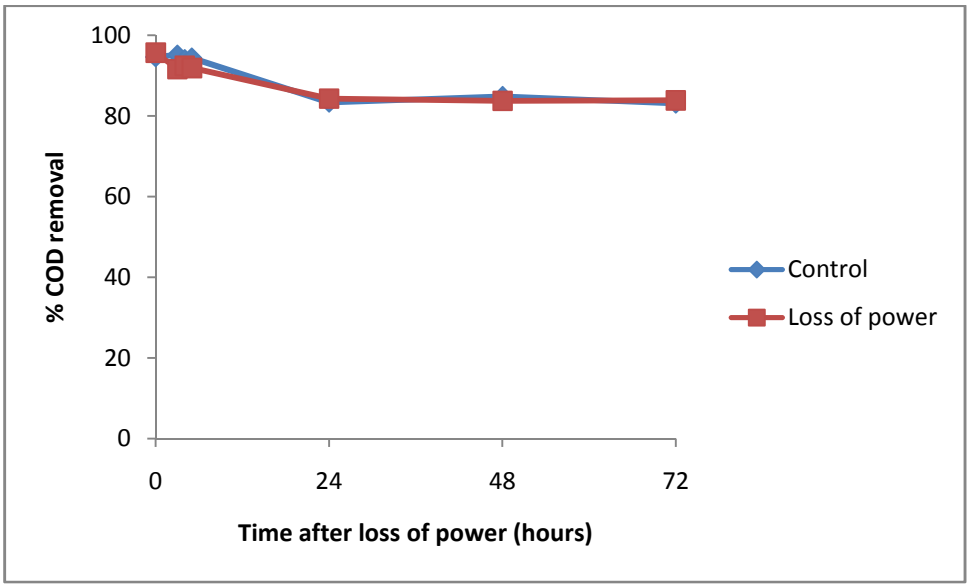


Figure 8.7 COD removal efficiency of the control and the loss of power reactors

MLSS and MLVSS concentrations of mixed liquor for the control and the loss of power reactors are presented in Figure 8.8 and Figure 8.9. Results show that MLSS concentrations in the loss of power reactor were slightly reduced from 4.7 $g.L^{-1}$ to 4.1 $g.L^{-1}$ after the loss of power period. The MLSS concentrations then increased to 4.3 $g.L^{-1}$ after the power had been returned for 24 h and 4.4 $g.L^{-1}$ after 48 h. The MLSS concentrations had recovered back to the pre-shock levels after 72 h. The MLVSS data had the same trend as the MLSS data. In general, the MLSS and MLVSS concentrations were not affected by the loss of power for a duration of 2 h.

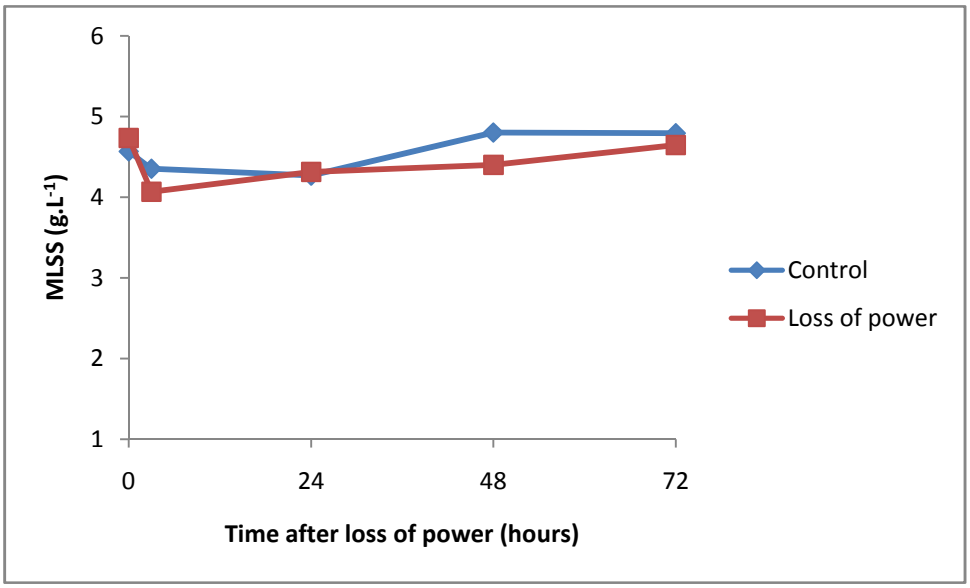


Figure 8.8 MLSS concentrations of the control and the loss of power reactors

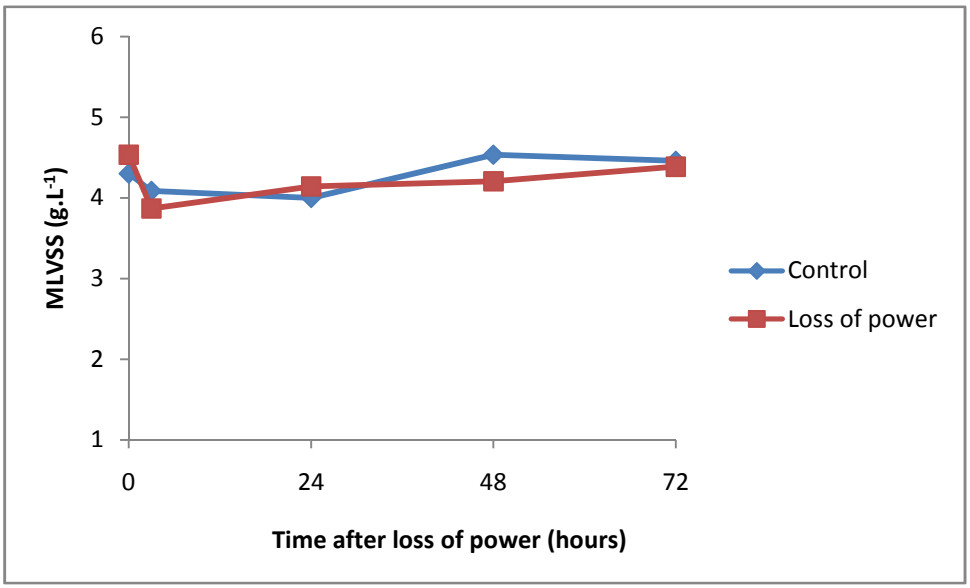


Figure 8.9 MLVSS concentrations of the control and the loss of power reactors

Mixed liquor CST of the control and the loss of power experiments are presented in Figure 8.10. Results show that CST for the mixed liquor from the loss of power reactor was slightly higher than that of the control, but the difference was within the variation of the measurement itself (±10%). TMP of the loss of power reactor was similar to that of the control, which increased gradually from 10 kPa to 23 kPa during the experiment.

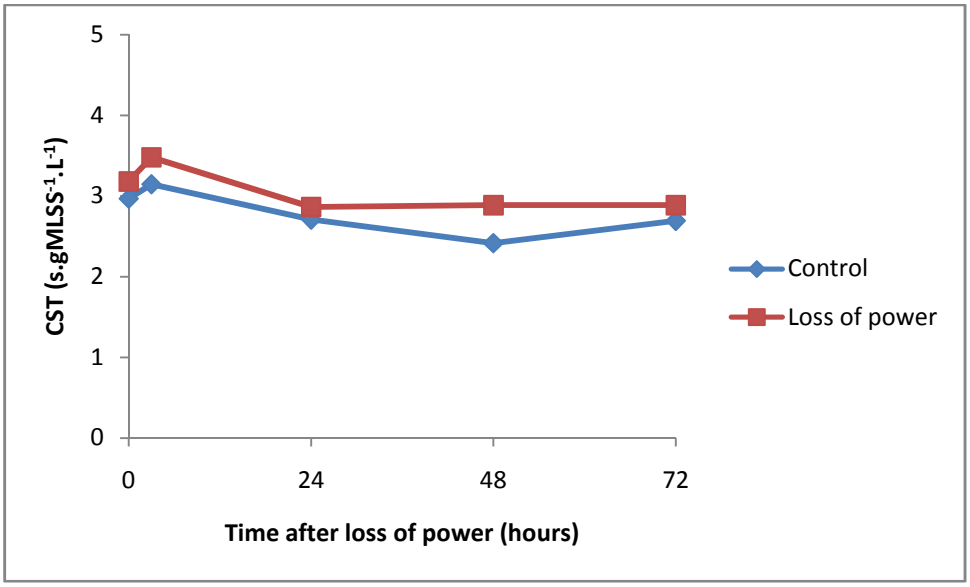


Figure 8.10 CST of the control and the loss of power reactors

8.3.2. Trace chemicals

Removal of trace chemicals by the MBRs under control, physical membrane damage and loss of power conditions are presented in this section. Among the analysed chemicals, 17α-estradiol, 17α-ethynylestradiol, mestranol, levonorgestrel, nonylphenol, 4-tert-octylphenol, diazepam, dilatin, meprobamate, enalapril, hydroxyzine, omeprazole, simvastatin, simvastatin hydroxy acid, atrazine and linuron were not detected in influent samples. Some trace chemicals (dihydrotestosterone, androstenedione, amitriptyline, risperidone, triamterene) were only detected in a few influent samples.

Similar to Chapters 6 and 7, the results of trace chemicals that were consistently detected in the influent during the experimental period were divided into 3 groups including hydrophilic chemicals (log D_{pH8} < 2), moderately hydrophobic chemicals (2 ≤ log D_{pH8} ≤ 3. 2), very hydrophobic chemicals (log D_{pH8} > 3.2) as previously described by Tadkaew et al. (2011).

Physical membrane damage experiment

The removals of hydrophilic, moderately hydrophobic and hydrophobic chemicals during the physical membrane damage experiment are presented in Figure 8.11, Figure 8.12 and Figure 8.13, respectively. The permeate samples for trace chemical analysis were collected after the samples for turbidity and COD analysis was taken (20 min after cutting the second fibre). Turbidity and COD analyses revealed that these

impacts were 'self-repaired' by blocking of the breach within approximately 15 min. Accordingly, the physical membrane damage conditions were shown to have an insignificant impact to overall trace chemical removals.

The overall removals of these chemicals were generally high, above 80% during the experiment. Removal via adsorption to biomass contributed up to 11% to the overall removal of triclosan. For triclocarban, the percentage removal via adsorption to biomass was 74%-81% in the control reactor and 69%-77% in the shock reactors. This variation was within the variation range observed during reproducibility experiment. Removal via adsorption to biomass contributed less than 5% to the overall removals of other chemicals including sulfamethoxazole, caffeine, ketoprofen, naproxen, ibuprofen, paracetamol, gemfibrozil, oestriol, testosterone, propylparaben, 2-phenylphenol, oestrone, etiocholanolone, androsterone and 17β-estradiol.

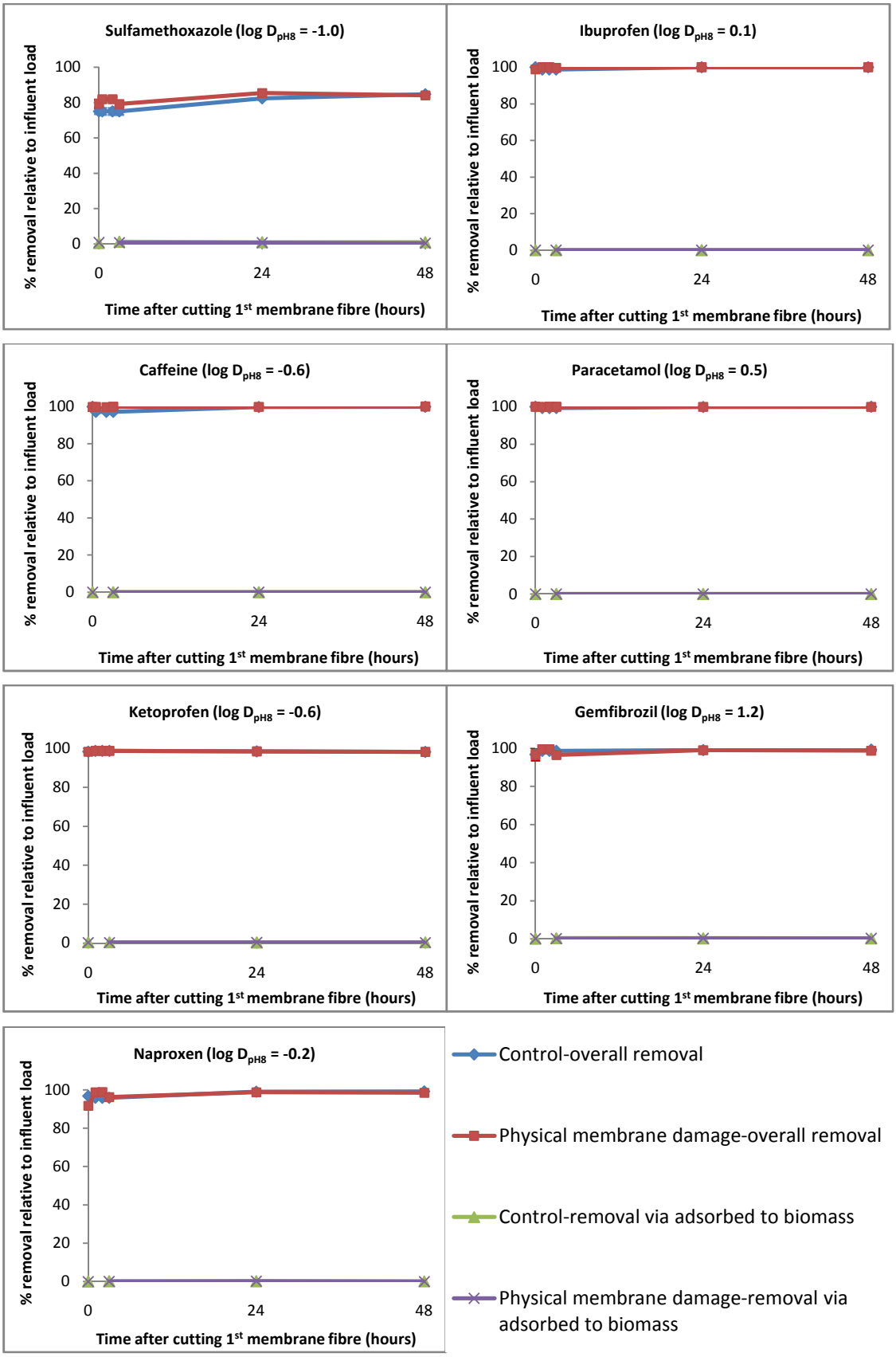


Figure 8.11 Removal of hydrophilic chemicals (log D_{pH8} < 2) by the control and the physical membrane damage reactors

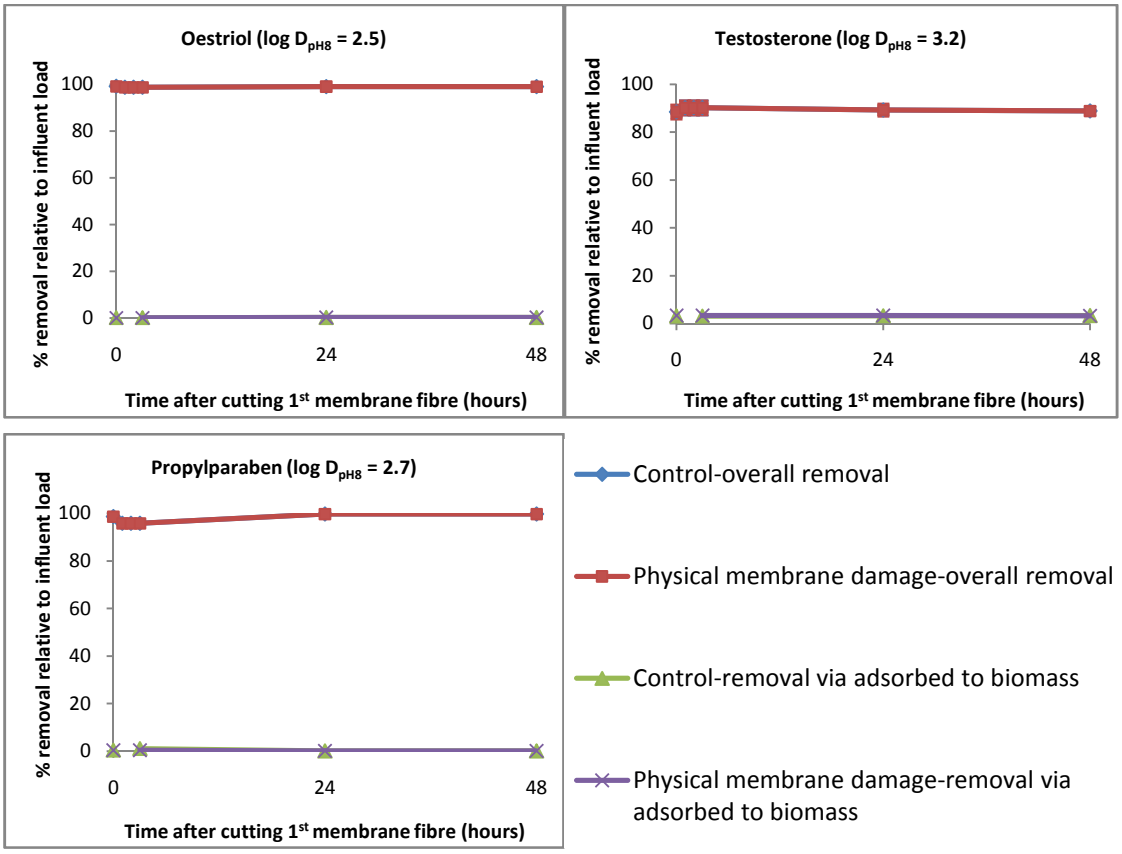


Figure 8.12 Removal of moderately hydrophobic chemicals (2 ≤ log D_{pH8} ≤ 3. 2) by the control and the physical membrane damage reactors

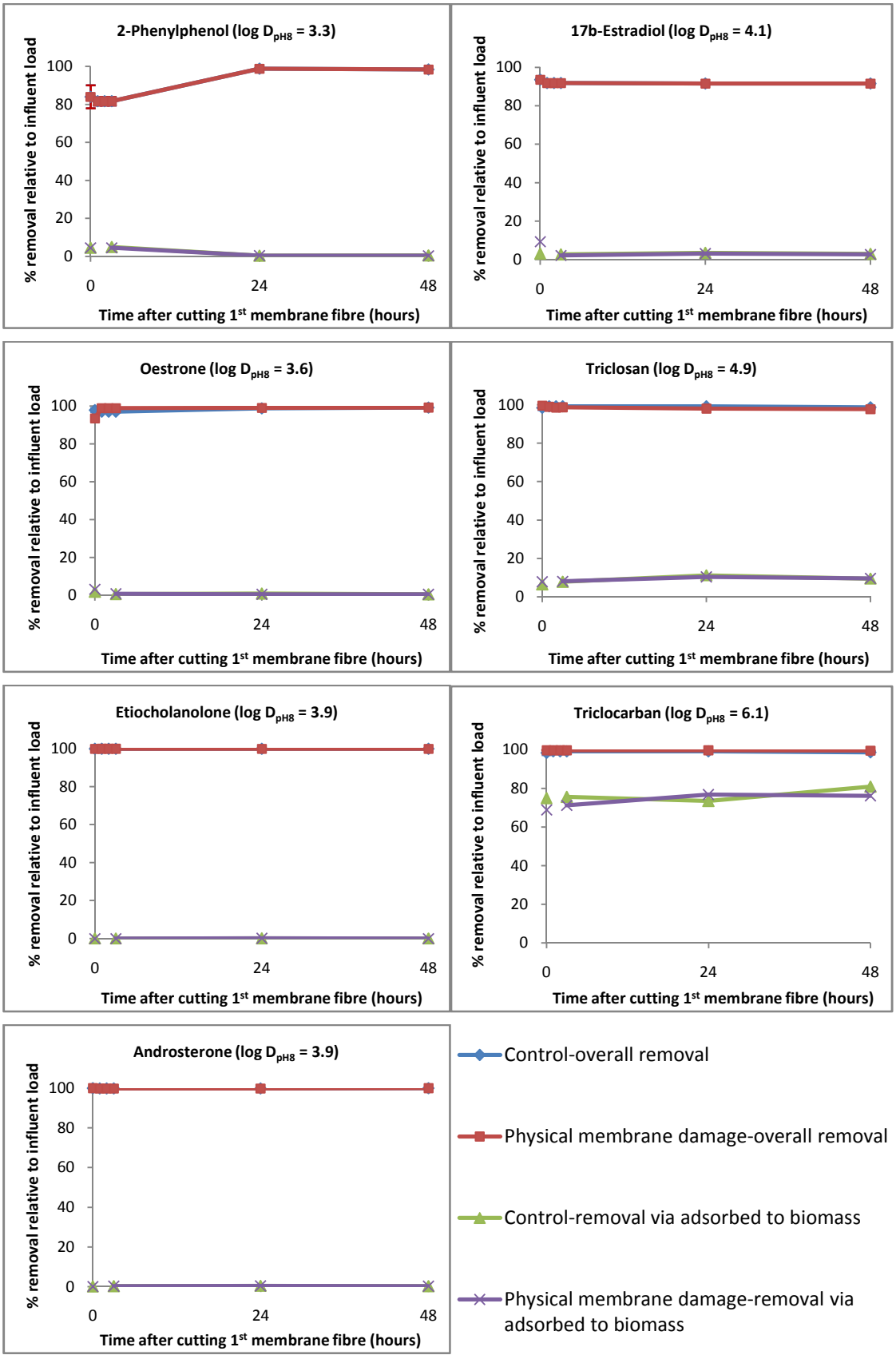


Figure 8.13 Removal of very hydrophobic chemicals (log D_{pH8} > 3.2) by the control and the physical membrane damage reactors

Loss of power experiment

Figure 8.14 presents removal efficiencies of hydrophilic trace chemicals by the control and the loss of power reactors. Results show that only removals of sulfamethoxazole, ketoprofen, gemfibrozil and naproxen were slightly reduced after the loss of power (for a 2 h duration) but these removal efficiencies recovered fully within 24 h. This reduction in removal efficiencies of these chemicals may be due to the anoxic conditions during the power loss period that affected the activity of specific organism groups responsible for degrading these compounds. Results from Figure 8.14 confirm that removal via adsorption to biomass was an insignificant removal mechanism (< 2%) for these hydrophilic chemicals and this was not changed under the loss of power conditions. Biotransformation was the main removal mechanism for these hydrophilic chemicals.

Figure 8.15 presents the removal efficiencies for moderately hydrophobic chemicals by the control and the loss of power reactors. Results show that the overall removals of the moderately hydrophobic chemicals oestriol, propylparaben and testosterone were high (> 90%) and not affected by loss of power conditions. Removal via adsorption to biomass was not a significant removal mechanism for these chemicals. It is noted that the concentration of testosterone in biomass was below the limit of quantification (LOQ), so the LOQ value was used to calculate the mass balance, revealing that the percentage of testosterone removed via adsorption to biomass was < 4%.

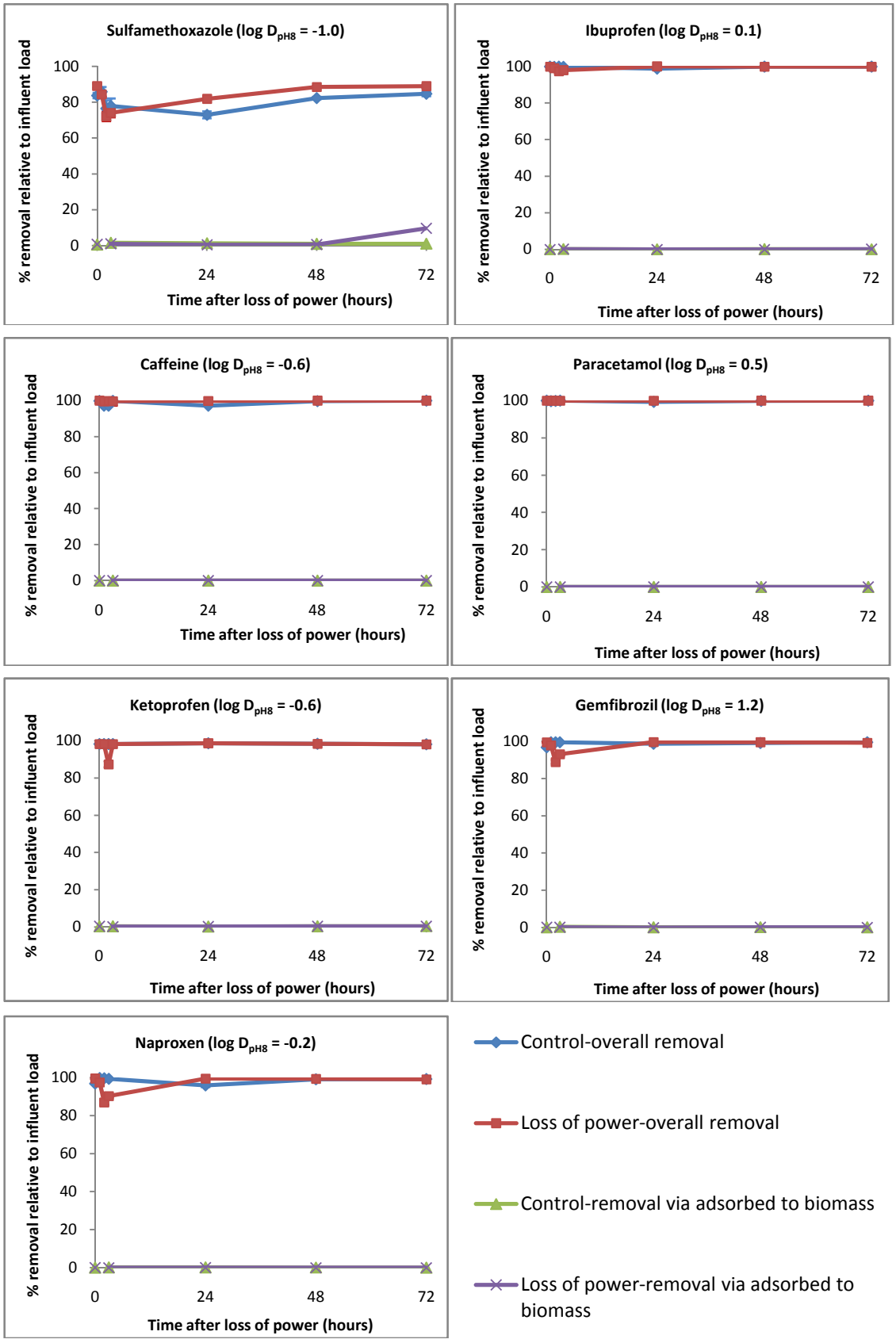


Figure 8.14 Removals of hydrophilic chemicals (log D_{pH8} < 2) by the control and the loss of power reactors

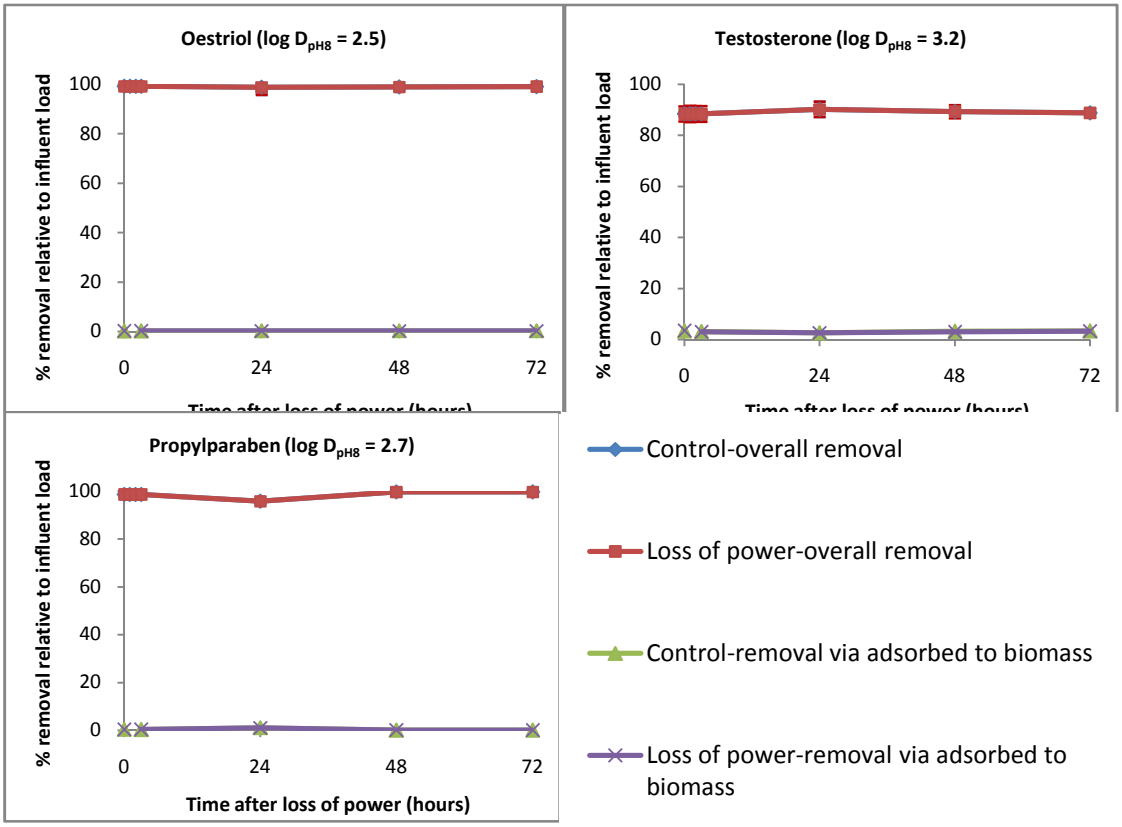


Figure 8.15 Removals of moderately hydrophobic chemicals (2 ≤ log DpH8 ≤ 3. 2) by the control and the loss of power reactors

Figure 8.16 presents the removal efficiencies for very hydrophobic chemicals by the control and the loss of power reactors. Similar to the moderately hydrophobic chemicals, results showed that the removal efficiencies for these very hydrophobic chemicals were not affected by the loss of power conditions. The overall removals of 2-phenylphenol, oestrone, etiocholanolone, androsterone, 17β-estradiol, triclosan and triclocarban were high, greater than 80% during the experiment. Removal via adsorption to biomass contributed up to 12% to the overall removal of triclosan. For triclocarban, percentage removal via adsorption to biomass of the loss of power reactor was similar with that of the control, varying from 67% to 81% during the experiment. Removals via adsorption to biomass contributed less than 4% for other chemicals.

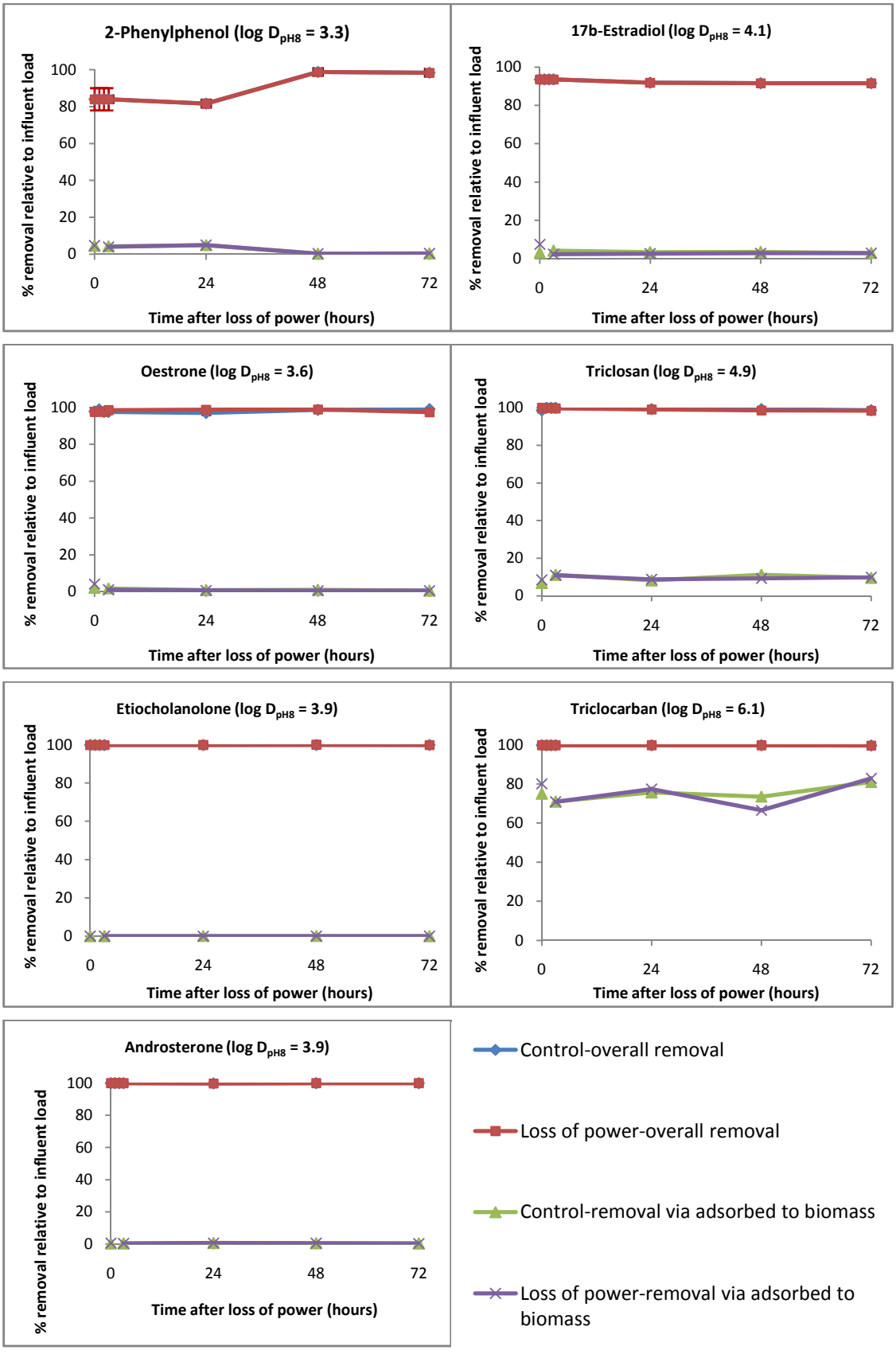


Figure 8.16 Removals of very hydrophobic chemicals (log DpH8 > 3.2) by the control and the loss of power reactors

8.4. CONCLUSIONS

The physical membrane damage experiment showed that after breaking 1 membrane fibre, permeate turbidity was not affected. However, after breaking 2 membrane fibres, turbidity and COD of the MBR permeate immediately increased. Turbidity and COD analyses revealed that these impacts were 'self-repaired' by the blocking of the breach within approximately 15 minutes. Accordingly, the physical membrane damage conditions were shown to have not a significant impact to overall removals of the trace chemicals. MLSS and MLVSS concentrations were also not affected by the physical membrane damage conditions. Results confirm that turbidity is an effective indicator for online monitoring and able to quickly detect physical membrane damage. Permeate COD is also a potentially indicator for monitoring physical membrane damage conditions, but is limited by the fact that it cannot currently be measured continuously.

A loss of power supply for a duration of 2 hours was observed not to significantly impact the performance of the MBR. This indicates that the MBR was relatively robust against this type of hazardous event. Nonetheless, results revealed that the removal efficiencies of some trace chemical contaminants were marginally impacted. These included the hydrophilic, less readily biotransformable chemicals sulfamethoxazole, ketorpofen, gemfibrozil and naproxen. As such, these chemicals appear to be sensitive indicators for loss of removal efficiencies for other chemicals with similar physicochemical properties during some (even relatively minor) hazardous event scenarios.

CHAPTER 9. APPLICATION OF INDICATORS AND SURROGATES FOR HAZARDOUS EVENT IDENTIFICATION AND ASSESSMENT

This chapter has been accepted for publication in part in the following book:

T. Trinh, A. Branch, B. van den Akker, P. Le-Clech, J. Drewes, S. Khan, Chapter 7: Impacts of hazardous events on performance of membrane bioreactors, In: F. I. Hai, K. Yamamoto, C-H. Lee (Eds), *Membrane Biological Reactors*. IWA Publishing, London, 2014, pages 207-221.

9.1. INTRODUCTION

The work described in this thesis has led to the identification of a number of specific chemical substances, as well as some bulk water quality measures, for which treatment performance is observably impacted by various hazardous events. It is proposed that monitoring the treatment performance of these chemicals and bulk parameters may, therefore, provide a sensitive measure of the occurrence and impacts of such events.

In this Chapter, a brief description of current relevant frameworks for risk assessment is provided. This is followed by a description of the current concept of using indicator chemicals are derived from the hazardous event simulation experiments in this study and surrogates to characterise water treatment process performance. It is then proposed that the current risk assessment framework for water recycling could effectively accommodate the expansion of the application of indicators and surrogates approach to the detection and characterisation of hazardous events.

9.2. RISK ASSESSMENT IN WATER AND WASTEWATER

Environmental risk assessment is the process of estimating the potential impact of a biological, chemical, physical or radiological hazard on a specified human population or ecological system under a specific set of conditions and for a certain time frame. In 1983, The US National Research Council published what became known as the “red book” (NRC, 1983), which laid the foundation for contemporary risk assessment processes. This document introduces four major steps to be taken for assessing risks to human health by chemicals from environmental or other sources. These steps included (1) hazard identification; (2) dose-response assessment; (3) exposure assessment and (4) risk characterisation. This risk assessment framework has since been adapted by Australian environmental health regulators and is described in the Australian EnHealth Council document ‘Environmental Health Risk Assessment: Guidelines for Assessing Human Health Risks from Environmental Hazards’ (EnHealth Council, 2012).

Although the documents from the US National Research Council and the EnHealth Council provide the essential fundamental approach to risk assessment, they do not fully capture the true assessment of risks unless hazardous events are considered, as real water quality and exposure risks tend to be posed when things go wrong (Hrudey and Hrudey, 2007). Risk assessment focused on hazardous events has been applied

for a wide variety of applications including managing water borne diseases (Mouchtouri et al., 2012), managing chemical accidents (Jang et al., 2011), modelling oil refinery accidents (Kalantarnia et al., 2010), preventing loss of containment of materials and energy from industrial processes (Dharmavaram and Klein, 2010), and probabilistic characterisation of possible future eruptive events of a volcano (Neri et al., 2008).

The assessment of hazardous events is a key philosophy in the approach to water quality risk assessment used by the World Health Organisation (WHO) for the development of Water Safety Plans (WHO, 2009) and is described in the WHO Guidelines for Drinking Water Quality (WHO, 2011).

Current Australian water quality management guidelines are based on risk assessment and risk management considerations. Examples include the Australian Drinking Water Guidelines (NHMRC & NRMMC, 2011) and the Australian Guidelines for Water Recycling (NRMMC & EPHC, 2006). As defined in these national guideline documents, a hazard is a biological, chemical, physical or radiological agent that has the potential to cause harm; and a hazardous event is an incident or situation that can lead to the presence of a hazard. Risk is then the likelihood of identified hazards causing harm in exposed populations in a specified timeframe, including the severity of the consequences.

In this context, potential hazardous events are identified and each is allocated a qualitative measure for both perceived 'likelihood' (Table 9.1) and 'consequence' or impact (Table 9.2). The examples given in Tables 9.1 and 9.2 are adopted from the Australian Guidelines for Water Recycling (NRMMC & EPHC, 2006).

Table 9.1 Qualitative measures of likelihood (NRMMC & EPHC, 2006)

Level	Descriptor	Example description
A	Rare	May occur only in exceptional circumstances. May occur once in 100 years
B	Unlikely	Could occur within 20 years or in unusual circumstances
C	Possible	Might occur or should be expected to occur within a 5- to 10-year period
D	Likely	Will probably occur within a 1- to 5-year period
E	Almost certain	Is expected to occur with a probability of multiple occurrences within a year.

Table 9.2 Qualitative measures of consequence or impact (NRMMC & EPHC, 2006)

Level	Descriptor	Example description
1	Insignificant	Insignificant impact or not detectable
2	Minor	Health – Minor impact for small population Environment – Potentially harmful to local ecosystem with local impacts contained to site
3	Moderate	Health – Minor impact for large population Environment – Potentially harmful to regional ecosystem with local impacts primarily contained to on-site.
4	Major	Health – Major impact for small population Environment – Potentially lethal to local ecosystem; predominantly local, but potential for off-site impacts
5	Catastrophic	Health – Major impacts for large population Environment – Potentially lethal to regional ecosystem or threatened species; widespread on-site and off-site impacts

Once a suitable qualitative measures of likelihood and consequences have been allocated to each identified (potential) hazardous event, a qualitative risk estimation or 'risk rating' can be applied according to the risk matrix presented in Table 9.3. The specific characterisation (e.g., low, moderate, high, very high) of risks relating to various combinations of likelihood and consequence measures may be adapted for particular systems and applications. The example given in Table 9.3 is that used in the Australian Guidelines for Water Recycling (NRMMC & EPHC, 2006) and is very similar to those presented in the Australian Drinking Water Guidelines (NHMRC & NRMMC, 2011) and the WHO Guidelines for Drinking Water Quality (WHO, 2011).

Table 9.3 Qualitative risk estimation (NRMMC & EPHC, 2006)

Consequences					
Likelihood	**1-Insignificant**	**2-Minor**	**3-Moderate**	**4-Major**	**5-Catastrophic**
A Rare	Low	Low	Low	High	High
B Unlikely	Low	Low	Moderate	High	Very high
C Possible	Low	Moderate	High	Very high	Very high
D Likely	Low	Moderate	High	Very high	Very high
E Almost Certain	Low	Moderate	High	Very high	Very high

This risk assessment process provides a basis for managing risks and applying preventive measures. In the context of wastewater and recycled water management, preventative measures most commonly refer to actions, activities and processes used to prevent significant hazards from being present in final effluents or to reduce the hazards to acceptable levels.

9.3. INDICATORS AND SURROGATES FOR WATER TREATMENT PROCESS PERFORMANCE ASSESSMENT

As defined by Drewes et al. (2008), an indicator chemical is an individual chemical, which represents certain physicochemical and biodegradable characteristics of a family of trace constituents that are relevant to fate and transport during treatment, providing a conservative assessment of removal. A surrogate is defined as a quantifiable change of a bulk parameter that can serve as a performance measure of individual unit processes or operation regarding the removal of trace chemicals (Drewes et al., 2008).

The development of indicator chemicals and surrogate parameters for monitoring treatment performance has been described in a number of publications (Drewes et al., 2008, Dickenson et al., 2009, Salveson et al., 2012). A summary of the basic approach to indicator chemicals and surrogate measures is presented in Table 9.4. According to Drewes et al (2008), the application of the surrogate/indicator framework to assess treatment performance should be divided into two phases: piloting/start-up and full-scale operation/compliance monitoring. Firstly, proper operational boundary conditions of each unit process need to be defined according to their technical specifications. Then, surrogate or operational parameters that demonstrate a measurable removal under normal operating conditions need to be identified for each unit process. In parallel, an occurrence study to confirm presence of viable indicator compounds in the feedwater of each unit process should be conducted. A challenge or spiking study with selected 5-10 indicator chemicals classified as “good removal” should be conducted during piloting or start-up of a new treatment process to determine the removal differentials under normal operating conditions. For full-scale operation, operational boundary conditions and removal differential ΔX and ΔY for selected surrogate, operational parameters and indicator chemicals need to be confirmed. Selected surrogate and operational parameters need to be measured on a daily or weekly basis to ensure proper treatment process performance. Selected indicator chemicals (3-6 indicators) should also be monitored on a semi-annually or annually basis.

Table 9.4 Application of the surrogate/indicator framework to an overall treatment train (Drewes et al., 2008)

	Surrogate parameters	Indicator compounds
Pilot or/and start-up		
Step 1	Define operational boundary conditions for each unit process comprising overall treatment train for proper operation according to technical specification	
Step 2	For each unit process, identify those surrogate or operational parameter that demonstrate a measurable removal under normal operating conditions and quantify their removal differential $\Delta X = (X_{in}-X_{out})/X_{in}$	Conduct occurrence study to confirm presence of viable indicator chemicals in the feedwater of each unit process
Step 3		Conduct challenge or spiking study with selected 5-10 indicator chemicals during pilot-or start-up to determine the removal differentials under normal operating conditions $\Delta Y = (Y_{in}-Y_{out})/Y_{in}$
Step 4	Select viable surrogate and operational parameters for each unit process	Select 3-6 indicator chemicals from categories classified as “good removal”
Full-scale operation/compliance monitoring		
Step 5	Confirm operational boundary conditions of full-scale operation and removal differential ΔX for selected surrogate and operational parameters	
Step 6	Monitor differential ΔX of selected surrogate and operational parameters for each unit process or/and the overall treatment train on a regular basic (daily, weekly)	Monitor differential ΔY of selected indicator chemicals for each unit process or/and the overall treatment train semi-annually/annually

Application of indicators and surrogates for assessing water treatment process performance is an important research area that has been developed. In the studies described above, indicator chemicals are described as being useful to assess treatment process performance in general. However, the idea of applying them specifically to hazardous event identification and assessment has not been previously investigated. These previous studies have not described how removals of particular indicator chemicals will change outside normal operating conditions.

9.4. INCORPORATION OF CHEMICAL INDICATORS FOR HAZARDOUS EVENT IDENTIFICATION AND ASSESSMENT

MBR process performance has been monitored in real-time by turbidity measurement, flow measurements, transmembrane pressure, bioreactor tank levels, dissolved oxygen concentration of the bioreactor, as well as status of pumps and critical valves (i.e., on/off). The results from hazardous event simulation experiments in this study confirm that turbidity is an effective indicator for online monitoring and able to quickly detect physical membrane damage. Permeate chemical oxygen demand (COD) is also a potentially indicator for monitoring physical membrane damage, but is limited by the fact that it cannot currently be measured continuously. The results also reveal that COD removal, capillary suction time (CST) and transmembrane pressure (TMP) are effective MBR operational parameters for monitoring impacts of organic shock and toxic shocks such as salinity, ammonia and 2,4 dinitrophenol (DNP) shocks on MBR performance. However, these parameters are not sufficiently sensitive to pick up treatment performance changes caused by relatively minor hazardous event scenarios (e.g a loss of power duration event of 2 hours). In our experiments, it was observed that the removal performance of some groups of chemicals was impacted under such scenarios. Therefore, more sensitive indicators are required for such hazardous event identification and assessment.

The results from salinity, ammonia, DNP and organic shock experiments consistently revealed that the hydrophobic chemicals and easily biotransformable hydrophilic chemicals are generally resistant to impacts from the shocks. However, the less readily biotransformable hydrophilic chemicals (e.g ketoprofen and gemfibrozil) were the least resistant to loss of biotransformability from the shocks. Even in relatively minor hazardous event scenarios (e.g. loss of power duration of 2 hours), where bulk parameter results revealed no significant impact to the MBR performance, trace chemical analysis results revealed that the removal efficiencies for some less readily biotransformable hydrophilic chemicals (e.g. sulfamethoxazole, ketoprofen, gemfibrozil and naproxen) were measurably impacted. As such, these chemicals appear to be sensitive indicators for identifying and assessing impacts of such hazardous events on MBR treatment performance including impacts on removal efficiencies for other chemicals with similar physicochemical properties.

9.5. RISK ASSESSMENT AND MANAGEMENT

In this section, the risk is that which is considered to result in poor removal of trace chemical contaminants by the MBR or, in other words, how reliable is the MBR for removing trace chemical contaminants.

9.5.1. Likelihood

The likelihood of each of these types of hazardous events will be specific to various treatment plants and their catchments. Important factors will include:

- Types of industries around the catchment,
- Types of other activities around the catchment (dairy farms etc.),
- Potential for stormwater dilution,
- Reliability of power supply,
- Size of the catchment,

For MBRs treating municipal wastewater, literature has reported that toxic shocks, particularly salinity shocks at coastal sites occur occasionally (Judd and Judd, 2011). In addition, hydraulic shock loads due to storm flows often occur during exreme wet weather events (Judd and Judd, 2011).

A workshop was conducted with the aim of characterising hazardous event scenarios for MBRs as part of this project. This was hosted at the AWA Water Reuse and Desalination conference in November, 2010 in Sydney. Workshop participants included representatives from MBR suppliers, MBR operators, MBR researchers and Australian health regulators. The outcomes of this workshop identified organic shock, starvation, salinity shock, ammonia shock, toxic shock, loss of power and physical membrane damage as the most common types of hazardous events expected to impact MBRs performance.

Case studies at water supply systems in Australia, Latin America and the United Kingdom have revealed that pump breakdown and power supply loss may also be expected to occur a few times a year in some systems (WHO, 2009).

Similarly, in the package MBRs in this project, pump and blower malfunctions were also known to occur a few times per year. Pump chokes have been found to occur more frequently at sites where pumps do not have an open impeller design or where pump stations have not been maintained by vacuuming or jetting. However, in these MBR plants, there are always standby pumps and blowers so these spare pumps and blowers can be changed immediately when such events occur (McLeod and Powell, 2013, Watkins, 2013). Loss of power supply has been reported to occur about once per year at the plant in Old Bar for a short period of 1-3 hours (Watkins, 2013) while the plants in Bega Valley have experienced fluctuating supplied voltage leading up to eight power outage events lasting from a few minutes to 4 hours across Bega Valley in a year (McLeod and Powell, 2013). Planned power outages due to upgrades are also reported up to 8 hours in duration (McLeod and Powell, 2013). Organic and ammonia shock has been reported to occur at coastal MBR plants in a peak holiday period during Christmas time and sometimes during Easter school holidays with likelihood/frequency of around once to twice per year. In addition, organic shock has also been found to occur about once per year to the MBRs receiving diary waste discharge (McLeod and Powell, 2013, Watkins, 2013). Salinity shock caused by broken sewer mains has been found to happen about once in 10 years for coastal plants only (McLeod and Powell, 2013, Watkins, 2013). Electrical conductivity of influent is monitored for this occurrence. In Old Bar, other toxic shocks were found to occur about once per year in catchments where the industrial component of the catchment is high or where the treatment plant is small with some industrial component (Watkins, 2013). In contrast, in Bega Valley, only one event of toxic shock occurred in one plant out of ten plants over the past 3 years (McLeod and Powell, 2013). Visible physical membrane damage was also reported to occur approximately once per year and turbidity of MBR permeate is monitored for this event. Starvation was rarely experienced in the studied package MBRs (McLeod and Powell, 2013, Watkins, 2013). However, the plant in Bega Valley experienced an event in 2008 when a new low pressure sewer system was brought online with a few connected properties. At that time, nitrification was achieved but the load was insufficient to also support biological phosphorous removal. As the load reached around 25% of design, the plant was able to achieve all design targets without chemical assistance (McLeod and Powell, 2013).

9.5.2. Consequences

The consequences in this chapter are defined in terms of impacts of the hazardous events to performance of MBR for removal of trace chemical contaminants.

For example, one might adopt the following consequences table or something similar to it to assess the consequences of hazardous events.

Table 9.5 Consequences of hazardous events

Level	Descriptor	Description
1	Insignificant	• Negligible loss of performance for all trace chemical contaminants
2	Minor	• Moderate loss of performance for some trace chemicals (≤ 70% normal removal) • Short recovery time (≤ 1 hour)
3	Moderate	• Significant loss of performance for most trace chemicals (≤ 50% normal removal) • Moderate recovery time (≤ 3 days)
4	Major	• Significant loss of performance for most of trace chemicals (≤ 50% normal removal) • Long recovery time (> 3 days)
5	Catastrophic	• Complete loss of performance for removal of all trace chemicals (≤10% of normal removal) • Long recovery time (> 3 days)

The results from the hazardous event simulation experiments in Chapters 6, 7 and 8 can benefit future risk monitoring and risk assessment for MBRs. In general, the results show that the less readily biotransformable hydrophilic chemicals (e.g ketoprofen and gemfibrozil) appear to be sensitive indicators for identifying and assessing impacts of such hazardous events on MBR treatment process performance including impacts on removal efficiencies for other chemicals with similar physicochemical properties. Therefore, ketoprofen and gemfibrozil which are the most sensitive indicators to hazardous events, are recommended as representative indicators for monitoring removal of trace chemical contaminants under various conditions. Table 9.6 presents the recommended indicators for MBR performance, especially for removal of trace chemical contaminants. If a hazardous event occurs at an MBR and if the MBR operators and managers require a detailed understanding of the consequences of the hazardous event, they could monitor the indicators presented in Table 9.6. The monitoring results of these indicators will provide information regarding the impacts of the hazardous event to MBR performance including impacts to removal of trace chemical contaminants.

Table 9.6 MBR performance indicators for trace chemical removals

Hazardous event	Description of impacts to trace chemical removal	Impacts to specific indicator chemical	Impacts to bulk parameters
Salinity shock	• Potential inhibited biotransformation of some hydrophilic chemicals	• Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal unaffected in minor circumstance[1] • Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal reduced significantly in major circumstance[2]	• Loss of COD removal • Increase in TMP and CST
2,4 DNP shock	• Possible reduced biological transformation of some hydrophilic chemicals	• Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal unaffected in minor circumstance[1] • Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal decreased significantly in major circumstance[2]	• Loss of COD removal • Increase in TMP and CST
Ammonia shock	• Potential hindered biotransformation of some hydrophilic chemicals	• Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal unaffected in minor circumstance[1] • Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal reduced significantly in major circumstance[2]	• Slightly reduce in COD removal • Rise in TMP and CST
Organic shock	• Possible hampered biological transformation of some hydrophilic chemicals	• Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal unaffected in minor circumstance[1] • Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal decreased significantly in major circumstance[2]	• Loss of COD removal • Increase in TMP and CST
Starvation	• Potential hindered	• Ketoprofen, naproxen, sulfamethoxazole and	• Potential inhibited

Hazardous event	Description of impacts to trace chemical removal	Impacts to <u>specific indicator chemical</u>	Impacts to bulk parameters
	biotransformation of some chemicals if the starvation period was long	gemfibrozil removal unaffected in minor circumstance[1] • Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal was possibly affected in major circumstance[2]	COD removal in major circumstance[2]
Loss of power	• Possible reduced biotransformation of some chemicals if the loss of power period was long	• Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal unaffected in minor circumstance[1] • Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal potential affected in major circumstance[2]	• Possible inhibited COD removal in major circumstance[2]
Physical membrane damage	• Potential decreased removal of trace chemical through membrane	• Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal unaffected in minor circumstance[1] • Ketoprofen, naproxen, sulfamethoxazole and gemfibrozil removal potential affected in major circumstance[2]	• Potential increased in permeate COD and turbidity.

[1]Short recovery time (≤ 1 hour); [2]Long recovery time (> 3 days)

9.5.3. Risk management

Certain hazardous events can be managed by planning ahead. For example, if spare blowers and pumps are available at the MBRs, then the MBR operators can respond to blower or pump break down by replacing the broken blower or pump immediately. Similarly, if an alternative power supply is planned, a loss of power incident can be well accommodated. The impacts of these hazardous events will be negligible if such rapid responses are in place.

However, it is generally not possible to guarantee the prevention of many types of hazardous events. Accordingly, systems must be designed with a degree of robustness to manage impacts to ongoing operation as well as risks to human health and the environment when hazardous events occur. Important concepts for managing hazardous events are the incorporation of multiple barriers in the design and establishment of a monitoring program that is suitable to constantly assess proper system performance. The selection of multiple barriers and a monitoring program will depend on the context in which an MBR is employed. Meeting effluent discharge standards will require a different management approach to potential hazardous events as compared to practices where MBR effluents are used for non-potable or potable reuse applications given the higher degree of potential exposure to public health.

Multiple barriers in water treatment and reclamation are aimed at ensuring that performance goals are met by (1) expanding the variety of contaminants a process train can effectively address by providing engineered redundancy (i.e., robustness) and (2) by improving the extent of consistent performance of a unit process to attenuate a contaminant (i.e., reliability) (NRC, 2012).

Even when true redundancy is not provided, multiple barriers can reduce the consequences of hazardous events when they do occur. For example, to mitigate the risk from pathogen exposure, all MBRs usually employ a disinfection step either using a chlorine-based disinfectant or UV irradiation, in addition to the MF or UF membrane that serves as a barrier to pathogens. A large balancing tank installed before the treatment process can help to equalise sudden spikes of shock chemicals if such hazardous events occur. This is especially useful for small plants where installation of online monitoring equipment for quick detection of hazardous events is not possible.

The extent of system performance and water quality monitoring will depend on project-specific water quality objectives and the potential impact from hazardous events. An

idealised monitoring program would measure critical process parameters and microbial and chemical contaminants in real time in the finished product water. However, real-time monitoring comes at significant capital and maintenance expenses and needs to be balanced against the estimated likelihood of certain hazardous events.

Monitoring requirements usually become more stringent (e.g., more frequent and broader in scope) as the potential for human contact with the reclaimed water increases (e.g., non-restricted irrigation of public parks; potable reuse). Monitoring programs to assure that water quality requirements are met most commonly include effluent turbidity and residual chlorine. Operational parameters that are measured in real-time include flow measurements, transmembrane pressure, bioreactor tank levels, dissolved oxygen concentration of the bioreactor, as well as status of pumps and critical valves (i.e., on/off). These parameters are recorded in the Supervisory Control and Data Acquisition System (SCADA) of the treatment facility and usually linked to certain threshold levels. An exceedance of these threshold levels might be caused by a hazardous event resulting in a shut-down of the system to mitigate the negative impact of that event.

Chemical indicators can play an important role in identifying hazardous events and assessing their significance in regard to any loss of performance for chemical removals. They, therefore, are useful for assessing and managing risks associated with hazardous events.

9.6. CONCLUSIONS

Application of indicators and surrogates for assessing water treatment process performance is a crucial research area. However, studies in previous publications have focused on development of indicator chemicals to assess treatment process performance under normal operational conditions. The application of indicator chemicals for hazardous event identification and assessment has not been previously investigated. This project has made an important contribution to this knowledge gap. This study has identified which indicator chemicals are sufficiently sensitive to hazardous events and thus can serve as representative indicator chemicals for identifying and assessing impacts of hazardous events on treatment process performance, including impacts on removals of other chemicals with similar physicochemical properties.

The less readily biotransformable hydrophilic chemicals (e.g ketoprofen, naproxen, sulfamethoxazole and gemfibrozil) appear to be sensitive indicators for identifying and assessing impacts of such hazardous events on MBR treatment process performance. These chemicals were measurably impacted even in relatively minor hazardous event scenarios (e.g. loss of power duration of 2 hours), where bulk parameter results revealed no significant impact to the MBR performance. Based on the results from the hazardous event simulation experiments in Chapters 6, 7 and 8, a consequences table for hazardous events and MBR performance indicators for trace chemical removals has been recommended. One might adopt the consequences table and performance indicators or something similar to it to assess the consequences of hazardous events to MBR systems.

Certain hazardous events such as blower or pump break down, loss of power can be managed by planning ahead and the impacts of these hazardous events will be negligible if appropriate planning and response is in place. However, it is generally not possible to guarantee the prevention of many types of hazardous events. Accordingly, systems must be designed with a degree of robustness to manage impacts to ongoing operation as well as risks to human health and the environment when hazardous events occur.

CHAPTER 10. CONCLUSIONS AND RECOMMENDATIONS

10.1. CONCLUSIONS

In Australia, performance of wastewater treatment processes for recycling such as MBRs requires validation in terms of a range of parameters and under a variety of operational conditions to ensure that water quality standards are achieved. One crucial aspect of this validation is to study the removals of trace organic chemical contaminants through MBRs during hazardous event conditions. The research presented in this thesis provides important contributions to understanding the impacts of several hazardous events including organic shock, salinity shock, ammonia shock, 2,4 dinitrophenol (DNP) shock, feed starvation, loss of power and physical membrane damage on MBRs performance. In addition, this research enables the identification and application of sensitive chemical indicators for hazardous event assessment. These findings are important for assessing and managing risks associated with hazardous events. The key conclusions from this research are summarised in the following sections, followed by some recommendations for future studies.

10.1.1. Conclusions from the development of an analytical method for steroid hormones

A rapid gas chromatography–tandem mass spectrometry (GC–MS/MS) analytical method was developed for the simultaneous analysis of 7 estrogenic hormones (17α-estradiol, 17β-estradiol, estrone, mestranol, 17α-ethynylestradiol, levonorgestrel, estriol) and 5 androgenic hormones (testosterone, androsterone, etiocholanolone, dihydrotestosterone, androstenedione) in aqueous matrices. This method is unique in its inclusion of all 12 of these estrogens and androgens and is of particular value due to its very short chromatographic run time of 15 min. The use of isotope dilution for all analytes ensures the accurate quantification, accounting for analytical variabilities that may be introduced during sample processing and instrumental analysis. Direct isotopically labelled analogues were used for 8 of the 12 hormones and satisfactory isotope standards were identified for the remaining 4 hormones. Method detection levels (MDLs) were determined to describe analyte concentrations sufficient to provide a signal with 99% certainty of detection. The established MDLs for most analytes were 1–5 $ng.L^{-1}$ in a variety of aqueous matrices. However, slightly higher MDLs were observed for etiocholanolone, androstenedione, testosterone, levonorgestrel and dihydrotestosterone in some aqueous matrices. Sample matrices were observed to have only a minor impact on MDLs and the method validation confirmed satisfactory

method stability over intra-day and inter-day analyses of surface water and tertiary treated effluent samples.

10.1.2. Conclusions from investigations at the full-scale package MBR in Wollumla, Bega Valley

The study at the full-scale package MBR in Wollumla, Bega Valley revealed that the removals of most trace organic chemical contaminants through the MBR were high (> 80%). In general, biotransformation was a dominant removal mechanism for most trace chemicals. However, removal via adsorption to biomass was a significant removal mechanism for amitriptyline, 17α-estradiol, triclosan and triclocarban with the percentage of removal via adsorption to biomass ranging from 35% to 86%. Removal via adsorption to biomass was reduced from 76% to 14% for 17β-estradiol from winter to summer sampling. This observation was attributed to the higher temperature in the bioreactor during the summer, which enhanced biotransformation of 17β-estradiol. Adsorption to biomass was a moderate removal mechanism for bisphenol A and estrone with the percentage of removal via adsorption to biomass being 10%. Adsorption to biomass was an insignificant (<5%) removal mechanism for the other trace chemicals including estriol, dihydrotetosterone, androsterone, etiocholanolone, propylparaben, 2-phenylphenol and 4-tert-octylphenol, atorvastatin, *o*-hydroxyatorvastatin, *p*-hydroxyatorvastatin, DEET, ibuprofen, ketoprofen, naproxen, paracetamol and caffeine.

Overall, the concentrations of trace chemicals detected in the MBR permeate were 1 to 5 orders of magnitude lower than the Australian guideline values for water recycling. The results of this study enhance the understanding of the levels, fate and removals of a comprehensive list of 48 trace chemical contaminants of concern through full-scale package MBR systems under normal operating conditions.

10.1.3. Conclusions from construction and testing of the experimental MBR system

Clean water testing of a constructed experimental MBR system suggested that bisphenol A was leaching from the materials used to build the experimental system (i.e. the plastic influent tank, effluent tank, plastic pipes, or plastic valves etc.). Bisphenol A was therefore removed from the list of analytes and not considered for the MBR

performance analysis using the experimental MBR system and the hazardous event simulation studies. DEET was also excluded from the studies due to quality control.

The reproducibility experiments revealed that key bulk water quality parameters, operational parameters and trace chemicals were generally reproducible with a maximum standard deviation of 8% between the four parallel experimental MBRs. The exception was triclocarban, which had a percentage removal via adsorption to biomass varying from 75% to 100% between the four MBRs. For atenolol, trimethoprim and diclofenac, although the overall removals of these chemicals were reproducible with a maximum standard deviation of 8% between the four MBRs, the standard deviations of overall removals of these chemicals between different sampling dates within the same MBR was larger, varied from 12 to 13 % for atenolol, from 7% to 12% for trimethoprim and from 9 to 14% for diclofenac.

For the very hydrophobic chemical triclocarban, the load of this chemical adsorbed to the suspended solids in the influent was as significant as the load in the filtered influent. Therefore, both contributions to the overall influent load need to be taken into account in the mass balance calculation. For other chemicals, the loads adsorbed to the suspended solids in the influent were negligible (< 1% of total load in the influent).

10.1.4. Conclusions from hazardous event simulation experiments

A significant reduction in chemical oxygen demand (COD) removal and a considerable increase in capillary suction time (CST) and transmembrane pressure (TMP) were observed under DNP, salinity, ammonia and organic shock conditions. These results revealed that COD removal, CST and TMP are effective MBR operational parameters for monitoring the impact of hazardous events such as DNP, salinity, ammonia and organic shocks on MBR performance.

The hazardous event simulation experiments revealed that removals of moderately and very hydrophobic chemicals (such as oestriol, testosterone, propylparaben, 2-phenylphenol, oestrone, etiocholanolone, androsterone, 17β-estradiol, triclosan, triclocanban) were not affected under DNP, salinity, ammonia and organic shock conditions. These observations suggest that biotransformation within the biomass structure itself was preserved as the chemicals were largely adsorbed to the biomass. Nevertheless, removals of hydrophilic chemicals were commonly affected by hazardous event conditions, implying the loss of the bioactivity in the aqueous phase.

These effects were mainly observed for hydrophilic chemicals with low or moderate readily biotransformability whereas highly biotransformable chemicals were still largely removed. These findings imply that the shock conditions seem to impact some specific organisms and/or metabolic processes, that are most severely affecting the less readily biotransformable chemicals (e.g. sulfamethoxazole, ketorpofen, gemfibrozil and naproxen), which rely upon specific organisms or metabolic pathways for their biotransformation. In contrast, readily biotransformable compounds (such as caffeine and paracetamol) may be more easily transformed by a wider range of organisms and/or metabolic pathways so they are still widely biotransformed under the shock conditions. Therefore, less readily biotransformable hydrophilic chemicals (e.g. sulfamethoxazole, ketorpofen, gemfibrozil and naproxen) may be sensitive indicators for monitoring the impact of toxic shock conditions on the performance of MBRs, especially for the removal of other trace chemicals with similar physicochemical properties.

Feed starvation seems to have significant impacts on MLSS concentrations but the overall system performance remained relatively resilient to the starvation shock as it continued to achieve effective COD and trace chemical removals. MLSS concentrations may be a sensitive indicator of fluctuations in feed compositions. However, this indication may not necessarily translate into immediate performance problems.

A loss of power hazardous event of 2 hour duration revealed no significant impact to the MBR performance. This indicates that the MBR was relatively robust against this type of hazardous event. However, results revealed that the removal efficiencies of the hydrophilic, less readily biotransformable chemicals sulfamethoxazole, ketorpofen, gemfibrozil and naproxen were measurably reduced. Therefore, these chemicals appear to be sensitive indicators for loss of removal efficiencies for other chemicals with similar physiochemical properties during some (even relatively minor) hazardous event scenarios.

Impacts from physical membrane damage were investigated by sequentially cutting two hollow-fibre membranes within the MBR. Turbidity and COD analyses revealed that these impacts were ‘self-repaired’ by blocking of the breach within approximately 15 minutes. Accordingly, these hazardous events were shown to have an insignificant impact to overall trace chemical removals. Results confirm that turbidity is an effective indicator for online monitoring and able to quickly detect physical membrane damage. Permeate COD is also a potentially indicator for monitoring physical membrane

damage conditions, but is limited by the fact that it cannot currently be measured continuously. The use of indicator chemicals in this case is not practical due to the rapid self-repair time.

10.1.5. Conclusions from application of indicators and surrogates for hazardous event identification and assessment

Assessing wastewater treatment process performance by chemical indicators and surrogates is an important current research area. Literature has shown that indicator chemicals are useful for assessing treatment process performance in general but that the application of these indicators for hazardous event identification and assessment has not been previously studied. In this research, trace chemicals that are more sensitive to hazardous events are identified. These trace chemicals can serve as representative indicator chemicals for identifying and assessing impacts of hazardous events on treatment process performance, including the impact on the removal of other chemicals with similar physicochemical properties.

The results of the hazardous event simulation experiments revealed that the less readily biotransformable hydrophilic chemicals (e.g. sulfamethoxazole, ketorpofen, gemfibrozil and naproxen) appear to be sensitive indicators for identifying and assessing impacts of hazardous events on MBR treatment process performance as they were measurably impacted even in relatively minor hazardous event scenarios (e.g. loss of power duration of 2 hours), where bulk parameter results revealed no significant impact to the MBR performance.

According to the Australian Guidelines for Water Recycling, to be able to assess the risk of a potential hazardous event, likelihood and consequences of the hazardous event need to be estimated. The likelihood of each of these types of hazardous events will be specific to various treatment plants and their catchments. Important factors will include: types of industries discharging to the catchment, types of other activities around the catchment (dairy farms etc.), potential for stormwater dilution, reliability of power supply and size of the catchment. Based on the results from the hazardous event simulation experiments, a consequences table of hazardous events and MBR performance indicators for trace chemical removals was recommended in Chapter 9. This approach can be adopted for hazardous event identification and assessment.

The impacts of certain hazardous events such as blower or pump failure can be minimised by planning for back-up blower or pump equipment to be replaced

immediately when necessary. Nevertheless, the frequencies of most of these types of hazardous events are not easily estimated. Accordingly, systems must be designed with a degree of robustness to manage their impact to ongoing operation as well as risks to human health and the environment when hazardous events occur.

10.2. RECOMMENDATIONS

During this research, a number of knowledge gaps were identified and future research areas are recommended below.

10.2.1. Identification of metabolic products of trace chemical biotransformation

In this study, biodegradation/transformation were grouped together since it is often difficult to distinguish between processes of chemically or biologically mediated transformation or degradation processes. This is largely due to current analytical limitations for the analysis of metabolites and other transformation products. However, the formation of specific by products or degradation products will ultimately determine the actual changes in environmental risks associated with the effluents from MBRs. Therefore, it is of value to study the metabolism processes and products of trace chemical biodegradation/transformation to assess whether the trace chemicals are completely biodegraded or transformed to other products. The challenge for this research is the requirements of considerable analytical method developments for new transformation products of trace chemical contaminants. Nonetheless, current developments in high resolution mass spectroscopy are rapidly increasing the viability of this objective.

10.2.2. Identifying and characterising specific microorganisms responsible for biotransformation of certain group of trace chemicals

The results from the hazardous event simulation experiments suggested that the toxic and organic shocks appear to impact some specific organisms and/or metabolic processes, that mostly affect the less readily biotransformable chemicals (e.g. sulfamethoxazole, ketorpofen, gemfibrozil and naproxen) which rely upon specific organisms or metabolic pathways for their biotransformation. However, the identification of the key species responsible remains largely unknown. Identifying and

characterising specific microorganisms responsible for biotransformation of certain group of trace chemicals would assist in improving our understanding of optimal operating conditions in order to facilitate growth and metabolism of these species. A challenge for this research includes the ability to isolate the specific microbial groups responsible for biotransformation of certain groups of trace chemicals from the MBR biomass.

10.2.3. Research on robustness of MBR under a wider range of hazardous event conditions

In this research, a range of key hazardous event scenarios was investigated. However, there is potentially a much wider range of conceivable events, which may be expected to impact MBR performance to some degree. On-going robustness of MBR needs to be confirmed under other hazardous event conditions, for examples, discharge of other toxic chemicals, extreme temperature fluctuation and hydraulic load etc. Studies on the responses of MBRs under various exposure levels of each hazardous event are also recommended to establish a more quantitative understanding of the relationship between event severity and impacts.

10.2.4. Characterisation of the likelihoods of hazardous events

The published literature on the likelihood of hazardous events that may occur to the MBR plants is still very limited. More study on likelihood of hazardous events is required to provide necessary information for future risk assessment. As the likelihood of each of these types of hazardous events will be specific to various treatment plants and their catchments, important factors such as types of industries around the catchment, types of other activities around the catchment (dairy farms etc.), potential for stormwater dilution, reliability of power supply, size of the catchment need to be carefully addressed in the study.

10.2.5. Assessment of the role of operational parameters in response to hazardous event conditions

There is conflicting evidence available about the role of operational parameters in response to some hazardous events (e.g. one study suggested that the impact of organic shock on treatment process performance depended mainly on the magnitude of the shock, not on the MLSS concentrations, while another study implied that higher MLSS concentrations may provide some resilience against the organic shock).

Therefore, more research is required to fully understand the relationships between some of the operational parameters and resilience of the systems to hazardous events. This research should include studying responses of MBRs under various operational conditions (different MLSS concentrations or various solid retention time (SRT) etc.) for each hazardous event.

10.2.6. Incorporating Bioassay methods for water quality assessment

Measuring trace chemical contaminants alone may be insufficient for water quality assessment. The limitations include the lack of ability to account for un-known chemicals and possible mixture interactions. In this aspect, Bioassay methods may represent an alternative analytical toolbox that can provide water quality information based directly on the biological effect of the water samples. However, Bioassay methods also have limitations as they provide information about the toxicity of the water matrix without detailed information on which contaminants are responsible. This lack of information makes it difficult to assess what changes to the treatment process may be required to improve performance. Research that incorporates Bioassay methods and trace chemical contaminant analysis is potentially a very powerful experimental approach for water quality assessment.

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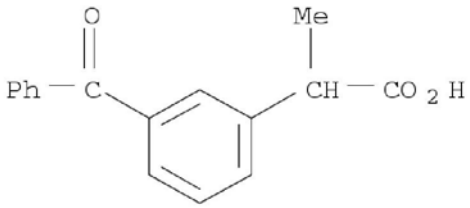
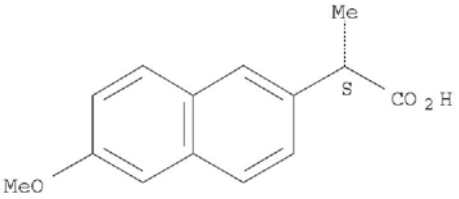
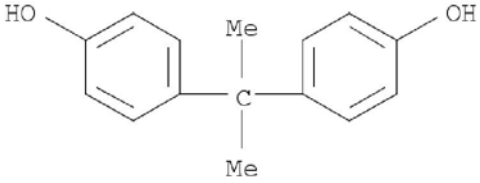
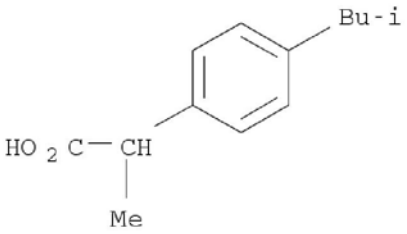
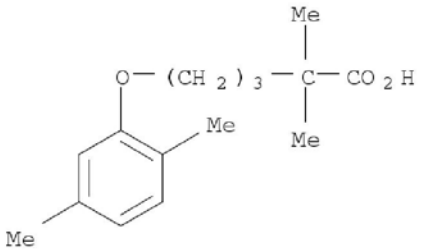
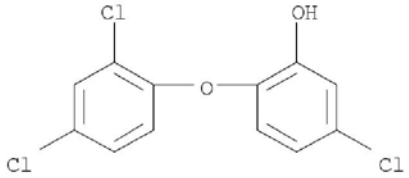
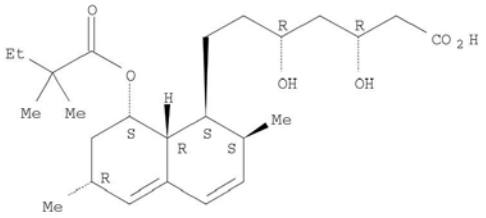
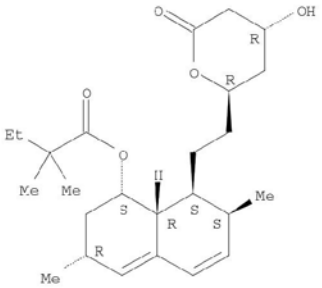
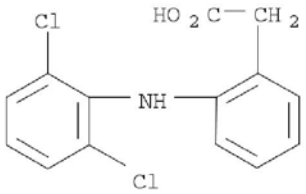
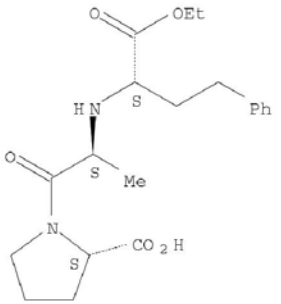
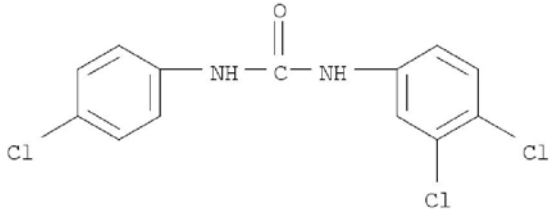
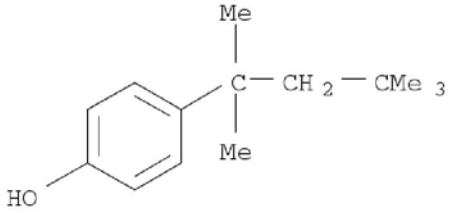
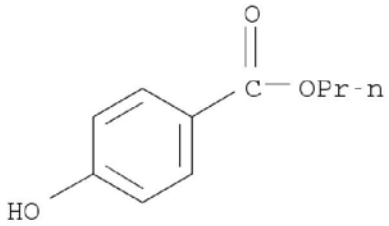
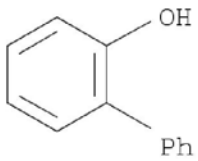
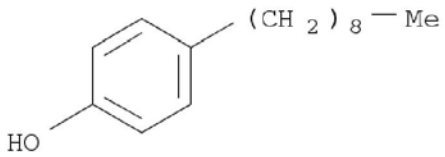
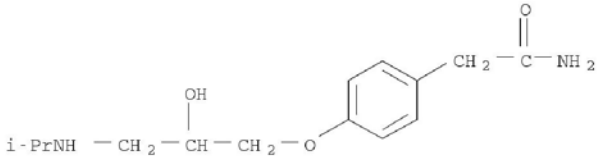
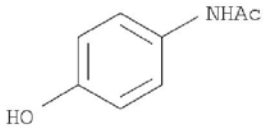
APPENDIX

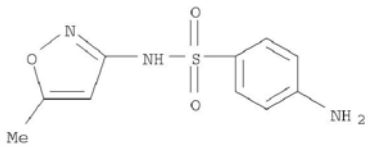
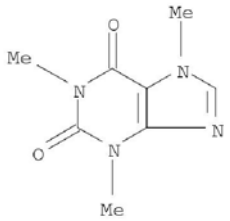
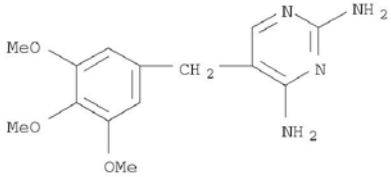
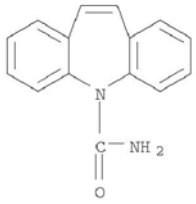
Table A-1 Physio-chemical properties of trace chemical contaminants

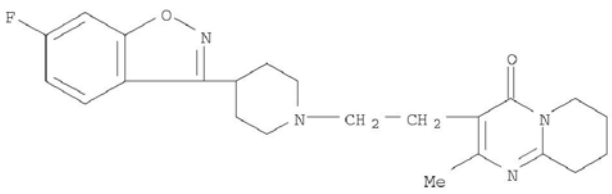
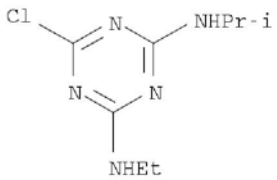
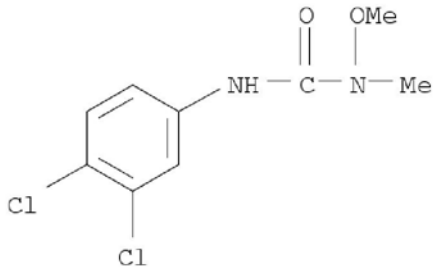
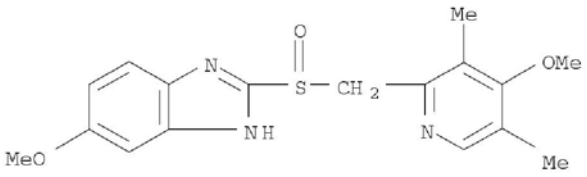
Compound	CAS number	Structure	Formula	MW (g/mol)	Log D (at pH 8)	pKa
Ketoprofen	22071-15-4		$C_{16}H_{14}O_3$	254.28	-0.55	4.23±0.10
Naproxen	22204-53-1		$C_{14}H_{14}O_3$	230.26	-0.18	4.84±0.30
Bisphenol A	80-05-7		$C_{15}H_{16}O_2$	228.29	3.64	10.29±0.10
Ibuprofen	15687-27-1		$C_{13}H_{18}O_2$	206.28	0.14	4.41±0.10

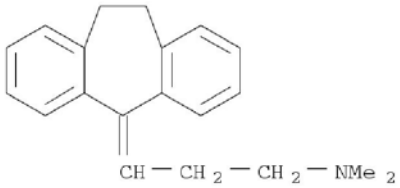
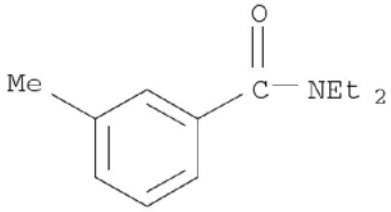
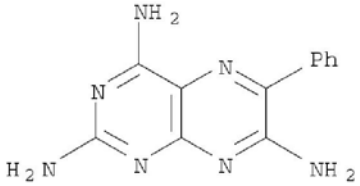
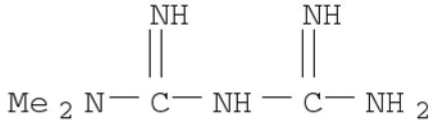
Compound	CAS number	Structure	Formula	MW (g/mol)	Log D (at pH 8)	pKa
Gemfibrozil	25812-30-0		$C_{15}H_{22}O_3$	250.33	1.18	4.75±0.45
Triclosan	3380-34-5		$C_{12}H_7Cl_3O_2$	289.54	4.93	7.80±0.35
Simvastatin hydroxy acid	121009-77-6		$C_{25}H_{40}O_6$	436.58	1.07	4.31±0.10
Simvastatin	79902-63-9		$C_{25}H_{38}O_5$	418.57	4.72	13.49±0.40

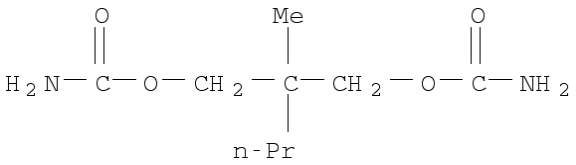
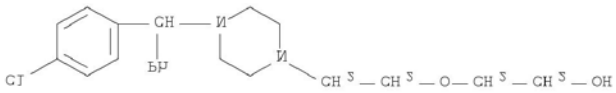
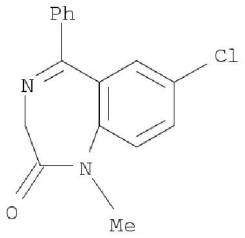
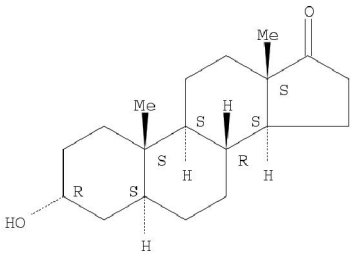
Diclofenac	15307-86-5		$C_{14}H_{11}Cl_2NO_2$	296.15	1.06	4.18±0.10
Enalapril	75847-73-3		$C_{20}H_{28}N_2O_5$	376.45	-0.45	3.15±0.20 5.43±0.39
Triclocarban	101-20-2		$C_{13}H_9Cl_3N_2O$	315.58	6.07	12.77±0.70
4-Tert-octylphenol	140-66-9		$C_{14}H_{22}O$	206.32	5.18	10.15±0.15

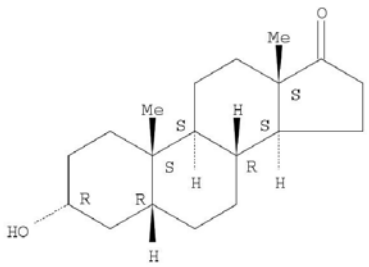
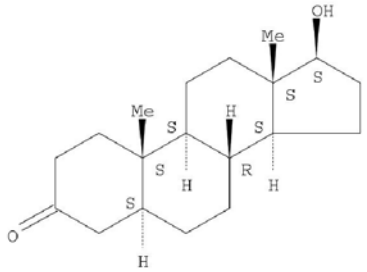
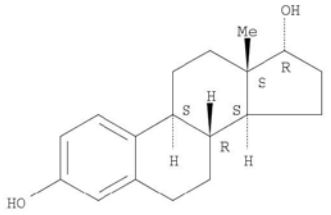
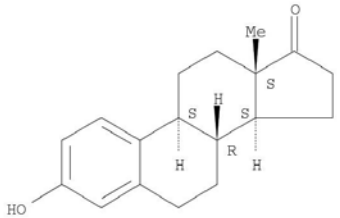
Propylparaben	94-13-3		$C_{10}H_{12}O_3$	180.20	2.70	8.23±0.15
2-Phenylphenol	90-43-7		$C_{12}H_{10}O$	170.21	3.29	10.00±0.10
4-Nonylphenol	104-40-5		$C_{15}H_{24}O$	220.35	6.14	10.15±0.15
Atenolol	29122-68-7		$C_{14}H_{22}N_2O_3$	266.34	-1.20	13.88±0.20 9.43±0.10
Paracetamol	103-90-2		$C_8H_9NO_2$	151.16	0.47	9.86±0.13 1.72±0.50

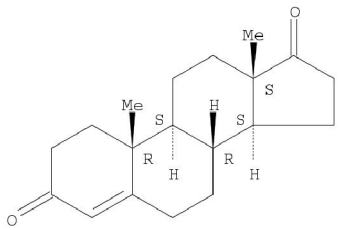
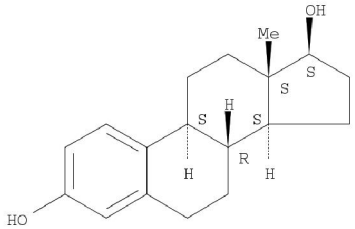
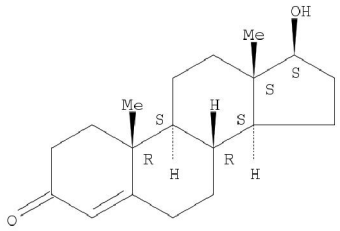
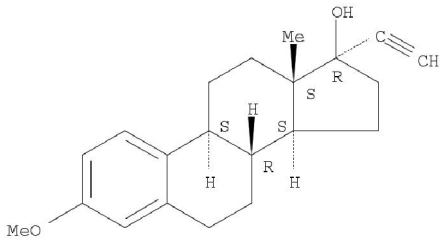
Sulfamethoxazole	723-46-6		$C_{10}H_{11}N_3O_3S$	253.28	-0.96	5.81±0.50 1.39±0.10
Caffeine	58-08-2		$C_8H_{10}N_4O_2$	194.19	-0.63	0.52±0.70
Trimethoprim	738-70-5		$C_{14}H_{18}N_4O_3$	290.32	0.55	7.04±0.10
Carbamazepine	298-46-4		$C_{15}H_{12}N_2O$	236.27	1.89	13.94±0.20 -0.49±0.20

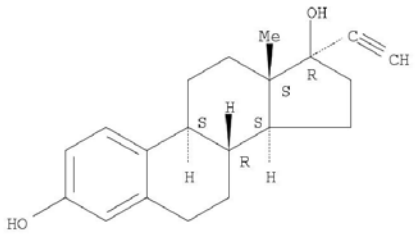
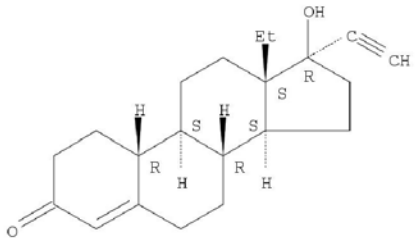
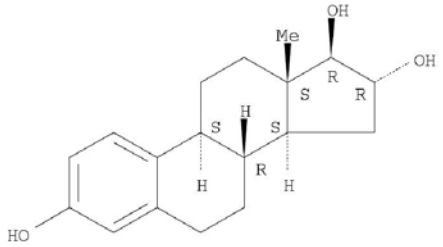
Risperidone	106266-06-2		$C_{23}H_{27}FN_4O_2$	410.48	2.31	8.07±0.10
Atrazine	1912-24-9		$C_8H_{14}ClN_5$	215.68	2.64	2.27±0.10
Linuron	330-55-2		$C_9H_{10}Cl_2N_2O_2$	249.09	3.12	12.13±0.70 -1.04±0.50
Omeprazole	73590-58-6		$C_{17}H_{19}N_3O_3S$	345.42	2.33	8.78±0.10 4.72±0.40

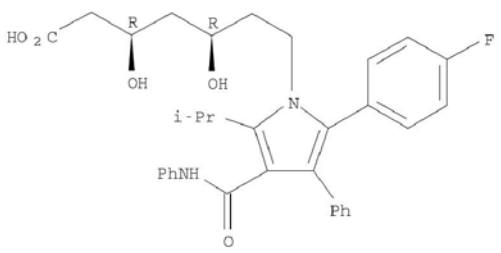
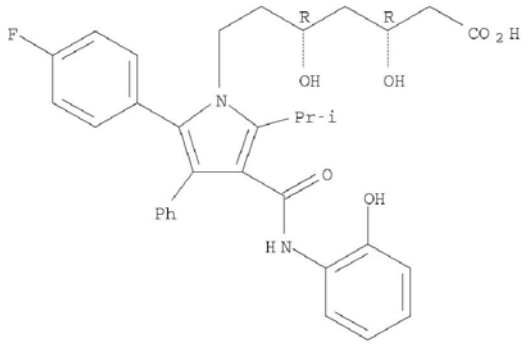
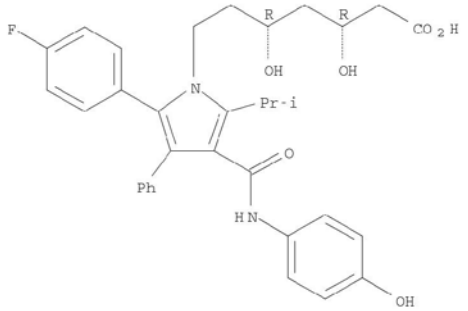
Amitriptyline	50-48-6		C_{20} H_{23} N	277.40	3.21	9.18±0.28
DEET (N,N-Diethyl-3-methylbenzamide)	134-62-3		C_{12} H_{17} N O	191.27	2.42	-1.37±0.70
Triamterene	396-01-0		C_{12} H_{11} N_7	253.26	1.15	6.28±0.10
Metformin	657-24-9		C_4 H_{11} N_5	129.16	-3.20	12.27±0.10

Meprobamate	57-53-4		$C_9 H_{18} N_2 O_4$	218.25	0.70	13.09±0.50 -1.09±0.70
Hydroxyzine	68-88-2		$C_{21} H_{27} Cl N_2 O_2$	374.90	2.30	14.41±0.10 6.62±0.10
Diazepam	439-14-5		$C_{16} H_{13} Cl N_2 O$	284.74	2.80	3.40±0.10
Androsterone	53-41-8		$C_{19} H_{30} O_2$	290.44	3.93	15.14±0.60

Etiocholanolone	53-42-9		$C_{19}H_{30}O_2$	290.44	3.93	15.14±0.60
Dihydrotestosterone	521-18-6		$C_{19}H_{30}O_2$	290.44	3.93	15.08±0.60
17α-Estradiol	57-91-0		$C_{18}H_{24}O_2$	272.38	4.14	10.27±0.60
Oestrone	53-16-7		$C_{18}H_{22}O_2$	270.37	3.62	10.25±0.40

Androstenedione	63-05-8		$C_{19}H_{26}O_2$	286.41	2.72	Not available
17β-Estradiol	50-28-2		$C_{18}H_{24}O_2$	272.38	4.14	10.27±0.60
Testosterone	58-22-0		$C_{19}H_{28}O_2$	288.42	3.18	15.06±0.60
Mestranol	72-33-3		$C_{21}H_{26}O_2$	310.43	4.94	13.10±0.40

17α-Ethynylestradiol	57-63-6		C_{20} H_{24} O_2	296.40	4.10	10.24±0.60
Levonorgestrel	797-63-7		C_{21} H_{28} O_2	312.45	3.37	13.09±0.40
Oestriol	50-27-1		C_{18} H_{24} O_3	288.38	2.53	10.25±0.70

Atorvastatin	134523-00-5		$C_{33}H_{35}FN_2O_5$	558.64	0.37	4.29±0.10 0.38±0.50
o-Hydroxyatorvastatin	214217-86-4		$C_{33}H_{35}FN_2O_6$	574.64	0.61	4.29±0.10 1.86±0.50
p-Hydroxyatorvastatin	214217-88-6		$C_{33}H_{35}FN_2O_6$	574.64	-0.28	4.29±0.10 1.59±0.50

Source: Scifinder, 21 January 2013

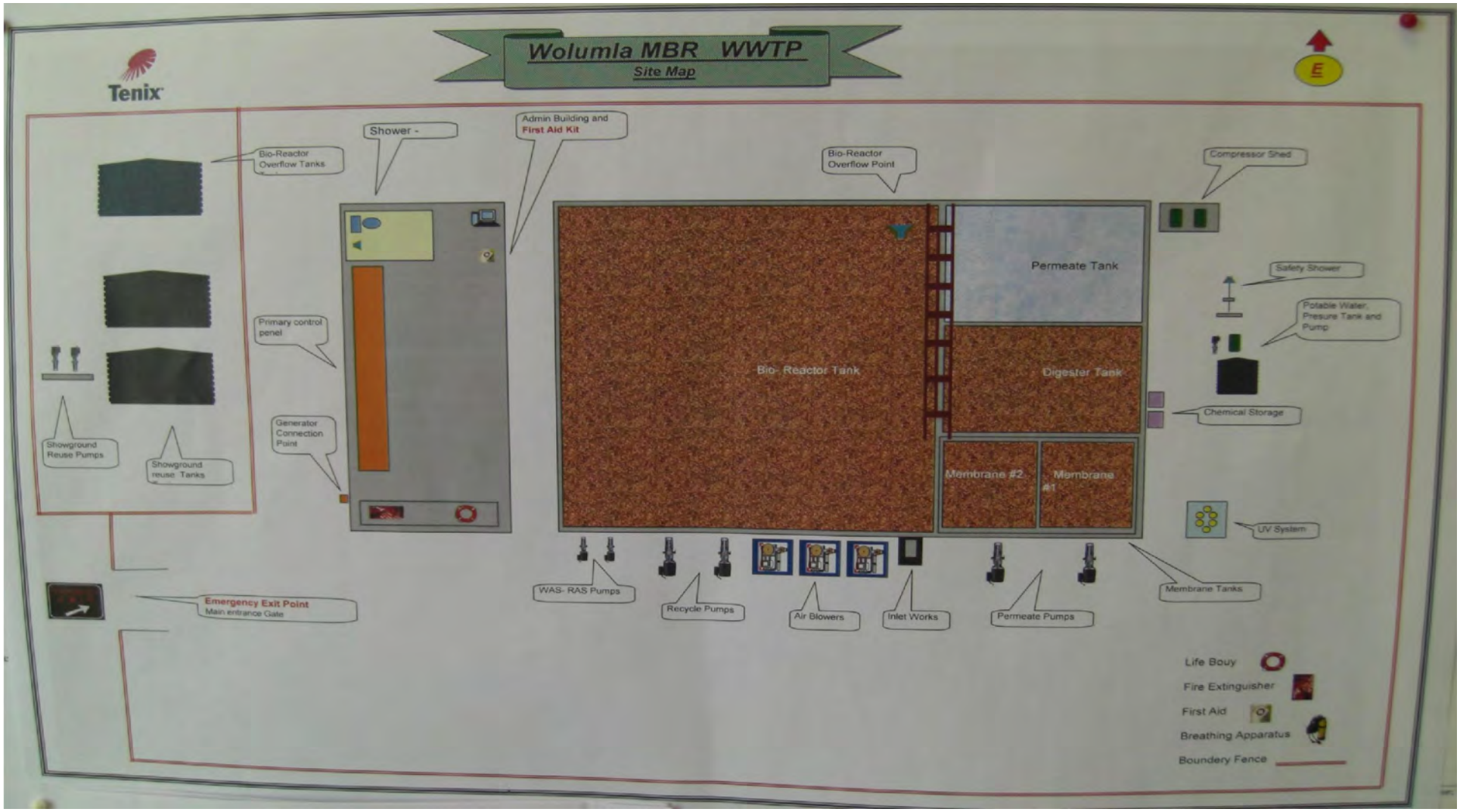


Figure A-1 Site map of Wolumla package MBR

Figure A-2 Picture of Wolumla package MBR

Figure A-3 Sampling of mixed liquor at the bioreactor in Wolumla package MBR

Figure A-4 After a loss of power event, the membrane modules were clogged due to biomass deposition and were taken out of the membrane chamber for manual cleaning.

Figure A-5 After the manual cleaning, the membrane modules were put back together and ready to be re-installed into the membrane chamber.

Figure A-6 The right membrane chamber shows the biomass status after a loss of power event (the membrane in the left chamber was taken out for manual cleaning)

Figure A-7 Sampling of membrane permeate at Wolumla package MBR

Figure A-8 The UV disinfection unit at Wolumla package MBR

Figure A-9 Storage of treated water for recycling at Wolumla package MBR

Figure A-10 Conducting solid phase extraction (SPE) onsite at Wolumla package MBR

Figure A-11 Trials to select suitable type of cistern valve and appropriate pressure for constructing experimental MBRs

Figure A-12 Trials to select suitable type of cistern valve and appropriate pressure for constructing experimental MBRs (continue)

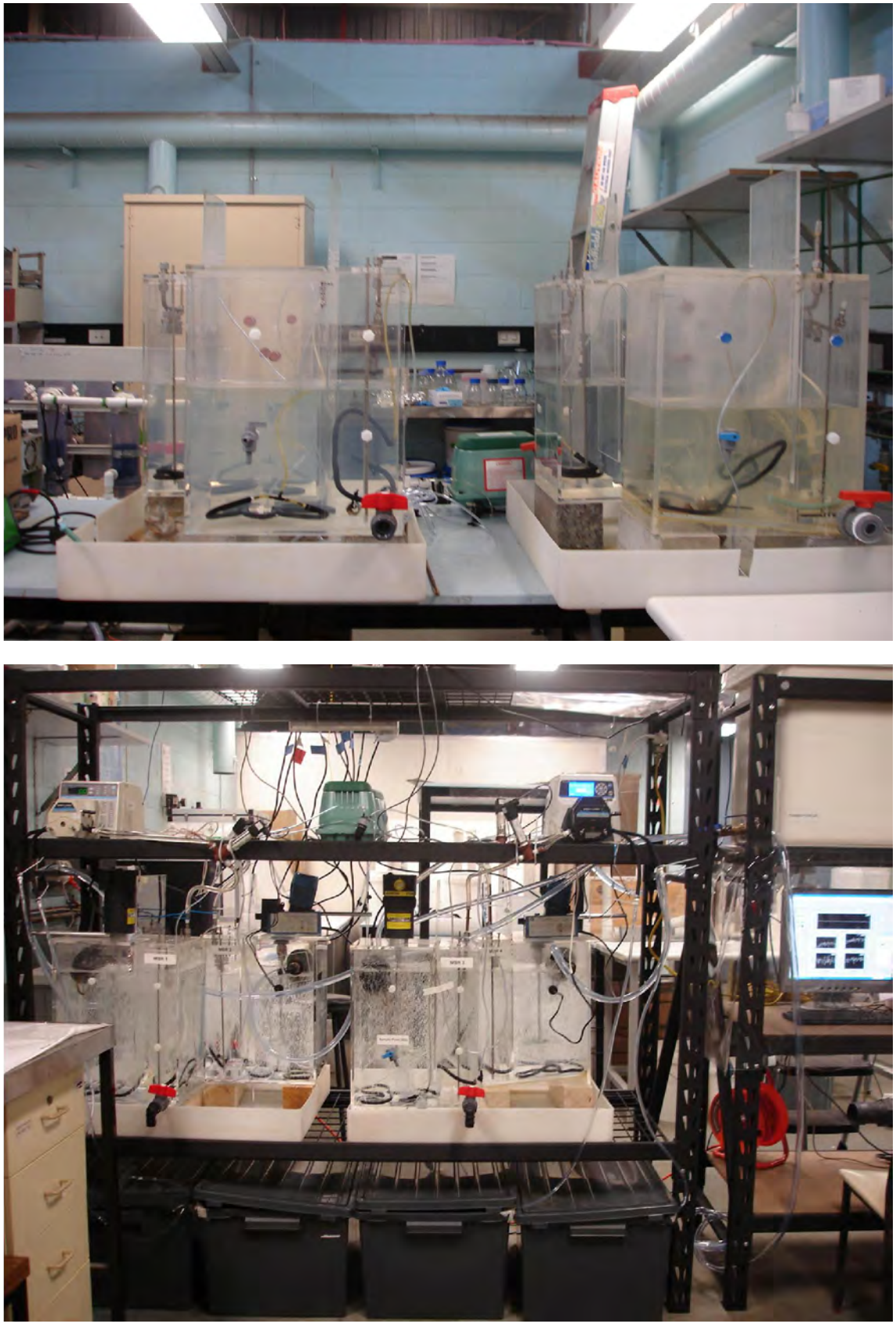


Figure A-13 Constructing experimental MBRs at a CIVENG lab, UNSW

Figure A-14 Installing experimental MBRs at a sewage treatment plant in NSW

Figure A-15 Experimental MBRs were operating at the plant

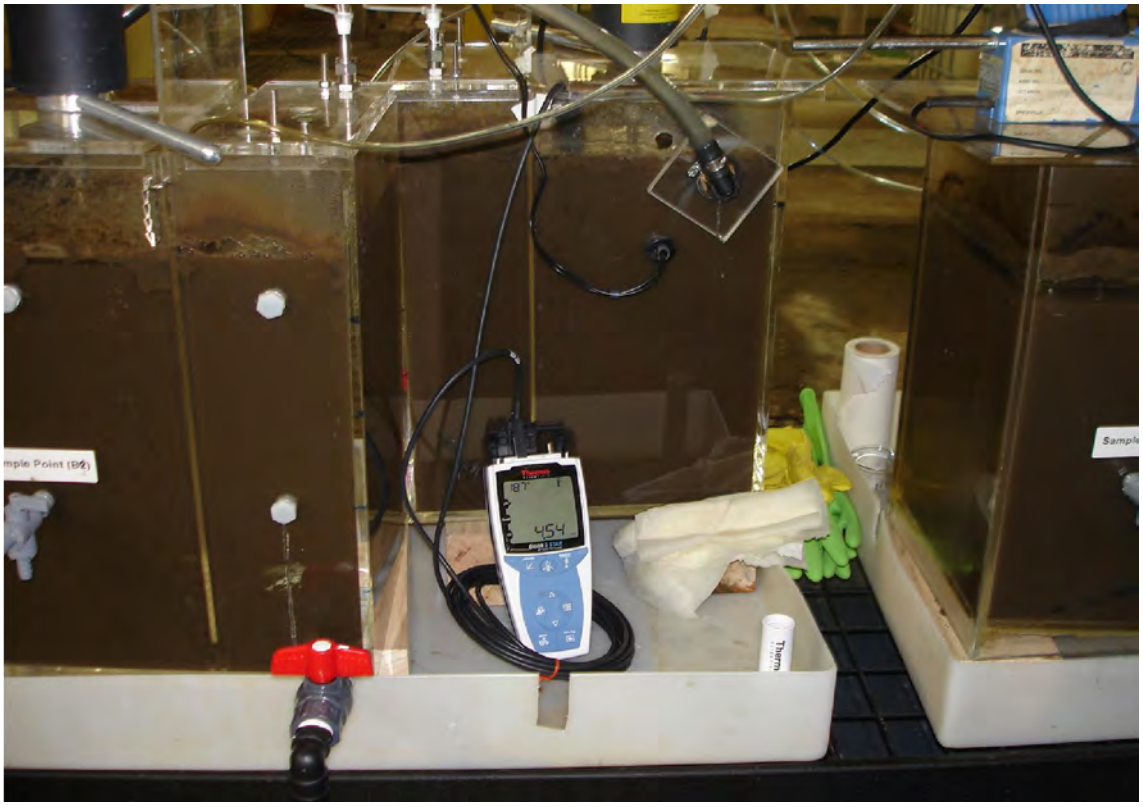


Figure A-16 Measuring pH in experimental MBRs

Figure A-17 Online transmembrane pressure (TMP) measurement for experimental MBRs

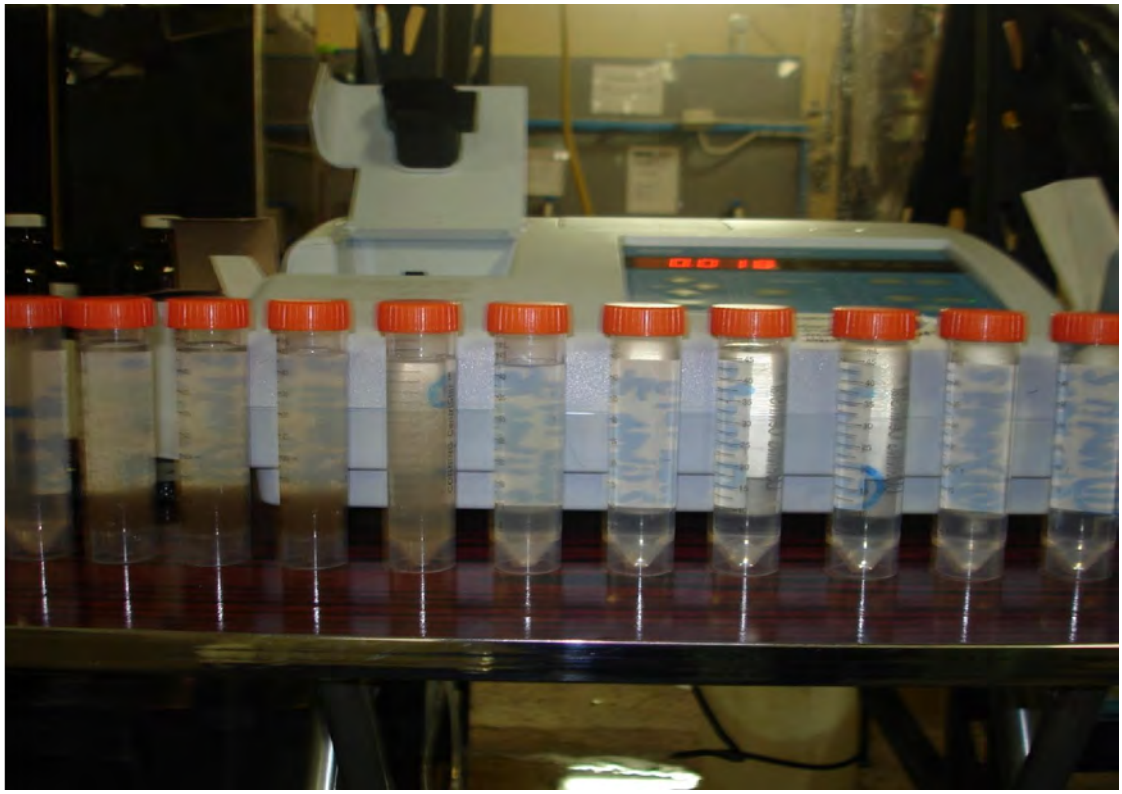


Figure A-18 Measuring turbidity onsite during physical membrane damage experiment

Figure A-19 Sampling permeate at experimental MBRs

Figure A-20 Extract biomass samples at CIVENG lab, UNSW

Figure A-21 Conducting solid phase extraction (SPE) at CIVENG lab, UNSW

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Simultaneous determination of estrogenic and androgenic hormones in water by isotope dilution gas chromatography–tandem mass spectrometry

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Surface water

ABSTRACT

A rapid gas chromatography–tandem mass spectrometry (GC–MS/MS) analytical method was developed for the simultaneous analysis of 7 estrogenic hormones (17α-estradiol, 17β-estradiol, estrone, mestranol, 17α-ethynylestradiol, levonorgestrel, estriol) and 5 androgenic hormones (testosterone, androsterone, etiocholanolone, dihydrotestosterone, androstenedione) in aqueous matrices. This method is unique in its inclusion of all 12 of these estrogens and androgens and is of particular value due to its very short chromatographic run time of 15 min. The use of isotope dilution for all analytes ensures the accurate quantification, accounting for analytical variabilities that may be introduced during sample processing and instrumental analysis. Direct isotopically labelled analogues were used for 8 of the 12 hormones and satisfactory isotope standards were identified for the remaining 4 hormones. Method detection levels (MDLs) were determined to describe analyte concentrations sufficient to provide a signal with 99% certainty of detection. The established MDLs for most analytes were 1–5 ng L^{-1} in a variety of aqueous matrices. However, slightly higher MDLs were observed for etiocholanolone, androstenedione, testosterone, levonorgestrel and dihydrotestosterone in some aqueous matrices. Sample matrices were observed to have only a minor impact on MDLs and the method validation confirmed satisfactory method stability over intra-day and inter-day analyses of surface water and tertiary treated effluent samples.

1. Introduction

Estrogenic and androgenic steroid hormones are environmental contaminants of increasing regulatory concern and attention. These include both natural and synthetic substances used for a variety of applications. Some steroidal hormones are used in medicine as contraceptives or in agriculture as growth promoters of meat-producing animals [1–3]. Natural and synthetic steroidal hormones are excreted by humans and animals and can be transferred to surface water by discharging treated municipal wastewaters [4,5] or through run-off from agricultural operations [6,7]. The application of digested municipal sewage sludge to agricultural fields may also be an important pathway for the transfer of steroidal hormones to soil and groundwater [8]. Estrogenic steroids have been reported in treated sewage effluents in many countries across Asia, Europe, Australia and North America [8–15]. Although they have not been subjected to the same degree of scrutiny, androgenic steroid hormones have also been reported in municipal wastewaters in a few studies [16–18].

The main constituent of the estrogenic contraceptive drug, 17α-ethynylestradiol, has been shown to result in localised extinction of some fish species due to reproductive disruption at concentrations of 5–6 ng L^{-1} [19]. The androgenic hormone testosterone can elicit pheromonal responses in fish at nanogram per litre concentrations [20]. Reported impacts of steroidal hormones to aquatic species include behaviour changes [21], morphological abnormalities [22–26], increased occurrence of hermaphrodite organisms and thus reduced reproductive success of fish [21–25,27–31]. Due to the widespread observations of these impacts and ongoing global concern, new analytical developments leading to improved sensitivity, faster analysis times and greater capability for simultaneous analysis of a large number of analytes in water are warranted.

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been reported for the analysis of a range of steroidal estrogens and androgens [18,32–37]. While this has proved highly sensitive for the analysis of relatively clean

Abbreviations: BSTFA (99%)+TCMS (1%), N,O-bis(trimethylsilyl) trifluoroacetamide (99%) + trimethylchlorosilane (1%); EI, eleactron ionisation; MW, molecular weight; MRM, multiple reaction monitoring; MDL, method detection level; MBR, membrane bioreactor; HLB, hydrophilic lipophilic balance; SPE, solid phase extraction; Q, quadrupole.

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environmental waters, ion suppression, leading to marked losses of sensitivity can be a significant problem for more complex matrices such as wastewaters [37]. This problem has been particularly observed for androgenic steroids [36].

Gas chromatography–mass spectrometry (GC–MS) has been a preferred technique for determination of steroidal hormones as it is generally able to achieve improved detection limits in more complex matrices [38–50]. Better sensitivity has been achieved by GC coupled with tandem mass spectrometry (GC–MS/MS). A number of GC–MS/MS methods have been developed for the analysis of estrogenic steroids in biological and environmental samples [51–56] and a few GC–MS/MS methods have been developed for the analysis of a wider range of steroidal hormones including a few androgens [16,57]. However, to the best of the author's knowledge, no published methods are currently available for the simultaneous determination of all 7 estrogens and 5 androgens as presented in the current method. Furthermore, the previously published GC–MS/MS methods that have included simultaneous analysis of both androgenic and estrogenic hormones have not incorporated isotope dilution for accurate quantification accounting for extraction losses and potential matrix effects [16,57].

In order to overcome the above limitations, we have developed a simple, reliable and sensitive analytical method for the simultaneous determination of the most common 7 steroidal estrogens and 5 androgens in aqueous environmental matrices. Water samples are extracted by solid phase extraction (SPE) followed by GC–MS/MS analysis using isotope dilution. All the analytes can be monitored in a single GC–MS/MS run with a rapid run time of 15 min.

2. Materials and methods

2.1. Materials and reagents

17α-Estradiol, 17β-estradiol, estrone, estriol, 17α-ethynylestradiol, levonorgestrel, mestranol, testosterone, etiocholanolone, androstenedione, androsterone, dihydrotestosterone, pyridine and 99% N,O-bis(trimethylsilyl)trifluoro-acetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (all analytical grade), Whatman glass fibre filters and filtering system were purchased from Sigma–Aldrich (Castle Hill, NSW, Australia). D3-estriol, D3-dihydrotestosterone, D2-testosterone, D4-17α-ethynylestradiol, D4-estrone, D4-17β-estradiol, D2-etiocholanolone were purchased from CDN isotopes Inc., Canada, and D3-androstenedione was purchased from National Measurement Institute, Australia.

Acetonitrile and methanol (anhydrous spectroscopy grade) were purchased from Ajax Finechem (Tarron Point, NSW, Australia). Ultrapure water was produced using a Driec-Q filtering system from Millipore (North Ryde, NSW, Australia). Kimble culture tubes (13 mm I.D. × 100 mm) and a Thermo Speedvac concentrator (Model No. SPD121P) were purchased from Biolab (Clayton, Vic, Australia). Oasis hydrophilic lipophilic balance (HLB) solid phase extraction cartridges (6 mL, 500 mg) were purchased from Waters (Rydalmere, NSW, Australia).

Stock standard solutions of steroidal hormones and isotope labelled steroidal hormones were initially prepared in acetonitrile (500 mg L^{-1}, 20 mL) in amber vials and then further serial diluted with acetonitrile to obtain working standard solutions of lower concentrations. All standard solutions were stored at −18 °C and prepared freshly every three months. Working solutions of steroidal hormones and isotope labelled steroidal hormones at lower concentrations were stored at 4 °C and freshly prepared from concentrated stock standards monthly. Chemical structures of target analytes and their isotope labelled standards used in this study are presented in Table 1.

2.2. Sample collection

All samples were collected in 500 mL amber glass bottles. Ultrapure water was produced using a Driec-Q filtering system from Millipore. Drinking water was collected from a regular potable water tap at UNSW. Membrane Bioreactor (MBR) effluent was the effluent produced by a laboratory-scale MBR treating a synthetic feed solution. The design characteristics, operational parameters and synthetic feed solution of this MBR have been previously described [58]. Surface water was collected from a pond in a large municipal park in Sydney. Tertiary treated effluent was a disinfected final effluent from a municipal wastewater treatment plant in western Sydney. The dissolved organic carbon (DOC) and total suspended solids (TSS) of each of these water matrices are presented in Table 2.

Samples were spiked with stock solutions of all analytes for method recovery and detection level determination. The target concentrations of analytes were dependent on the specific experiments as described in the method validation studies (Section 2.7) below. All samples were then further spiked with isotopically labelled standards for accurate isotope dilution quantification. The target concentrations of the isotope standards were selected to be within an order of magnitude of the spiked analyte concentrations.

Spiked ultrapure water, drinking water and synthetic MBR effluents were extracted without any further treatment or processing. Surface water samples and tertiary treated effluent samples were filtered by 0.75 μm Whatman filter paper prior to extraction. All samples were extracted within 24 h of collection and spiking.

2.3. Solid phase extraction (SPE)

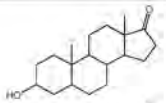
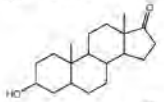
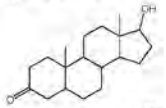
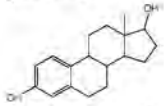
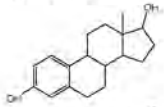
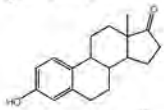
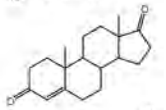
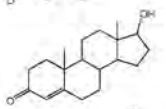
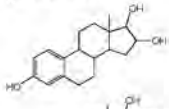
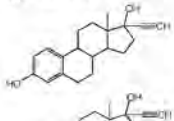
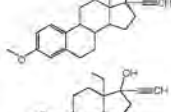
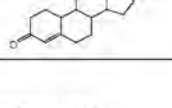
The Oasis HLB SPE cartridges were pre-conditioned prior to extraction with methanol (5 mL), followed by ultrapure water (5 mL). SPE cartridges were loaded by drawing through 500 mL of the aqueous samples under vacuum, maintaining a consistent loading flow rate of less than 5 mL min^{-1}. The SPE cartridges were rinsed with 10 mL of ultrapure water before drying by passing through a flow of nitrogen gas until visibly dry (approximately 1 h). If required, dried cartridges were stored at −18 °C prior to elution and quantitative analysis. Analytes were eluted from the cartridges with methanol (2× 5 mL) into Kimble culture tubes. The extracts were centrifugally evaporated under vacuum at 35 °C using a Thermo Speedvac (Biolab) concentrator. The concentrator was set to an 'auto vacuum' run, with a final pressure of 0.5 Torr. This evaporation process took approximately 1–4 h, depending on the number of samples and the types of matrices (a maximum of 32 samples can be dried in a single batch). The evaporated samples were reconstituted with anhydrous acetonitrile (1 mL) and transferred to amber GC autosampler vials and dried under a gentle nitrogen stream until visibly dry (approximately 3–15 min depending on the types of matrices).

2.4. Trimethylsilyl derivatisation

In preparation for GC–MS/MS analysis, all samples underwent chemical derivatisation. 50 μL of BSTFA (99%)–TCMS (1%), 50 μL of pyridine and 400 μL of acetonitrile (anhydrous grade) were added to the dried samples, then the vials were sealed and heated at 60 °C for 30 min. The derivatised samples were then allowed to cool to room temperature.

It should be noted that this derivatisation process is sensitive to the presence of any moisture. Accordingly, it is important to ensure that the samples are fully dried (as described in the previous section) before the addition of the derivatising reagents and anhydrous acetonitrile. Furthermore, the smallest commercially available bottles of pyridine (100 mL) and anhydrous acetonitrile (100 mL) were

Table 1
Chemical structures of target analytes and their corresponding isotope labelled standards in this study.

Target analytes (corresponding isotope labelled standards)	MW of target analytes (MW of corresponding isotope labelled standards)	Structure of target analytes
Androsterone (16,16-D2-etiocholanolone)	290.4 (292.5)	
Etiocholanolone (16,16-D2-etiocholanolone)	290.4 (292.5)	
Dihydrotestosterone (16,16,17-D3-dihydrotestosterone)	290.4 (293.5)	
17α-Estradiol (2,4,16,16-D4-17β-estradiol)	272.4 (276.4)	
17β-Estradiol (2,4,16,16-D4-17β-estradiol)	272.4 (276.4)	
Estrone (2,4,16,16-D4-estrone)	270.4 (274.4)	
Androstenedione (19,19,19-D3-androstenedione)	286.4 (289.4)	
Testosterone (1,2-D2-testosterone)	288.4 (290.4)	
Estriol (2,4,17-D3-estriol)	288.4 (291.4)	
17α-Ethynylestradiol (2,4,16,16-D4-17α-ethynylestradiol)	296.4 (300.4)	
Mestranol (2,4,16,16-D4-17α-ethynylestradiol)	310.4 (300.4)	
Levonorgestrel (2,4,16,16-D4-17α-ethynylestradiol)	312.5 (300.4)	

used to avoid long storage times of these moderately hygroscopic solvents. Similarly, the mixed derivatising reagent was purchased in 1 mL packs and used only on the same day that they were opened.

Table 2
DOC and TSS of water matrices used for method validation.

	DOC ($mg\,L^{-1}$)	TSS ($mg\,L^{-1}$)
Ultrapure water	0.1	N/A
Drinking water	2	N/A
MBR effluent	8	N/A
Surface water	16	14
Tertiary treated effluent	15	3

N/A: not applicable.

2.5. Gas chromatography–tandem mass spectrometry

Samples were analysed on an Agilent 7890A gas chromatograph (GC) coupled with an Agilent 7000B triple quadrupole mass spectrometer (MS/MS). The GC injection port was operated in splitless mode. The inlet temperature and the GC/MS interface temperature were maintained at 250 °C. An injection volume of 1 μL was used. The inlet was used in splitless mode with a purge time of 1.5 min. Analytes were separated on an Agilent HP5-MS (30 m × 250 μm × 0.25 μm) column using a 0.8 mL min^{-1} helium flow. The GC oven temperature was initiated at 130 °C and held for 0.5 min, then increased by 40 °C min^{-1} to 240 °C, and increased by 5 °C min^{-1} to 280 °C and held at 280 °C for 3.75 min. The total run time was 15 min.

Mass spectrometric ionisation was undertaken in electron ionisation (EI) mode with an EI voltage of 70 eV and a source temperature of 280 °C. The triple quadrupole MS detector was operated in multiple reaction monitoring (MRM) mode with the gain set to 100 for all analytes. In order to identify the most suitable transitions for MRM, analytical standards were initially analysed in scan mode to identify suitable precursor ions in MS1 with a scan range of m/z 30 to m/z M+10 (where M is the derivatised mass of the compound of interest). Fragmentation of the precursor ions in the collision cell was assessed by performing a product ion scan using the same mass range and scan time. All samples were run with a solvent delay of 5 min and the analytes were separated into 3 discrete time segments for MRM aquisition with dwell times ranging from 3 to 25 ms, depending on the time segment, to achieve 10–20 cycles across each peak for good quantification. All ions were monitored at wide resolution (1.2 amu at half height).

The ion transitions monitored for all analytes and isotope standards, as well as the specific dwell times and collision energies for the method are presented in Table 3. The first MRM transition shown for each molecule was used for quantification, while the second transition shown was monitored only for confirmation of molecular identification. A chromatogram showing quantifier peaks of 12 analytes in tertiary treated effluent matrix at a spiking concentration of 10 ng L^{-1} is presented in Fig. 1.

2.6. Identification and quantification

As described in the previous section, two MRM transitions of a single precursor ion were monitored for each target compound. Analysis of the acquired data was undertaken using Agilent MassHunter software. The confirmed identification of a target compound was only established once the analysis met all of the identification criteria. These included the observed presence of the two expected transitions at the same retention time, the area ratio of two transitions within a range of 20% variability with respect to the mean area ratio of all calibration solutions, and a consistent analyte-surrogate relative retention time as that of calibration solutions with relative standard deviation of less than 0.1 min.

2.7. Method validation studies

Isotope labelled compounds were used as surrogate standards to correct for matrix effects, SPE recovery variabilities and instrumental variations for the steroid analytes. Direct analogue isotopic standards were used for etiocholanolone, dihydrotestosterone, 17β-estradiol, estrone, androstenedione, testosterone, estriol and 17α-ethynylestradiol. However, for four of the target analytes, alternative isotope standards were used based on their structural similarity and confirmed suitability (see Section 3.1). Accordingly, D2-etiocholanolone was selected for its stereoisomer androsterone, and D4-17β-estradiol was selected for its stereoisomer 17α-estradiol, and D4-17α-ethynylestradiol was selected as the isotopic standard for mestranol and levonorgestrel. Method recoveries of the target analytes were validated in a variety of matrices including ultrapure water, drinking water, synthetic MBR effluent, natural surface water and tertiary treated effluent. The method recoveries of target analytes in various matrices are presented in Table 4.

SPE absolute recoveries were assessed using the spiked ultrapure water, surface water and tertiary treated effluent samples at both a high concentration (100 ng L^{-1}) and a low concentration (10 ng L^{-1} except for dihydrotestosterone, which was spiked at 20 ng L^{-1} since it has an MDL of 15.8 ng L^{-1}). Because the aim was to assess the loss of the target analytes during SPE extraction, the isotope standards (50 ng) were added to the SPE extracts only after the elution step for direct relative comparison to the analytes. The results of this experiment are presented in Table 5.

To assess potential analyte losses occurring specifically during the drying by Speedvac concentrator and reconstitution steps, 3 centrifuge tubes containing 10 mL anhydrous grade methanol were spiked with 100 ng of the target analytes before being vacuum dried for 3 h and reconstituted in anhydrous acetonitrile. The results of this assessment are presented in Table 6. Further potential losses after reconstitution in anhydrous acetonitrile and during drying under nitrogen gas were also assessed with various drying times of 5 min, 30 min and 1 h. The results of these assessments are also presented in Table 6.

Finally, the impact of any potential sample volume-specific effects, such as SPE breakthrough, was assessed by extracting larger sample volumes (1 L, 2 L, 3 L and 4 L) of tertiary treated effluent, each spiked with 20 ng of each analyte, and comparing the recoveries.

MDLs were determined in each of the matrices described above according to Method 1030C from Standard Methods for the Analysis of Water and Wastewater [59]. For each matrix, seven samples of 500 mL were spiked with target analytes at concentrations close to the expected MDLs. The samples were then spiked with isotopic standards, extracted and analysed through all of the above sample processing and data quantification steps. The seven samples were not analysed sequentially, but were divided into two batches and processed independently on different days to better represent day-to-day variability. MDLs were calculated by multiplying the standard deviation of seven replicates by Student's T value of 3.14 (one-side T distribution for six degrees of freedom at the 99% level of confidence). Where the calculated MDLs were greater than the actual spiked concentration of any target analytes, a further seven replicates spiked with higher concentrations were analysed to calculate revised MDLs for those analytes. Alternatively, where the calculated MDLs were 5 or more times smaller than the actual spiked concentrations, a further seven replicates spiked with lower concentrations were analysed to calculate revised MDLs. This procedure was repeated until MDLs of all target analytes were determined with a signal-to-variability ratio within the bounds of the above criteria. Final MDL values are presented in Table 7.

Instrument stability was assessed on an intra-day and inter-day basis by injecting a standard solution containing all analytes (100 ng mL^{-1}) onto the column three times per day over two separate days and comparing the variation in the signal intensity of each analyte standard from these injections. This variation was expressed at the coefficient of variation (C_v) determined as the ratio of the standard deviation (σ) to the mean (μ). The results of this assessment are presented in Table 8. The absolute stability of the whole method for measuring surface water and tertiary treated effluent samples was also assessed by processing three samples of each matrix at various times within a day and three additional samples for each matrix on a different day. The coefficients of variation for these samples are presented in Table 8. Note that the instrument stability calculation does not include correction by isotope dilution, but the method stability calculation does.

Matrix assessment was undertaken by spiking all of the target analytes (and isotopic standards) into extracted and reconstituted surface water and tertiary treated effluent matrix samples. These spiked matrix samples were then derivatised and analysed by the GC–MS/MS. The absolute signal of each analyte was compared to a standard solution (prepared in acetonitrile) of the same concentration in order to calculate a percentage signal enhancement or suppression. The mean values and standard deviations for triplicate samples are presented in Table 9. Note that these experiments did not include correction of measured ion intensities by isotope dilution.

Quantitative determination of the target analytes was undertaken using external calibration principles combined with the

Table 3
Optimal analyte dependent parameters for tandem mass spectrometry.

Segment start time	Analytes and isotope labelled standards	MRM transitions	Retention time (min)	Dwell time (ms)	Optimum collision energy (V)
7.00 min	Androsterone	347.2 → 271.2	8.58	25	6
		347.2 → 175.1		25	8
	Etiocholanolone	347.2 → 271.2	8.70	25	6
		347.2 → 175.1		25	8
	D2-Etiocholanolone	349.2 → 273.3	8.68	25	6
		349.2 → 175.0		25	8
9.20 min	Dihydrotestosterone	347.2 → 213.2	9.70	3	10
		347.2 → 271.2		3	10
	D3-Dihydrotestosterone	350.1 → 215.1	9.67	3	10
		350.1 → 273.2		3	10
	17α-Estradiol	416.0 → 285.1	9.79	3	10
		416.0 → 326.2		3	5
	17β-Estradiol	416.0 → 285.1	10.25	3	10
		416.0 → 326.2		3	5
	D4-17β-Estradiol	420.0 → 287.2	10.23	3	10
		420.0 → 330.3		3	5
	Estrone	342.1 → 257.1	9.82	3	15
		342.1 → 243.9		3	15
	D4-Estrone	346.3 → 261.2	9.79	3	15
		346.3 → 246.2		3	15
	Androstenedione	286.1 → 109.1	10.10	3	5
		286.1 → 124.1		3	5
	D3-Androstenedione	289.3 → 110.0	10.07	3	5
		289.3 → 127.0		3	5
	Testosterone	360.2 → 174.1	10.41	3	11
		360.2 → 162.1		3	11
	D2-Testosterone	362.1 → 176.1	10.40	3	11
		362.1 → 164.1		3	11
	Mestranol	367.0 → 193.2	10.82	3	17
		367.0 → 173.1		3	17
11.15 min	17α-Ethynylestradiol	425.0 → 193.1	11.45	9	20
		425.0 → 231.2		9	20
	D4-17α-Ethynylestradiol	429.1 → 195.1	11.43	9	20
		429.1 → 233.1		9	20
	Levonorgestrel	355.0 → 167.0	12.13	9	20
		355.0 → 193.0		9	20
	Estriol	504.2 → 324.3	12.58	9	11
		504.2 → 386.3		9	9
	D3-Estriol	507.3 → 327.0	12.55	9	11
		507.3 → 389.4		9	9

isotope dilution technique. Calibration curves were comprised of at least 5 points out of nine calibration points for the non-labelled standards (1.0, 2.5, 5.0, 10, 25, 50, 100, 250, 500 ng mL^{-1} in GC autosampler vials). The lowest calibration point used for each analyte was that corresponding to the lowest concentration above the analyte-specific MDL as shown in Table 7). Isotope standards were added to all calibration solutions in a mass equivalent to the mass of isotope standards added to the samples to be analysed.

3. Results and discussion

3.1. Analyte recovery experiments

The calculated method recoveries of the target compounds in ultrapure water, drinking water, synthetic MBR effluent, surface water and tertiary treated effluent matrices are shown in Table 4. It was observed that the use of isotope dilution satisfactorily corrected for any loss during sample processing,

Table 4
Method recoveries of analytes in various water matrices from a spiking concentration of 100 ng L^{-1}, μ ($\pm\sigma$) %.

Analytes	Method recoveries				
	Ultrapure water (n = 9)	Drinking water (n = 9)	MBR effluent (n = 9)	Surface water (n = 9)	Tertiary treated effluent (n = 9)
Androsterone	110 (±10)	104 (±3)	105 (±4)	103 (±6)	114 (±4)
Etiocholanolone	101 (±5)	98 (±3)	97 (±3)	106 (±5)	100 (±7)
Dihydrotestosterone	98 (±5)	97 (±8)	92 (±7)	93 (±7)	95 (±7)
17α-Estradiol	102 (±2)	101 (±2)	102 (±2)	106 (±5)	96 (±4)
Estrone	116 (±5)	100 (±4)	96 (±2)	100 (±4)	96 (±4)
Androstenedione	104 (±3)	105 (±3)	103 (±3)	105 (±7)	104 (±7)
17β-Estradiol	100 (±2)	98 (±2)	94 (±3)	98 (±7)	98 (±6)
Testosterone	101 (±2)	100 (±4)	100 (±3)	104 (±4)	106 (±7)
Mestranol	90 (±15)	90 (±4)	84 (±2)	80 (±10)	86 (±10)
17α-Ethynylestradiol	112 (±5)	88 (±4)	83 (±2)	81 (±5)	90 (±3)
Levonorgestrel	100 (±15)	100 (±8)	99 (±7)	107 (±7)	120 (±10)
Estriol	101 (±3)	92 (±5)	98 (±3)	94 (±5)	96 (±5)

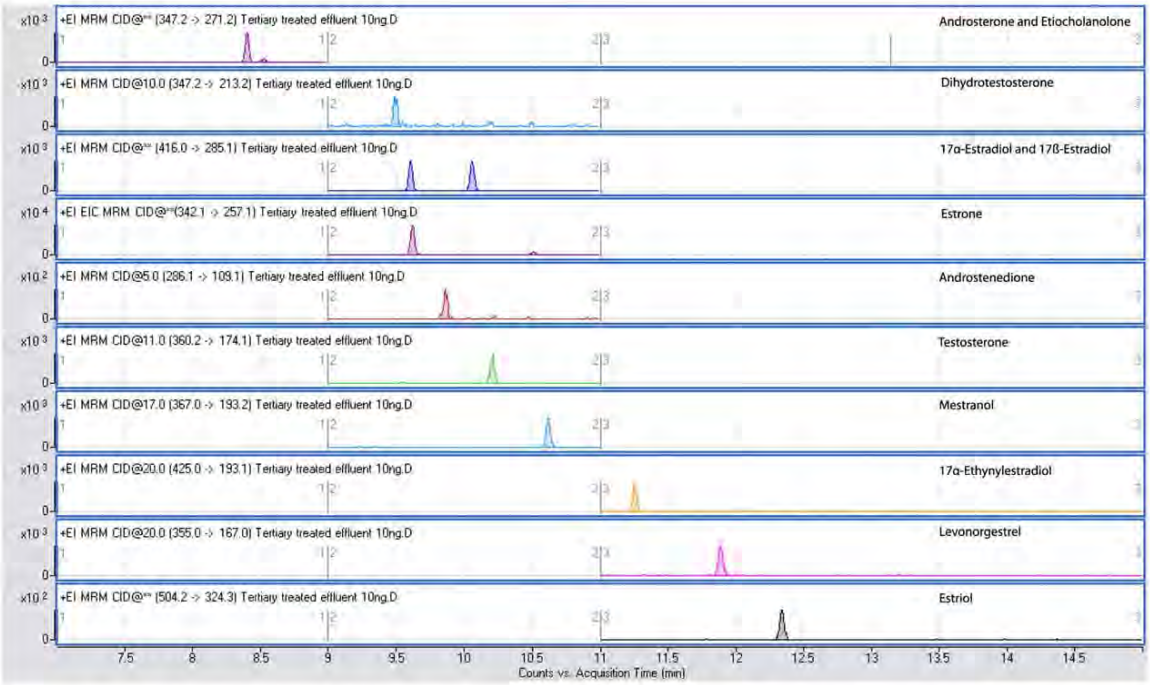


Fig. 1. A chromatogram showing quantifier peaks of 12 analytes in tertiary treated effluent matrix (on column mass = 10 pg).

Table 5
SPE absolute recoveries of analytes from low spiking concentration (10 ng L^{-1}) and high spiking concentration (100 ng L^{-1}), μ ($\pm\sigma$) %.

Analytes	SPE recoveries, 100 ng L^{-1} spiked			SPE recoveries, 10 ng L^{-1} spiked[a]	
	Ultrapure water (n = 3)	Surface water (n = 3)	Tertiary treated effluent (n = 3)	Surface water (n = 3)	Tertiary treated effluent (n = 3)
Androsterone	90 (±3)	101 (±1)	106 (±3)	107 (±3)	102 (±3)
Etiocholanolone	87 (±5)	89 (±7)	102 (±3)	106 (±1)	100 (±3)
Dihydrotestosterone	92 (±7)	92 (±7)	100 (±5)	100 (±3)	104 (±5)
17α-Estradiol	97 (±3)	92 (±7)	93 (±1)	98 (±4)	87 (±5)
Estrone	95 (±2)	95 (±6)	99 (±5)	105 (±4)	104 (±4)
Androstenedione	86 (±7)	92 (±7)	97 (±2)	103 (±7)	109 (±7)
17β-Estradiol	95 (±2)	95 (±5)	92 (±2)	98 (±5)	102 (±4)
Testosterone	95 (±6)	96 (±6)	102 (±6)	96 (±7)	97 (±7)
Mestranol	52 (±6)	95 (±2)	96 (±3)	96 (±2)	97 (±5)
17α-Ethynylestradiol	98 (±2)	92 (±5)	99 (±2)	92 (±5)	98 (±6)
Levonorgestrel	68 (±2)	105 (±7)	109 (±6)	104 (±7)	107 (±7)
Estriol	97 (±3)	98 (±6)	95 (±2)	91 (±7)	93 (±4)

[a] Except for dihydrotestosterone, which was spiked at 20 ng L^{-1} since it has an MDL of 15.8 ng L^{-1}.

Table 6
Recoveries during drying/reconstituting by Speedvac concentrator and drying by nitrogen gas from a spiking concentration of 100 ng L^{-1}, μ ($\pm\sigma$) %.

Analytes	Recoveries during drying and reconstituting by Speedvac concentrator		Recoveries during drying by nitrogen gas	
	Dry 3 h (n = 3)	Dry 5 min (n = 3)	Dry 30 min (n = 3)	Dry 60 min (n = 3)
Androsterone	99 (±2)	97 (±4)	100 (±2)	90 (±5)
Etiocholanolone	96 (±5)	97 (±6)	93 (±5)	90 (±7)
Dihydrotestosterone	105 (±3)	110 (±8)	112 (±2)	104 (±9)
17α-Estradiol	99 (±4)	105 (±3)	108 (±3)	107 (±5)
Estrone	97 (±2)	100 (±4)	110 (±5)	106 (±3)
Androstenedione	100 (±7)	95 (±5)	103 (±9)	105 (±7)
17β-Estradiol	100 (±2)	106 (±4)	104 (±4)	106 (±3)
Testosterone	107 (±9)	106 (±9)	107 (±8)	108 (±6)
Mestranol	99 (±5)	108 (±5)	110 (±3)	102 (±3)
17α-Ethynylestradiol	100 (±4)	107 (±4)	104 (±3)	101 (±5)
Levonorgestrel	104 (±7)	90 (±10)	91 (±11)	89 (±9)
Estriol	101 (±5)	102 (±3)	109 (±5)	105 (±9)

matrix effects and instrument variation leading to accurate quantification in all tested matrices. D2-etiocholanolone and D4-17β-estradiol were confirmed to be suitable isotope standards for the quantification of their stereoisomers androsterone and 17α-estradiol, respectively, with method recoveries in all tested matrices between 96% and 114% (max σ = 10%). Similarly, D4-17α-ethynylestradiol was confirmed to be a reasonable isotopic standard for quantification of mestranol and lev-

Table 7
MDLs of target analytes in various water matrices.

Analytes	MDLs ($ng\,L^{-1}$)				
	Ultrapure water ($n=7$)	MBR effluent ($n=7$)	Drinking water ($n=7$)	Surface water ($n=7$)	Tertiary treated effluent ($n=7$)
Androsterone	1.0	1.0	1.0	1.2	1.4
Etiocholanolone	5.0	5.0	5.0	5.8	6.4
Dihydrotestosterone	8.9	11.3	15.2	15.8	15.0
17α-Estradiol	0.8	0.9	1.2	0.9	1.0
Estrone	0.7	0.7	0.7	0.7	0.8
Androstenedione	5.0	5.0	5.0	5.0	5.5
17β-Estradiol	1.1	1.3	1.2	1.2	1.3
Testosterone	5.0	5.0	5.0	5.0	6.0
Mestranol	1.0	1.3	1.0	1.0	1.2
17α-Ethynylestradiol	1.0	1.0	1.0	1.3	1.2
Levonorgestrel	5.0	6.0	7.5	5.0	7.0
Estriol	2.5	2.5	2.5	2.6	3.0

Note: Injection volume is 1 μL, thus 1 $ng\,L^{-1}$ is equal to 1 pg on column mass.

Table 8
Coefficient of variation $C_v = \sigma/\mu$ for instrument stability and method stability of target analytes in various water matrices.

Analytes	Instrument stability[a]		Method stability[b]			
	Standard 100 $ng\,mL^{-1}$		Surface water 100 $ng\,L^{-1}$		Tertiary treated effluent 100 $ng\,L^{-1}$	
	Intra-day ($n=3$)	Inter-day ($n=6$)	Intra-day ($n=3$)	Inter-day ($n=6$)	Intra-day ($n=3$)	Inter-day ($n=6$)
Androsterone	0.08	0.11	0.06	0.07	0.05	0.07
Etiocholanolone	0.03	0.12	0.04	0.04	0.04	0.04
Dihydrotestosterone	0.08	0.10	0.04	0.07	0.02	0.02
17α-Estradiol	0.02	0.08	0.03	0.02	0.02	0.05
Estrone	0.04	0.10	0.01	0.06	0.01	0.06
Androstenedione	0.10	0.12	0.02	0.07	0.01	0.07
17β-Estradiol	0.02	0.09	0.02	0.04	0.04	0.05
Testosterone	0.06	0.11	0.02	0.05	0.02	0.05
Mestranol	0.05	0.12	0.03	0.06	0.06	0.10
17α-Ethynylestradiol	0.04	0.12	0.01	0.06	0.03	0.04
Levonorgestrel	0.07	0.13	0.05	0.09	0.05	0.10
Estriol	0.05	0.10	0.01	0.05	0.01	0.05

[a] Instrument stability not corrected by isotope dilution.
[b] Method stability includes correction by isotope dilution.

Table 9
Signal enhancement/suppression in surface water and tertiary treated effluent matrices from a spiking concentration of 20 $ng\,L^{-1}$, μ ($\pm\sigma$) %.

	Surface water matrix ($n=3$)	Tertiary treated effluent matrix ($n=3$)
Androsterone	−4 (±9)	−13 (±7)
Etiocholanolone	−18 (±10)	−25 (±8)
Dihydrotestosterone	+9 (±9)	+15 (±2)
17α-Estradiol	−5 (±10)	−8 (±8)
Estrone	−8 (±6)	−1 (±5)
Androstenedione	−10 (±9)	+19 (±10)
17β-Estradiol	−8 (±10)	−7 (±9)
Testosterone	+15 (±11)	+24 (±10)
Mestranol	−3 (±9)	+5 (±9)
17α-Ethynylestradiol	−5 (±7)	+12 (±7)
Levonorgestrel	−9 (±10)	+11 (±10)
Estriol	−1 (±7)	+9 (±9)

onorgestrel with method recoveries from 80% to 120% (max $\sigma = 15\%$).

The results of SPE absolute recoveries of the target compounds from low concentration (10 $ng\,L^{-1}$) and high concentration (100 $ng\,L^{-1}$) spiking tests are presented in Table 5. In surface water and tertiary treated effluent matrices, the absolute SPE recoveries ranged from 89% to 109% when spiked at 100 $ng\,L^{-1}$ and from 87% to 109% when spiked at 10 $ng\,L^{-1}$. Interestingly, the absolute recoveries from ultrapure water spiked at 100 $ng\,L^{-1}$, were somewhat lower (52–97%) suggesting that dissolved organic carbon in the matrix may enhance the SPE recovery. A possible explanation may be that the organic matrix materials improve the method performance by competing for active adsorption sites on glassware and the GC inlet liner. This would improve quantitative steroid transfer through to the MS detector. Regardless of the cause, these observed matrix differences emphasise the importance of isotope dilution for SPE recovery correction among diverse matrices.

The mean analyte recoveries from spiked methanol samples after drying by the Speedvac concentrator are shown in Table 6. This table also shows the recoveries of the analytes from evaporation of anhydrous acetonitrile samples after evaporation under nitrogen with various drying times (5 min, 30 min and 60 min). The results of these two experiments confirm that negligible losses of all analytes occurred under all of the tested drying conditions.

The results of the recovery experiments from larger sample volumes of tertiary treated effluent (not shown) indicate that recovery efficiencies for all analytes were not detrimentally affected for sam-

ple volumes up to 1 L. This suggests that the MDLs may be driven somewhat lower by the use of 1 L samples instead of 0.5 L samples in some circumstances. However, recoveries of most of the analytes were diminished by up to 50% for sample volumes of 2 L or greater.

3.2. Method detection levels

The MDLs in the different water matrices are presented in Table 7. These results show that in ultrapure water, drinking water, synthetic MBR effluent, surface water and tertiary treated effluent, MDLs typically ranged between 1 and 5 ng L^{-1}. However, slightly higher MDLs were observed for etiocholanolone (up to 6.4 ng L^{-1}), androstenedione (up to 5.5 ng L^{-1}), testosterone (up to 6.0 ng L^{-1}), levonorgestrel (up to 7.5 ng L^{-1}) and dihydrotestosterone (up to 15.8 ng L^{-1}) in some aqueous matrices. Numerous previous studies have reported the presence of estrogenic hormones in effluents of sewage treatment plants at concentrations of 1–70 ng L^{-1} [8,16,54]. Furthermore, estrogenic hormones have been reported at up to 6 ng L^{-1} in impacted surface waters [9]. Much fewer data are available for androgenic hormones, but some have been reported in surface water at concentrations up to 12 ng L^{-1} [18].

Dihydrotestosterone was the least sensitive target compound with generally higher MDLs in ultrapure water (8.9 ng L^{-1}), synthetic MBR effluent (11.3 ng L^{-1}), drinking water (15.2 ng L^{-1}), surface water (15.8 ng L^{-1}) and tertiary treated effluent (15.0 ng L^{-1}). However, these elevated MDLs were the consequence of a decision to base the quantification of this analyte on the most specific (but not most intense) ion transition at m/z 347.2 → m/z 213.2. This decision was made in order to facilitate the clear distinction of dihydrotestosterone from androsterone and etiocholanolone. If required, reduced MDLs for dihydrotestosterone can be achieved by alternatively basing the quantification on the more intense m/z 347.2 → m/z 271.2 transition.

The fact that the MDLs were not significantly reduced from ultrapure water to more complex matrices highlights the robustness of this method against potential impacts of matrix-specific ion suppression during mass spectral analysis.

Some previous studies have quoted lower detection limits for some of the analytes presented in this paper. While the approach taken to determine these detection limits has been variable (and often not explicitly stated), the most common procedure has been to identify an analyte concentration for which a signal-to-noise ratio (S/N) of 3 can be obtained. The concentration obtained by this approach is most correctly termed the 'lower level of detection' (LLD) or the 'level of detection' (LOD) [59]. This approach is intended to set the probability of both false positives and false negatives at 5%. However, the LLD method is not well suited to GC–MS/MS analysis since it is commonly not possible to observe any 'noise' (for example, see Fig. 1). A more robust (but somewhat more conservative) approach for defining detection limits is adopted in this paper, as has been referred to as the 'method detection level' (MDL). The MDL is used to describe the analyte concentration that, when processed through the complete method, produces a signal with a 99% probability that it is different from the blank [59].

The better sensitivity of the estrogens compared to the androgens is assumed to be largely due to differences in EI fragmentation at 70 eV. Fragmentation of estrogens generally resulted in the production of around 6–10 highly stable ion fragments (as observed in full scan mass spectra, not shown). However, the androgens were typically fragmented into a much larger number of ion fragments, thus the overall signal was distributed (or diluted) between a larger number of m/z values. The stable ion formation for many of the estrogens (with the exception of mestranol) may be partially due to the TMS-derivatised phenol group, which the androgens lack.

3.3. Instrument stability, matrix effects and calibration range

The results of instrument and method stability assessments are presented in Table 8. The coefficients of variability ($C_V = \sigma/\mu$) for instrument variability on an intra-day basis ranged from 0.02 to 0.10. Slightly greater coefficients of variability for instrument variability were observed on an inter-day basis, from 0.08 to 0.13. However, the coefficients of variability for the full method analysis of spiked surface water and tertiary effluent samples, on both an intra-day and inter-day basis were observably lower. These varied from 0.01 to 0.07 for analytes with direct isotope labelled analogue correction and up to 0.10 for analytes with alternative isotope labelled analogue correction. This observation emphasises the importance of the isotope dilution process to ensure a high level of analytical reproducibility.

The results of the signal enhancement/suppression assessment in surface water and tertiary treated effluent matrices are presented in Table 9. These data represent the means and standard deviations of three samples assessed in each of the two matrices. Some degree of signal suppression may be evident for a few analytes (e.g. etiocholanolone) and enhancement for others (e.g. testosterone). However, these results reveal a high degree of variability between samples, thus obscuring any real trends. This variability again reinforces the importance of isotope dilution for accurate quantification in real sample matrices.

Blank (unspiked) matrix samples were run to assess background concentrations of the analytes in ultrapure water, drinking water, synthetic MBR effluent, surface water and tertiary treated effluent. The only observed analyte in these matrix samples was estrone, which was measured in tertiary treated effluent at a concentration of 1 ng L^{-1}. Accordingly, all validation experiments on this matrix were calculated after correcting for a background concentration of 1 ng L^{-1} estrone.

The linear calibration range for the target compounds was determined to be from their identified MDLs to 500 ng L^{-1}, thus the upper quantification limit is 500 ng L^{-1} for all analytes. The calibration points for each of the analytes were fitted to linear regressions and the calibration curve regression correlation coefficients were always at least 0.99 for all sample batches.

4. Conclusion

An analytical method was developed for the simultaneous analysis of 12 natural and synthetic hormones in aqueous matrices. No previous GC–MS method is known that encompasses this full range of estrogenic and androgenic analytes. Furthermore, the use of GC–MS/MS has enabled unambiguous identification and non-interfering quantification of closely eluting chromatographic peaks in a very short analysis time of only 15 min.

The use of isotope dilution for all analytes ensures the accurate quantification, accounting for analytical variabilities that may be introduced during sampling, extraction, derivatisation, chromatography, ionisation or mass spectrometric detection. Direct isotopically labelled analogues were used for 8 of the 12 hormones. However, satisfactory isotope standards were determined for the remaining 4 hormones, based on structural similarity and observed method recoveries of 80–120% in all sample matrices.

The established MDLs for most analytes were 1–5 ng L^{-1} in a variety of aqueous matrices. However, slightly higher MDLs were observed for etiocholanolone, androstenedione, testosterone, lev-

onorgestrel and dihydrotestosterone in some aqueous matrices. Sample matrices were observed to have only a minor impact on MDLs indicating that interferences such as ion suppression, which is a common problem for HPLC–MS (or HPLC–MS/MS) methods, did not have a significant impact on sensitivity for this method. The method validation confirmed very good method stability over intra-day and inter-day analyses.

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Fate of Pharmaceuticals during Wastewater Treatment by a Membrane Bioreactor

Wastewater treatment, membrane bioreactor, trace organic contaminants, pharmaceuticals

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This study provides a comprehensive insight into the levels and fate of nine commonly used pharmaceuticals (amitriptyline, atenolol, gemfibrozil, ibuprofen, ketoprofen, metformin, naproxen, paracetamol and simvastatin) through a full-scale membrane bioreactor (MBR) in New South Wales, Australia. Seven out of the nine studied pharmaceuticals were detected in the raw sewage with average concentrations in the range of 1.29–33.3 µg.L^{-1}, while gemfibrozil and simvastatin were below the analytical detection limit (1 ng.L^{-1}). The MBR consistently achieved high removal efficiencies of the detected pharmaceuticals, in the range of 77.2–99.9 %. A mass-balance showed that sorption to biomass was the dominant removal mechanism for amitriptyline while biodegradation/transformation was responsible for removing all other pharmaceuticals. This study revealed that log D (Log K_{ow} corrected for ionisation at the ambient pH) provides an effective estimation of the sorption capacity of these pharmaceuticals to biomass.

1. Introduction

Submerged membrane bioreactors (MBRs) are favoured technology for sewage treatment in small communities. This is due to their small footprint and the ability to produce high quality effluent, which is suitable for water reuse. MBRs have achieved excellent removal efficiencies with respect to pathogens, suspended solids, organics and nitrogen [1]. Recently, interest into the ability of MBRs to eliminate trace organic chemicals such as pharmaceuticals has increased [2–5]. Pharmaceuticals are of concern for water recycling schemes because of their widespread use by the public and their potential for adverse environment and public health effects [6-10]. The fate of pharmaceuticals during MBR treatment is complex and includes biodegradation/transformation and/or partitioning to either solid or liquid phases.

To date, the majority of studies which have characterised the removal of pharmaceuticals by MBRs have focused on measuring pharmaceuticals within the aqueous phase, with little consideration of the solid phase (i.e. biomass). To better understand the fate and removal mechanisms of pharmaceuticals through MBRs, both aqueous and solid phases of the MBR need to be investigated.

Accordingly, the aim of this study was to investigate the fate and removal of pharmaceuticals through a full-scale, package MBR plant treating municipal wastewater in New South Wales, Australia. In this study, both aqueous (influent and effluent) and biomass samples were analysed for nine pharmaceuticals. A simple mass balance was calculated to estimate the contribution of biodegradation/transformation and sorption to the removal of pharmaceuticals by the MBR. The nine target pharmaceuticals in this study include lipid-lowering drugs gemfibrozil and simvastatin; a beta-adrenoceptor blocking drug atenolol; anti-inflammatory/analgesic drugs ibuprofen, ketoprofen, naproxen, paracetamol; an antidiabetic drug metformin and a common antidepressant amitriptyline. These particular pharmaceuticals were selected considering the following factors: their high annual consumption in Australia [11], their diversity in terms of physio-chemical characteristics (e.g. neutral, acidic, ionic, hydrophobic and hydrophilic) and the analytical capacity of the laboratory.

2. Materials and Methods

2.1 Description of the package MBR

Samples were collected from a full-scale package MBR plant (800 equivalent persons) located in Wolumla, Bega Valley, New South Wales, Australia. The treatment process is comprised of a fine screen (3 mm), a bioreactor tank, two parallel-submerged membrane modules and a medium pressure ultra-violet (UV) disinfection unit. The sludge retention time (SRT) of the bioreactor is 10-15 days, the hydraulic retention time (HRT) is 1 day and the mixed liquor suspended solids (MLSS) concentration is 7.5–8.5 g.L^{-1}. The bioreactor tank was operated

with a 10 minute cyclic on/off aeration (dissolved oxygen (DO) set-point of 1 mg.L^{-1}). The submerged membrane modules are comprised of hollow fibre membranes (Koch Puron) which have an effective pore size of 0.1–0.2 µm. The final effluent is used for irrigation. The mean water quality values in the raw sewage and MBR permeate are presented in **table 1**.

Table 1. Quality of the raw sewage and MBR permeate (mean values reported, n=5).

Quality parameters	Raw sewage	MBR permeate
DOC (mg.L^{-1})	114	13.2
NH_3 (mg.L^{-1})	43.2	0.1
Total N (mg.L^{-1})	81.5	4.5
Total P (mg.L^{-1})	Unavailable	6.2
pH	7.0	7.9

2.2 Sample collection

Daily composite aqueous samples of raw sewage (0.5 L), MBR permeate (1 L) and grab samples of mixed liquor (0.5 L) were taken in triplicates over a 5-day-period in March 2011 (giving a total of 15 raw sewage samples, 15 MBR permeate samples and 15 mixed liquor samples). After collection, the mixed liquor was immediately filtered through 0.7 µm Millipore glass fibre prefilters and the solid biomass was stored in 60 mL plastic containers and frozen. These frozen biomass samples were transported to the laboratory for further analysis. The raw sewage was immediately filtered through 0.7 µm Millipore glass fibre prefilters. All aqueous samples were then spiked with isotopically labelled standards of trace chemicals of interest for accurate isotope dilution quantification. These aqueous samples were stored in ice and extracted onsite using solid phase extraction (SPE) within 24 hours of collection.

2.3 Extraction of biomass

Frozen biomass samples were freeze dried over a period of up to four days. The freeze-dried samples were then subjected to ultrasonic solvent extraction following an adaptation of a method previously reported for extraction of sewage sludge samples [5, 12]. Freeze dried samples were ground to fine powder using mortar and pestle. Ground samples were then weighed (0.5 g) into 13 mL glass culture tubes. The isotope standards stock solution (1 mg/L) was added to the culture tube (200 µL). Methanol (5 mL) was then added and the solution thoroughly mixed using a vortex mixer. Each sample was then ultrasonicated (10 minutes, 40 °C) followed by gravity settling and decanting of the supernatant. The ultrasonication step was repeated with addition of 5 mL of acetone and the two supernatants combined in an acid-washed 500 mL bottle. The combined supernatant was then diluted with ultrapure water (500 mL) and filtered through 0.7 µm Whatman filter paper in preparation for SPE.

2.4 Solid phase extraction

The Oasis HLB SPE cartridges were pre-conditioned with methanol (5 mL), followed by ultrapure water (5 mL). SPE cartridges were loaded by drawing through 500 mL (for raw sewage samples) or 1000 mL (for MBR permeate samples) of the aqueous samples under vacuum, maintaining a consistent loading flow rate of less than 10 mL. min^{-1}. The SPE cartridges were rinsed with 10 mL of ultrapure water before drying with air for approximately 30 min. The dried cartridges were stored at –18 °C prior to elution and quantitative analysis. Analytes were eluted from the cartridges with methanol (2 x 5 mL) into Kimble culture tubes. The extracts were centrifugally evaporated under vacuum at 35 °C using a Thermo Speedvac (Biolab) concentrator. The evaporated samples were reconstituted with anhydrous methanol (0.5 mL) and transferred to amber autosampler vials before analysis by liquid chromatography - tandem mass spectrometry (LC-MS/MS).

2.5 LC/MS-MS analysis

The concentrations of pharmaceuticals in the samples were determined using isotope dilution LC-MS/MS. The target compounds were analysed by two different LC-MS/MS methods using positive mode electrospray ionisation (ESI+) and negative mode electrospray ionisation (ESI-) following an adaptation of a previously published method [13]. The ESI+ method was used to determine ibuprofen, ketoprofen, naproxen, gemfibrozil and simvastatin. The ESI- method was used to determine amitriptyline, atenolol, metformin, paracetamol. The analytical instrument includes the Agilent series 1200 LC system coupled with an Applied Biosystems QTrap API 4000 mass spectrometer. LC separation was carried out with a Luna C18, 5 µm, 150 mm · 4.6 mm, 100A column with a security guard cartridge C18, 5 µm, 4 mm x 3 mm, 100A (Biolab). Mobile phases were HPLC grade methanol (100 %) and ultrapure water with 5mM ammonium acetate. Direct isotopically labelled analogues were used as internal standards for all investigated compounds for accurate quantification.

3. Results and Discussion

3.1 Concentrations of pharmaceuticals in raw sewage

The mean concentrations of the investigated pharmaceuticals in the raw sewage are presented in **table 2**. Gemfibrozil and simvastatin were not detected above their analytical detection limits of 1 ng.L^{-1}. Atenolol, ibuprofen, ketoprofen, naproxen, paracetamol and metformin were detected in the range of 4.79-33.3 µg.L^{-1}. The concentrations of atenolol and paracetamol were of

Table 2. Mean concentrations of pharmaceuticals in raw sewage, MBR permeate and biomass (samples taken in triplicates over a 5-day-period).

Pharmaceutical	Raw sewage Mean (stdev)	MBR permeate Mean (stdev)	Biomass Mean (stdev)
	($\mu g.L^{-1}$)	($\mu g.L^{-1}$)	($\mu g.kg^{-1}$ dried biomass)
Amitriptyline	1.29 (± 0.83)	0.18 (±0.04)	$1.5x10^3$ (± 0.19 $x10^3$)
Atenolol	4.79 (± 0.68)	0.09 (±0.03)	2.70 (± 0.86)
Gemfibrozil	<LOD	<LOD	<LOD
Ibuprofen	19.3 (± 7.30)	0.05 (± 0.01)	36.5 (± 18.3)
Ketoprofen	8.20 (± 4.66)	0.07 (± 0.03)	2.40 (± 1.05)
Metformin	16.7 (± 12.8)	4.65 (± 2.23)	200 (± 38.1)
Naproxen	33.3 (± 10.9)	0.07 (± 0.04)	10.6 (± 5.16)
Paracetamol	29.0 (± 7.43)	0.03 (± 0.01)	40.0 (± 6.18)
Simvastatin	<LOD	<LOD	<LOD

<LOD: lower than limit of detection.

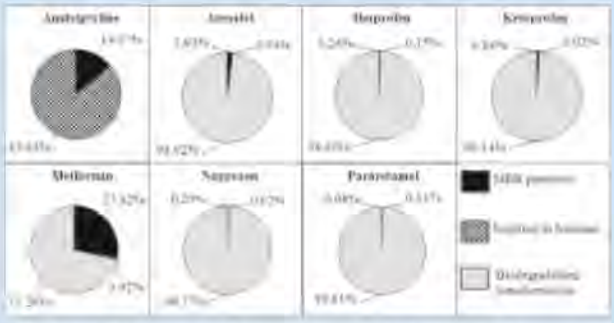

Figure 1. Relative removal mechanisms of the investigated pharmaceuticals through MBR.

Table 3. Log K_{ow} Log $D_{pH=8}$ and % sorption to biomass of detected pharmaceuticals.

Pharmaceutical	Log K_{ow}	Log $D_{pH=8}$	% sorption to biomass
Amitriptyline	4.92[b]	3.72[b]	85.93
Atenolol	0.10[b]	−1.09[b]	0.04
Ibuprofen	3.72[b]	0.36[b]	0.15
Ketoprofen	2.81[b]	−0.64[b]	0.03
Metformin	−2.64[c]	−2.64[d]	0.92
Naproxen	3.00[b]	−0.06[b]	0.02
Paracetamol	0.33[b]	0.33[b]	0.11

[b]: Tadkaew et al., 2011
[c]: US EPA, 2011
[d]: calculated from log K_{ow} and pK_a

the same order of magnitude as data from Spain [7] and the UK [8] while the concentration of naproxen was two orders of magnitude higher than the values reported in these countries. The concentrations of ibuprofen and ketoprofen detected were similar with the levels detected in Spain but were one to two orders of magnitude higher than values reported in the UK [7, 8]. These results may reflect the different pharmaceutical usage in these countries.

Amitriptyline has not been intensively studied in wastewater and reports on levels in raw sewage are still limited. However, a similar concentration of amitriptyline has been reported in one study from the UK [8].

3.2 Fate of pharmaceuticals through the MBR process

The removal mechanisms for pharmaceuticals through MBRs include biodegradation/transformation, sorption to biomass, volatilisation and physical retention by the membrane. As the molecular weight cut off for ultra-filtration MBR membranes is about 100–200 kDa, these membranes are not expected to retain pharmaceuticals, unless they were adsorbed to larger particles. In addition, the low Henry's constant for the targeted pharmaceuticals ($H < 10^{-6}$) suggests that volatilisation is an insignificant removal mechanism for these compounds [14, 15]. Therefore, biodegradation/transformation and sorption to biomass are the two most important removal pathways for these pharmaceuticals. Biodegradation/transformation are grouped together since it is often difficult to distinguish between processes of chemically or biologically mediated transformation or degradation processes. This is largely due to current analytical limitations for the analysis of metabolites and other transformation products.

The concentrations of pharmaceuticals within the MBR's permeate and biomass are also presented in **table 2**. These data were used together with the aqueous and biomass flow data to establish a mass balance for the fate of each pharmaceutical. These mass balances were calculated based on Equation 1:

$$\text{Influent load} = \text{effluent load} + \text{biomass load} + \text{biodegradation load} \quad \text{(Equation 1)}$$

The results of the mass balance calculations are presented in **figure 1**. The output loads (MBR permeate, biodegradation/transformation and sorption to biomass) are expressed as proportions relative to the influent load. Results show that amitriptyline was not significantly biodegraded or transformed through the MBR process. The overall removal efficiency (86 %) of this compound was solely attributed to sorption to biomass, given that high concentrations of up to $1.5x10^3$ $\mu g.kg^{-1}$ were detected within the biomass (**table 2**). A previous study based on a laboratory-scale MBR treating a synthetic feed solution reported 98 % removal of amitripty-

line [16]. This same study hypothesised that sorption to biomass was a dominant removal mechanism since amitriptyline is a very hydrophobic compound (log K_{ow} = 4.92). Another study on sorption potentials of trace organic compounds onto wastewater sludge indicated that high sorption potentials of positively-charged pharmaceuticals like amitriptyline were attributed to both hydrophobic sorption interactions and electrostatic interactions [14].

Figure 1 shows that MBR can remove atenolol effectively with an average removal efficiency of 98%. This result is slightly higher than reported values in previous studies on MBR treating municipal wastewater [7, 17]. This can be explained by the higher HRT in the current study compared to that of the previous studies, given that removal of atenolol has been shown to increase with an increase in HRTs [18]. The results from **figure 1** also show that biodegradation/transformation was the dominant removal mechanism for atenolol while sorption to biomass only contributed 0.04% to the overall removal. This result was expected as atenolol is a very hydrophilic drug with log K_{ow} = 0.10 [16].

The results in **figure 1** also show excellent removal efficiencies (>99%) of the anti-inflammatory/analgesic drugs ibuprofen, ketoprofen, naproxen and paracetamol by the MBR. These results are in agreement with previous studies on MBRs treating municipal wastewater [3, 5, 7]. Although these compounds vary from highly hydrophilic (paracetamol, log K_{ow} = 0.33) to moderately hydrophobic (ketoprofen, log K_{ow} = 2.8 and naproxen, log K_{ow} = 3) and to highly hydrophobic (ibuprofen, log K_{ow} = 3.7) [16], sorption to biomass only contributed to less than of 0.2% to the overall removal of these compounds and biodegradation/transformation was the main removal mechanism. These results are consistent with other studies where sorption to biomass was identified as a minor removal pathway and biodegradation was found to be a dominant removal mechanism for these pharmaceuticals [5, 7, 19]. Low concentrations of these moderate and highly hydrophobic pharmaceuticals in biomass may possibly explain the high degree of biodegradability [2]. A recent study indicated that the biodegradation process of paracetamol, ketoprofen and naproxen could result in transformation products which are more stable than these precursor drugs [3]. The biotransformation products of these pharmaceuticals through MBRs need to be investigated further.

This study was the first to investigate the removal of the antidiabetic drug metformin by MBRs. **Figure 1** shows that sorption of metformin to the biomass only contributed to 0.92% of the overall removal, while biodegradation/transformation was responsible for removing 71% of this drug. This low sorption potential to biomass was expected since metformin is highly "hydrophilic".

Table 3 presents the log K_{ow} (partition coefficient), log $D_{pH=8}$ (distribution coefficient) and percentage sorption to biomass through the MBR treatment process of the detected pharmaceuticals in this study. No clear relationship between Log K_{ow} and percentage of sorption to biomass is apparent. For example, relatively high Log K_{ow} values for ibuprofen and naproxen do not lead to correspondingly high levels of sorption. On the other hand, log $D_{pH=8}$ appears to provide a more reliable predictive indication of sorption. In this case, log $D_{pH=8}$ for ibuprofen and naproxen are significantly reduced, accounting for their high degree of acid dissociation at pH=8. After accounting for pK_a, amitriptyline would be correctly identified as the only analyte with Log $D_{pH=8}$ > 3.2 and hence the only one expected to be significantly adsorbed to biomass [16].

4. Conclusions

This study investigated the fate and levels of nine widely used pharmaceuticals through a full-scale, package MBR plant treating municipal wastewater in Australia. Detectable levels of seven out of the nine studied pharmaceuticals were observed in the raw sewage and these pharmaceuticals were effectively removed by the MBR. For amitriptyline, the main removal mechanism was sorption to biomass, whereas for the other pharmaceuticals, biodegradation/transformation was the dominant removal mechanism. The sorption capacity of these pharmaceuticals to biomass can be estimated based on log $D_{pH=8}$ values. The study enhances our understanding on the key removal mechanisms of investigated pharmaceuticals through MBR process. This knowledge can be used to optimise the performance of MBRs in removing pharmaceuticals to achieve the best possible effluent quality for water reuse applications.

5. Acknowledgements

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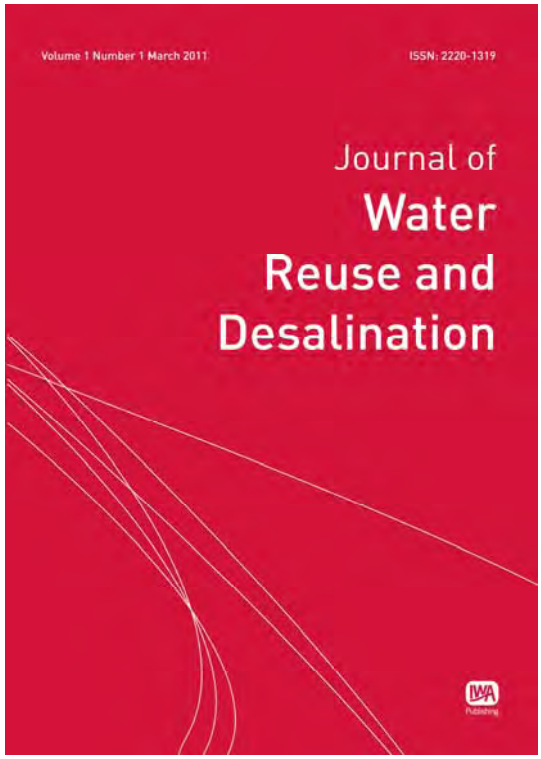

Removal of endocrine disrupting chemicals and microbial indicators by a decentralised membrane bioreactor for water reuse

T. Trinh, B. van den Akker, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan

ABSTRACT

Submerged membrane bioreactors (MBRs) have attracted a significant amount of interest for decentralised treatment systems due to their small footprint and ability to produce high quality effluent, which is favourable for water reuse applications. This study provides a comprehensive overview of the capacity of a full-scale decentralised MBR to eliminate 17 endocrine disrupting chemicals (EDCs) and five indigenous microbial indicators. The results show that the MBR consistently achieved high removal of EDCs (>86.5%). Only 2 of the 17 EDCs were detected in the MBR permeate, namely two-phenylphenol and 4-tert-octylphenol. Measured $\log_{10}$ reduction values of vegetative bacterial indicators were in the range of 5–5.3 $\log_{10}$ units, and for clostridia, they were marginally lower at 4.6 $\log_{10}$ units. Removal of bacteriophage was in excess of 4.9 $\log_{10}$ units. This research shows that MBRs are a promising technology for decentralised water reuse applications.

Key words | membrane bioreactor, microbial indicators, steroidal hormones, trace organic contaminants, wastewater treatment

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INTRODUCTION

In regional and rural communities where connection to centralised sewer networks is not possible or is economically unfeasible, decentralised wastewater treatment systems (or package plants) are becoming the preferred option for sewage treatment. Recently, submerged membrane bioreactors (MBRs) have attracted a significant amount of interest for decentralised treatment systems due to their small footprint and ability to produce high quality effluent, which is favourable for water reuse applications (Coleman *et al.* 2009; Le-Minh *et al.* 2010).

In Australia, implementation of water recycling processes such as MBRs requires validation to demonstrate that the process is capable of achieving the required water quality objectives (Australian Guidelines for Water Recycling 2008). Validation is most frequently based on characterising the removal of contaminants with health effects associated with acute or single dose exposures and therefore the majority of research on MBRs has focused on the removal of human pathogens or their surrogates (e.g. faecal coliforms, bacterial spores and bacteriophage).

Over the past decade, interest in the ability of MBRs to eliminate trace organic chemicals, such as endocrine disrupting chemicals (EDCs), has increased – particularly for water reclamation schemes that have potential for chronic human exposure (e.g. direct or indirect potable reuse). In contrast to microbial constituents, the efficiency of MBR technology as a barrier for EDCs is less clear and most of the data available have been derived from pilot- or laboratory-scale MBRs (e.g. Chen *et al.* 2008; Tadkaew *et al.* 2011). These studies show high removal of EDCs within the order of 90.4–>99.5%; however, without complementary research at the field scale, it can only be assumed that these values reflect the performance of larger-scale systems.

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Accordingly, the aim of this study was to investigate the removal of EDCs through a full-scale package MBR plant treating municipal wastewater in New South Wales, Australia. The removal of microbial indicators was also characterised in parallel to provide a comprehensive overview of the MBR's overall capacity to remove key contaminants of concern. The selected EDCs included seven natural and synthetic steroidal estrogens (17α-estradiol, 17β-estradiol, estrone, mestranol, 17α-ethynylestradiol, levonorgestrel, estriol), five steroidal androgens (testosterone, androsterone, etiocholanolone, dihydrotestosterone, androstenedione) and five xenoestrogens (bisphenol A, nonylphenol, 2-phenylphenol, propylparaben, 4-tert-octylphenol). As such, they represent the full suite of EDCs that have been subject to most environmental concerns internationally. Five indigenous microbial indicators were monitored: total coliforms, *Escherichia coli*, enterococci, sulphite-reducing clostridia (SRC) and F-RNA bacteriophage. These microbial indicators were selected because they are commonly used as surrogates for estimating the removal of pathogens in wastewater treatment systems (Wen *et al.* 2009).

MATERIALS AND METHODS

Description of the decentralised MBR

Samples were collected from a decentralised full-scale MBR plant (800 equivalent persons) located in Wolumla, Bega Valley, New South Wales, Australia. A schematic diagram of the MBR is presented in Figure 1, which summarises the key components, flow direction and sample sites. The treatment process comprises a fine screen (3 mm), a bioreactor tank, two parallel-submerged membrane modules and a medium pressure ultra-violet (UV) disinfection unit. The sludge retention time of the bioreactor was 10–15 d, the hydraulic retention time was 1 d and the mixed liquor suspended solids concentration was 7.5–8.5 g L^{-1}. The bioreactor tank was intermittently aerated in 10 min cycles (dissolved oxygen set-point of 1 mg L^{-1}) to achieve simultaneous nitrification and denitrification. The submerged membrane modules were made of hollow fibre membranes (Koch Puron), which have an effective pore size of 0.1–0.2 μm and a surface area of 235 m^2 (each). For cleaning, scour air was applied to the membranes using a positive displacement blower and backwashing occurred for a period of 60 s every 360 s. Chemical backwashing occurred automatically every 3 weeks, in accordance with the manufacturer's recommendations, to maintain a transmembrane pressure of <20 kPa. The membrane unit was designed to achieve an average flux of 25 L m^{-2} h. All of the final effluent is used for irrigation. The water quality values in the raw sewage and MBR permeate are presented in Table 1.

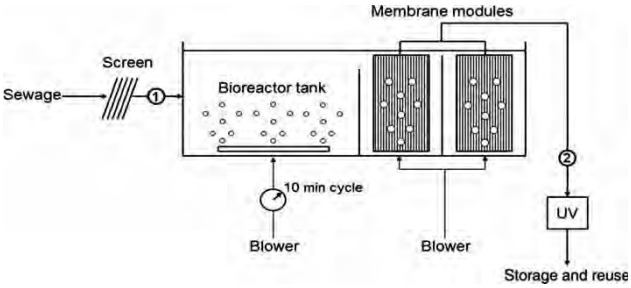

Figure 1 | Schematic diagram of the full-scale membrane bioreactor summarising the key components, flow directions and sample sites: (1) raw sewage and (2) permeate.

Analysis of EDCs

Sample collection

Daily composite aqueous samples of raw sewage (0.5 L) and MBR permeate (1 L) were taken in triplicate over a 5-day period in March 2011 (giving a total of 15 raw sewage samples and 15 MBR permeate samples). After collection, raw sewage was immediately filtered through 0.7 μm

Table 1 | Quality of raw sewage and MBR permeate

Quality parameters	Raw sewage range (mean) (n=5)	MBR permeate range (mean) (n=5)
DOC (mg L^{-1})	106.7–120.8 (114)	12.5–13.8 (13.2)
NH_3 (mg L^{-1})	35.7–50.7 (43.2)	0–0.2 (0.1)
Total N (mg L^{-1})	77.3–92.5 (81.5)	3.1–6.2 (4.5)
Total P (mg L^{-1})	Unavailable	5.0–7.4 (6.2)
pH	6.8–7.2 (7.0)	7.7–8.1 (7.9)

DOC: dissolved organic carbon.

Millipore glass fibre prefilters. All samples were then spiked with isotopically labelled standards of trace chemicals of interest for accurate isotope dilution quantification. The samples were stored in ice and extracted on site using solid phase extraction (SPE) within 24 h of collection. The SPE procedure was reported in a previous publication (Trinh *et al.* 2011b).

LC/MS-MS analysis

The concentrations of nonylphenol, 2-phenylphenol, bisphenol A, 4-tert-octylphenol and propylparaben in the samples were analysed by LC-MS/MS method using negative mode electrospray ionisation, following an adaptation of a previous published method (Vanderford & Snyder 2006). Direct isotopically labelled analogues were used for nonylphenol (D4-nonylphenol), 2-phenylphenol (phenylphenol-13C6-1) and bisphenol A (D6-bisphenol A). No direct isotopically labelled compound is available for 4-tert-octylphenol and propylparaben, therefore D17-n-octylphenol was used for quantification of 4-tert-octylphenol and D6-bisphenol A was used for quantification of propylparaben.

Trimethylsilyl derivatisation and GC/MS-MS analysis

After analysis by LC-MS/MS, the same samples were processed for GC-MS/MS analysis of steroidal hormones using a previously published method (Trinh *et al.* 2011a). The physicochemical properties of the EDCs are presented in Table 2.

Analysis of microbial indicators

Refrigerated time-proportional composite sampling of the raw sewage and membrane permeate (pre-UV disinfection) was performed to assess the MBR's overall capacity to remove microbial indicators. Slanetz and Bartley Agar plates (Oxoid CM0377) were used to enumerate enterococci and incubated at 44 °C for 44 h. Brilliance agar (Oxoid CM1046) was used to enumerate both *E. coli* and total coliforms, which were incubated at 37 °C for 24 h. These

Table 2 | Physicochemical properties of the EDCs

Compound	CAS number	Formula	Molecular weight (g/mol)	Partition coefficient Log K_{ow}	Distribution coefficient Log D pH=8	pK_a
17α-Estradiol	57-91-0	$C_{18}H_{24}O_2$	272.4	4.13	4.13	10.27
17β-Estradiol	50-28-2	$C_{18}H_{24}O_2$	272.4	4.13	4.13	10.27
17α-Ethynylestradiol	57-63-6	$C_{20}H_{24}O_2$	296.4	4.52	4.52	10.24
Estriol	50-27-1	$C_{18}H_{24}O_3$	288.4	2.94	2.94	10.25
Estrone	53-16-7	$C_{18}H_{22}O_2$	270.4	3.69	3.68	10.25
Lenovorgestrel	797-63-7	$C_{21}H_{28}O_2$	312.4	Unavailable	3.37	13.09
Mestranol	72-33-3	$C_{21}H_{26}O_2$	310.4	Unavailable	4.94	13.10
Androstenedione	63-05-8	$C_{19}H_{26}O_2$	286.4	2.90	2.90	8.78
Etiocholanolone	53-42-9	$C_{19}H_{30}O_2$	290.4	3.75	3.75	15.13
Androsterone	53-41-8	$C_{19}H_{30}O_2$	290.4	3.93	3.93	15.14
Dihydrotestosterone	521-18-6	$C_{19}H_{30}O_2$	290.4	Unavailable	3.93	15.08
Testosterone	58-22-0	$C_{19}H_{28}O_2$	288.4	3.47	3.47	15.06
Bisphenol A	80-05-7	$C_{15}H_{16}O_2$	228.3	3.43	3.43	9.73
Nonylphenol	104-40-5	$C_{15}H_{24}O$	220.4	Unavailable	6.19	10.14
2-Phenylphenol	90-43-7	$C_{12}H_{10}O$	170.2	Unavailable	3.29	10.00
Propylparaben	94-13-3	$C_{10}H_{12}O_3$	180.2	Unavailable	2.70	8.23
4-Tert-octylphenol	140-66-9	$C_{14}H_{22}O$	206.3	4.93	4.93	10.15

Source: Scifinder Scholar (2011); Tadkaew *et al.* (2011).

indicators were selected because they are commonly used as surrogates for estimating the removal of pathogenic bacteria in wastewater treatment systems (Wen *et al.* 2009). SRC were enumerated using the tryptose sulphite cycloserine agar for *Clostridium perfringens* (Oxoid CM0587), and incubated anaerobically at 35 °C for 24 h. F-RNA bacteriophage were quantified using the double agar layer technique as per the method of Noble *et al.* (2004), using *E. coli* F-amp (ATCC No. 700891) as the host and MS2 bacteriophage as the positive control. SRC and F-RNA bacteriophage were included because they are widely used as surrogates for measuring the inactivation of protozoa and enteric human viruses respectively (Wen *et al.* 2009; van den Akker *et al.* 2011). All bacterial indicators measured within the permeate were quantified using membrane filtration (Standard Methods for the Examination of Water and Wastewater 1992), whereby a desired quantity of sample (typically 5, 50 and 100 mL) was filtered through a 47 mm diameter, 0.45 μm gridded filter membrane (Millipore, S-Pak, type HA). The filter membrane was then transferred onto the surface of a well-dried plate of selective agar.

RESULTS AND DISCUSSION

Endocrine disrupting chemicals

Levels of EDCs in raw sewage

Concentrations of EDCs in raw sewage are presented in Table 3. The main components of the contraceptive pill (17α-ethynylestradiol, mestranol and levonorgestrel) and the breakdown product of the chemical used in detergents and personal care products (nonylphenol) were not detected. Natural estrogenic hormones detected include 17α-estradiol, 17β-estradiol and its metabolised products estrone and estriol. The androgenic hormone, testosterone and its androgenic metabolised products androsterone, etiocholanolone, androstenedione and dihydrotestosterone were also detected. The levels of androgenic hormones detected were higher than those of estrogenic hormones, which may be due to the higher excretion rates of androgens compared to estrogens in humans (Le-Minh *et al.* 2010). Generally, the levels of steroidal hormones within the sewage were comparable to

Table 3 | Concentrations and removals of the EDCs by the MBR (samples taken in triplicate over a 5-day period)

Contaminant	Raw sewage range (mean) (ng L^{-1})	MBR permeate range (ng L^{-1})	Removal range (%)
17α-Estradiol	3.7–6.5 (5.0)	<0.5	>86.5–>92.3
17β-Estradiol	26.5–41.7 (32.6)	<0.7	>97.4
17α-Ethynylestradiol	<1.2	<0.6	n/a
Estriol	291–1,053 (574)	<1.5	>99.5
Estrone	88–173 (127)	<0.4	>99.6
Lenovorgestrel	<7.0	<3.5	n/a
Mestranol	<1.2	<0.6	n/a
Androstenedione	99–465 (216)	<2.8	>97.2
Etiocholanolone	6,884–9,162 (7,682)	<3.2	>99.9
Androsterone	2,090–2,565 (2,360)	<0.7	>99.9
Dihydrotestosterone	450–1,453 (716)	<7.5	>98.3
Testosterone	88–541 (215)	<3.0	>96.6
Bisphenol A	453–1,200 (842)	<10.0	>97.8
Nonylphenol	<1.0	<0.5	n/a
2-Phenylphenol	2,150–4,290 (3,057)	11.2–15.6	99.5–99.6
Propylparaben	2,270–5,260 (4,053)	<0.5	>99.9
4-Tert-octylphenol	2,170–8,190 (5,175)	18.0–33.8	99.2–99.6

n/a: not applicable.

values reported in previous Australian research (Coleman *et al.* 2009, 2010; Le-Minh *et al.* 2010), with the exception of testosterone and dihydrotestosterone, which were found to be one to two orders of magnitude higher in the current study. This may be due to the higher sensitivity of the analytical method used here compared to other studies (Coleman *et al.* 2009, 2010; Le-Minh *et al.* 2010).

The detected estrogenic phenolic compounds include bisphenol A, 2-phenylphenol and 4-tert-octylphenol. Bisphenol A is used to produce polycarbonate plastic and epoxy resins (Staples *et al.* 1998) and 2-phenylphenol is used as an agriculture fungicide and household disinfectant (Tumah 2005). 4-tert-octylphenol is the breakdown product of octylphenol ethoxylate, which is widely used in detergents, emulsifiers, solubilisers, wetting agents and dispersants (Staples *et al.* 1999). The level of bisphenol A detected was comparable with previous studies (Lee *et al.* 2005; Cases *et al.* 2011) while the level of 4-tert-octylphenol detected was one order of magnitude higher than values reported previously (Coleman *et al.* 2009; Cases *et al.* 2011). This may again be due to the highly sensitive method used for analysis. Literature on the level of 2-phenylphenol in raw sewage is still limited but a previous study reported similar values to those found in this study (Lee *et al.* 2005).

Propylparaben is a preservative typically found in many water-based cosmetics, such as creams, lotions and some bath products. This compound was detected at concentrations of 2,270–5,260 ng L^{-1} which is comparable with previous reported values in the raw sewage (Regueiro *et al.* 2009).

Removal of EDCs by the MBR

The percentage removal of the EDCs investigated are presented in Table 3. The results show that the MBR removed the studied EDCs effectively with most removal rates being close to 100%. 2-phenylphenol and 4-tert-octylphenol were the only chemicals detected in the MBR permeate at concentrations of 11.2–15.6 and 18.0–33.8 ng L^{-1} respectively. However, removal efficiencies were still high (99.2–99.6%). The concentrations of 2-phenylphenol and 4-tert-octylphenol were two to three orders of magnitude lower than Australian guideline values for water recycling (Australian Guidelines for Water Recycling 2008). All other studied EDCs were undetectable in the MBR permeate. This indicates that MBRs are extremely promising for water reuse applications in terms of removal of EDCs.

These excellent removal efficiencies of steroidal hormones are consistent with previous studies on MBRs (Coleman *et al.* 2009; Le-Minh *et al.* 2010). The mechanisms responsible for removing these steroidal hormones in MBR plants typically include a combination of particulate adsorption and biodegradation (Cirja *et al.* 2008; Abegglen *et al.* 2009; Coleman *et al.* 2009). The estrogenic hormones are classified as having moderate hydrophobicity to high hydrophobicity with log $D_{pH=8}$ from 2.9 to 4.9 therefore having medium to high sorption potential to biomass (Rogers 1996; Cirja *et al.* 2008). Information on fate and removal of androgenic hormones through treatment processes is limited compared to that of estrogenic hormones. However, the log $D_{pH=8}$ values of androgenic hormones suggesting that these compounds are moderately to highly absorbed to the biomass (Liu *et al.* 2009).

The high removal efficiencies of bisphenol A and 4-tert-octylphenol were comparable with other studies on MBRs (Coleman *et al.* 2009; Tadkaew *et al.* 2010, 2011; Cases *et al.* 2011). A previous study found high concentrations of 4-tert-cotylphenol in biomass which indicated that adsorption to biomass was the main pathway of removal for this compound (Coleman *et al.* 2009). This can be explained by its hydrophobicity with high distribution coefficient (log $D_{pH=8}$=4.93) (Tadkaew *et al.* 2011). In contrast, bisphenol A has been found at low concentration in the biomass suggesting that biodegradation is the main mechanism responsible for the removal of this compound (Chen *et al.* 2008) since bisphenol A is a moderately hydrophobic compound with log $D_{pH=8}$=3.43 (Tadkaew *et al.* 2011).

This is the first reported study to investigate the removal of 2-phenylphenol and propylparaben by MBRs, which was >99%. Limited data concerning the removal of these compounds through wastewater treatment processes are available, with the exception of Regueiro *et al.* (2009) who reported removal efficiencies above 90% by a conventional wastewater treatment process.

Microbial indicator organisms

The numbers of indicators in the raw sewage and permeate including their reductions are summarised in Table 4.

Table 4 | Densities of indigenous microbial indicators within sewage and permeate (log_{10} cfu or pfu 100 mL^{-1}) and their log_{10} reductions (n=10 samples)

Microbial indicator	Raw sewage range (mean)	MBR permeate range (mean)	Mean log_{10} removal
E. coli	6.2–7.4 (6.8)	1.1–2.3 (1.7)	5.1
Total coliforms	7.5–8.7 (8.2)	2.2–3.4 (2.9)	5.3
Enterococci	5.5–6.2 (6.0)	0–1.6 (1.0)	5.0
SRC	5.2–7.2 (5.9)	0.3–1.8 (1.3)	4.6
F-RNA phage	4.3–5.7 (4.9)	BDL (<1)	>4.9

BDL=below detection limit; cfu=colony forming units; pfu=plaque forming units.

The mean log_{10} reduction values of all microbial indicators are comparable to those reported in pilot-scale studies (Ottoson *et al.* 2006; Zhang & Farahbakhsh 2007; Marti *et al.* 2011). The log_{10} reduction of SRC (4.6 log_{10} units) was marginally lower than all vegetative bacterial indicators (5.0–5.3 log_{10} units) and may be viewed as a useful worst-case performance benchmark. Removal values for F-RNA phage reached >5.7 log_{10} units; however, a reliable estimate of their removal was not obtained because they were not detected in the permeate. The failure to detect F-RNA phage within the permeate can be attributed to a combination of: (i) poor sensitivity of the assay, which was constrained by the low sample volume (10 mL); and (ii) low density within the sewage.

CONCLUSIONS

This study provides a comprehensive overview of a full-scale package MBR's ability to remove 17 different types of EDCs and five microbial indicators. The results of chemical analysis show that MBR treatment was highly effective in removing all of the studied EDCs. Of the 17 studied EDCs, only 2-phenylphenol and 4-tert-octylphenol were detected in the MBR permeate. The removal of all microbial indicators was in the range of 4.6–5.3 log_{10} units. This study highlights the applicability of MBRs as decentralised systems for water reuse.

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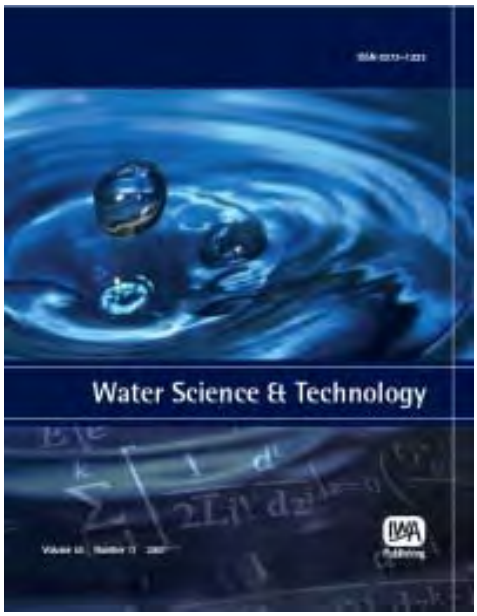

Removal of trace organic chemical contaminants by a membrane bioreactor

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ABSTRACT

Emerging wastewater treatment processes such as membrane bioreactors (MBRs) have attracted a significant amount of interest internationally due to their ability to produce high quality effluent suitable for water recycling. It is therefore important that their efficiency in removing hazardous trace organic contaminants be assessed. Accordingly, this study investigated the removal of trace organic chemical contaminants through a full-scale, package MBR in New South Wales, Australia. This study was unique in the context of MBR research because it characterised the removal of 48 trace organic chemical contaminants, which included steroidal hormones, xenoestrogens, pesticides, caffeine, pharmaceuticals and personal care products (PPCPs). Results showed that the removal of most trace organic chemical contaminants through the MBR was high (above 90%). However, amitriptyline, carbamazepine, diazepam, diclofenac, fluoxetine, gemfibrozil, omeprazole, sulphamethoxazole and trimethoprim were only partially removed through the MBR with the removal efficiencies of 24–68%. These are potential indicators for assessing MBR performance as these chemicals are usually sensitive to changes in the treatment systems. The trace organic chemical contaminants detected in the MBR permeate were 1 to 6 orders of magnitude lower than guideline values reported in the Australian Guidelines for Water Recycling. The outcomes of this study enhanced our understanding of the levels and removal of trace organic contaminants by MBRs.

Key words | decentralised treatment system, membrane bioreactor, pesticides, pharmaceuticals and personal care products, steroidal hormones

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INTRODUCTION

The presence of trace organic chemical contaminants such as steroidal hormones, xenoestrogens, pesticides, pharmaceuticals and personal care products (PPCPs) in municipal wastewater has been the subject of increasing concern throughout recent decades (Jjemba 2006; Wright-Walters & Volz 2007). Some of these trace organic chemical contaminants are known to have endocrine disrupting effects on aquatic organisms at low concentrations and others have been linked to ecological impacts due to acute and chronic toxicity mechanisms (Purdom *et al*. 1994; Hotchkiss *et al*. 2008). Investigating the removal of these trace organic chemical contaminants through treatment processes and assessing the risks associated with these chemicals to public health and the surrounding environment are particularly important for water reuse applications.

Recently, membrane bioreactors (MBRs) have attracted a significant amount of interest internationally due to their ability to produce high quality effluent over conventional activated sludge systems (Coleman *et al*. 2009; Le-Minh *et al*. 2010). MBRs comprise a combination of a conventional activated sludge process with microfiltration/ultrafiltration membrane separation, which enables these systems to produce effluents that could be recycled. In addition, this combination has the advantage of a small footprint which is favourable for small decentralised water reuse systems. However, like centralised wastewater treatment systems, there remains concern as to the fate and removal of trace chemical contaminants by MBR treatment processes.

This research investigated the removal of a comprehensive set of 48 trace organic chemical contaminants through a

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decentralised package-plant MBR treating municipal wastewater in New South Wales, Australia. This research is unique because it includes a wide range of studied trace organic chemical contaminants covering steroidal hormones, xenoestrogens, pesticides, caffeine and PPCPs.

MATERIALS AND METHODS

Description of the package MBR

Samples were collected from a decentralised full-scale MBR plant (800 equivalent persons) located in Wolumla, Bega Valley, New South Wales, Australia. A schematic diagram of the MBR is presented in Figure 1, which summarises the key components, flow direction and sample sites. The treatment process comprises of a fine screen (3 mm), a bioreactor tank, two parallel-submerged membrane modules and a medium pressure ultra-violet (UV) disinfection unit. The sludge retention time (SRT) of the bioreactor was 10–15 d, the hydraulic retention time (HRT) was 1 d and the mixed liquor suspended solids (MLSS) concentration was 7.5–8.5 g L^{-1}. The bioreactor tank was intermittently aerated in 10 minute cycles (dissolved oxygen set-point of 1 mg L^{-1}) to achieve simultaneous nitrification and denitrification. The submerged membrane modules were made of hollow fibre membranes (Koch Puron), which have an effective pore size of 0.1–0.2 μm and a surface area of 235 m^2 (each). For cleaning, scour air was applied to the membranes using a positive displacement blower and backwashing occurred every 360 seconds for a period of 60 seconds. Chemical backwashing occurred automatically every three weeks, in accordance with the manufacturer's recommendations, to maintain a transmembrane pressure of <20 kPa. The membrane unit was designed to achieve an average flux of 25 L m^{-2} h.

A medium pressure UV disinfection unit was installed after the membrane units to provide an extra barrier for removal of pathogens to ensure that high quality effluent standards are met. All of the final effluent (approximately 40 L d^{-1}) is used for irrigation. The water quality values in raw sewage and MBR permeate are presented in Table 1.

Sample collection and extraction

Daily composite aqueous samples of raw sewage (0.5 L) and MBR permeate (1 L) were taken in triplicates over a one week period in September 2010. After collection, the raw sewage was immediately filtered through 0.7 μm Millipore glass fibre prefilters. All aqueous samples were then spiked with isotopically labelled standards of trace chemicals of interest for accurate isotope dilution quantification. The samples were stored in ice and extracted onsite using solid phase extraction (SPE) within 24 hours of collection. The SPE procedure is reported in a previous publication (Trinh *et al.* 2011a).

Liquid chromatography-tandem mass spectrometry (LC/MS-MS) analysis

The concentrations of xenoestrogens, pesticides, caffeine and PPCPs in the samples were determined using isotope dilution LC-MS/MS. The target compounds were analysed by two different LC-MS/MS methods using positive mode electrospray ionisation (ESI+) and negative mode electrospray ionisation (ESI–) following an adaptation of a previous published method (Vanderford & Snyder 2006). Target compounds analysed by LC-MS/MS in this study include 36 chemicals. Direct isotopically labelled analogues were used for 35

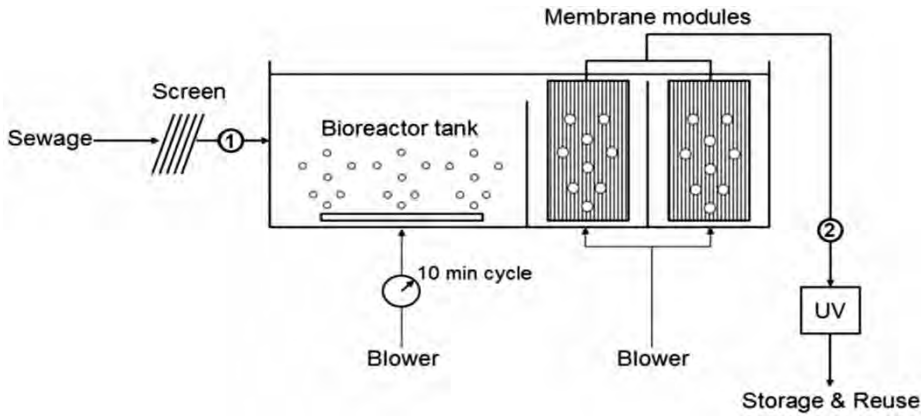

Figure 1 | Schematic diagram of the full-scale membrane bioreactor summarising the key components, flow directions and sample sites: (1) raw sewage and (2) permeate.

Table 1 | Quality of raw sewage and MBR permeate (mean values reported, $n = 6$)

Quality parameters	Raw sewage	MBR permeate
DOC (mg L^{-1})	147.1	14.9
NH_3 (mg L^{-1})	22.4	0.10
Total N (mg L^{-1})	71.6	1.9
Total P (mg L^{-1})	Unavailable[a]	2.7
pH	7.0	7.7

[a]Total P was not measured in raw sewage as the colourimetric method used onsite was not suitable for such coloured samples.

compounds including 31 PPCPs (amitriptyline, atenolol, atorvastatin, carbamazepine, diazepam, DEET (*N*,*N*-Diethyl-*meta*-toluamide), diclofenac, dilantin, enalapril, fluoxetine, norfluoxetine, gemfibrozil, hydroxyzine, ibuprofen, ketoprofen, meprobamate, metformin, naproxen, omeprazole, *o*-hydroxyatorvastatin, *p*-hydroxyatorvastatin, paracetamol, primidone, simvastatin, simvastatin hydroxy acid, sulphamethoxazole, triamterene, triclocarban, triclosan, trimethoprim, risperidone), two pesticides (atrazine, linuron), a xenoestrogen (bisphenol A) and caffeine. For another xenoestrogen propylparaben, no direct isotopically labeled analogue was able to be found, therefore quantification for this compound was based on external calibration only. $^{15}N^{13}C$-paracetamol and D5-diazepam were purchased from Cambridge Isotope Laboratories Inc., USA. D4-sulphamethoxazole, D6-trimethoprim, D5-atorvastatin, D5-*p*-hydroxyatorvastatin, D5-*o*-hydroxyatorvastatin, D4-risperidone, D5-enalapril, D6-simvastatin, D6-simvastatin hydroxy acid, D3-triclosan, D5-triamterene, D3-meprobamate and D8-hydroxyzine were purchased from Toronto Research Chemicals Inc., Canada. D6-amitriptyline, D7-atenolol, D5-atrazine, D7-bisphenol A, D9-caffeine, D10-carbamazepine, D4-DEET, D4-diclofenac, D10-dilatin, D6-gemfibrozil, D5-fluoxetine, D5-norfluoxetine, D3-ibuprofen, D3-ketoprofen, D6-linuron, D6-metformin, D3-naproxen, D3-omeprazole, D5-primidone and D4-triclocarban were purchased from Dr. Ehrenstorfer GmbH, Germany. Atorvastatin, fluoxetine, norfluoxetine, *o*-hydroxyatorvastatin, *p*-hydroxyatorvastatin, risperidone, simvastatin hydroxy acid were purchased from Toronto Research Chemicals Inc., Canada and other analytes were purchased from Sigma Aldrich. The limit of quantification (LOQ) for all analytes is 1 ng L^{-1}.

Gas chromatography-tandem mass spectrometry (GC/MS-MS) analysis

After analysis by LC-MS/MS, the same samples were processed for GC-MS/MS analysis of steroidal hormones using a previously published method (Trinh *et al.* 2011b). The studied steroidal hormones include seven estrogens and five androgens. Direct isotopically labelled analogues were used for eight hormones (17β-estradiol, estrone, 17α-ethynylestradiol, estriol, testosterone, etiocholanolone, dihydrotestosterone, androstenedione) and satisfactory isotope standards were applied for the remaining four hormones (17α-estradiol, mestranol, levonorgestrel and androsterone).

RESULTS AND DISCUSSION

Concentration of trace organic chemical contaminants in raw sewage

Steroidal hormones

The concentrations of steroidal hormones in raw sewage are presented in Figure 2. The natural estrogen, 17α-estradiol and the main components of the contraceptive pills (17α-ethynylestradiol, mestranol and levonorgestrel) were not detected. The estrogens that were detected included the natural estrogen, 17β-estradiol and its metabolised products estrone and estriol. The results show that the androgenic hormones were detected at higher concentrations than estrogenic hormones which may be due to the higher excretion rates of androgens compared with estrogens in humans (Leusch *et al.* 2006). Testosterone and its metabolised products, androsterone, etiocholanolone and dihydrotestosterone were all detected. In general, the concentrations of steroidal hormones are consistent with previous Australian studies; with the exception of testosterone and dihydrotestosterone, which were one to two orders of magnitude higher than values reported in the literature (Coleman *et al.* 2010; Le-Minh *et al.* 2010). This may be due to higher sensitivity of the analytical method used here compared with other studies (Coleman *et al.* 2009, 2010; Le-Minh *et al.* 2010).

Xenoestrogens, pesticides, caffeine and PPCPs

The concentrations of xenoestrogens, pesticides, caffeine and PPCPs that were detected in raw sewage are shown in Figure 2. Of the 36 studied chemicals, 12 were not detected in the raw sewage. These included 10 PPCPs (dilatin, enalapril, norfluoxetine, hydroxyzine, meprobamate, primidone, simvastatin, simvastatin hydroxy acid, triamterene, risperidone) and two pesticides (atrazine, linuron).

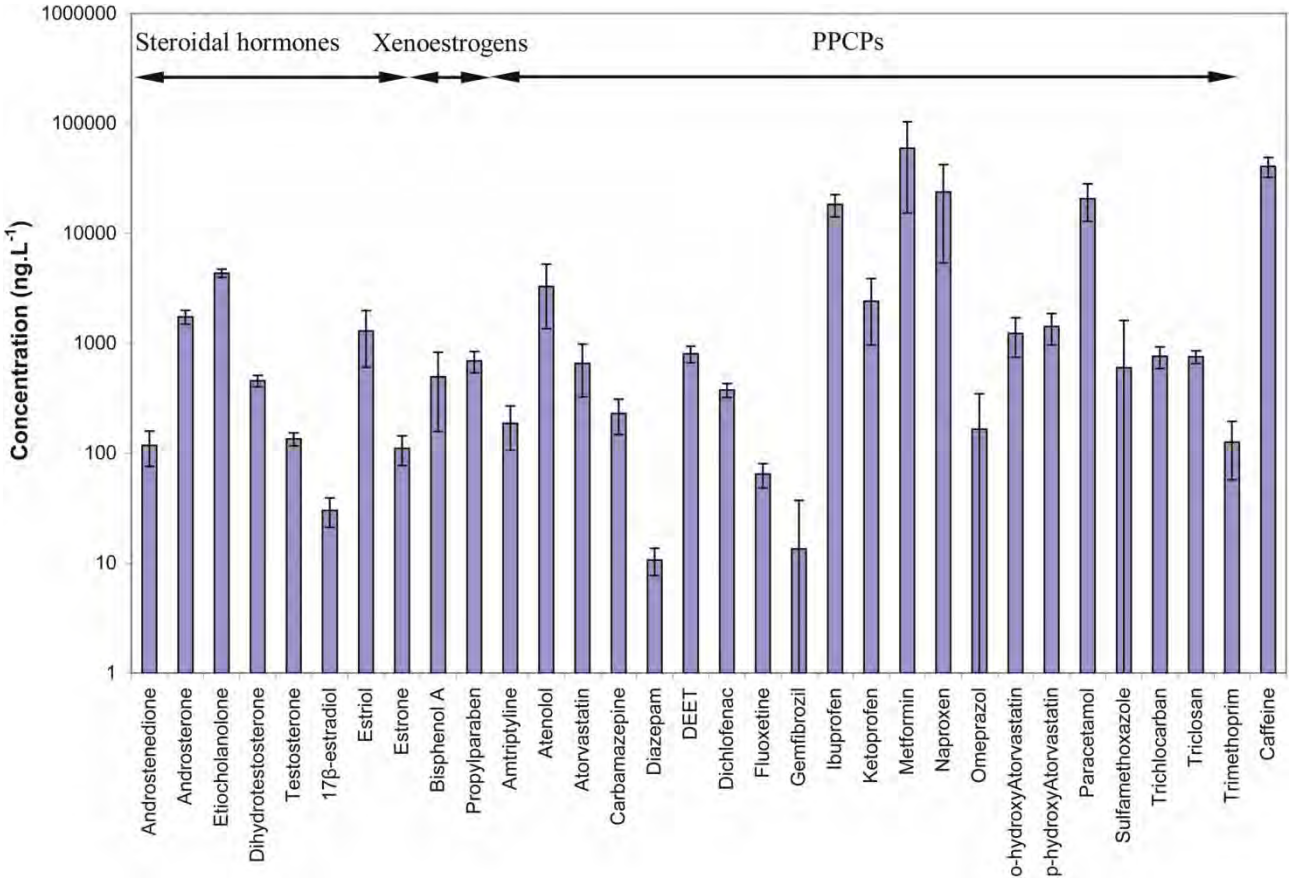

Figure 2 | Concentrations of trace organic contaminants in raw sewage.

Caffeine was found at concentrations of up to 40.5 μg L^{-1} which was four times higher than values reported in raw sewage in the literature (Kim *et al*. 2007). Pharmaceuticals including ibuprofen, metformin, naproxen and paracetamol were all detected in the raw sewage at concentrations in the range of 18.3–59.5 μg L^{-1}, which was not surprising given that these pharmaceuticals are used extensively in Australia (Khan & Ongerth 2004). The concentrations of carbamazepine, diclofenac and sulphamethoxazole are consistent with published Australian data while ketoprofen was found to be five times higher (Al-Rifai *et al*. 2007, Le-Minh *et al*. 2010). The remaining pharmaceuticals (trimethoprim, fluoxetine, omeprazole, amitriptyline, gemfibrozil and diazepam) were found in the raw sewage at concentrations of less than 100 ng L^{-1}. High day-to-day variability in concentrations of some chemicals, including gemfibrozil, omeprazole and sulphamethoxazole was observed. Such variability may be the expected result for relatively low prescription rate drugs in a very small wastewater catchment (800 equivalent persons).

Removals of trace organic chemical contaminants by the package MBR

Removal of steroidal hormones

The percentage removal of steroidal hormones through the package MBR is presented in Figure 3. Results from this study show that steroidal hormones were effectively removed by the package MBR, with the removal efficiencies in the order of 97–100%. These results are consistent with previous studies on MBRs (Kim *et al*. 2007; Spring *et al*. 2007; Lee *et al*. 2008; Coleman *et al*. 2010; Le-Minh *et al*. 2010; Trinh *et al*. 2012). The mechanisms responsible for removing these steroidal hormones in MBR plants typically include particulate adsorption and biodegradation (Ternes *et al*. 1999; Servos *et al*. 2005; Leusch *et al*. 2006; Abegglen *et al*. 2009; Coleman *et al*. 2010).

Removal of xenoestrogens, pesticides, caffeine and PPCPs

The removal of xenoestrogens, pesticides, caffeine and PPCPs through the package MBR is presented in Figure 3.

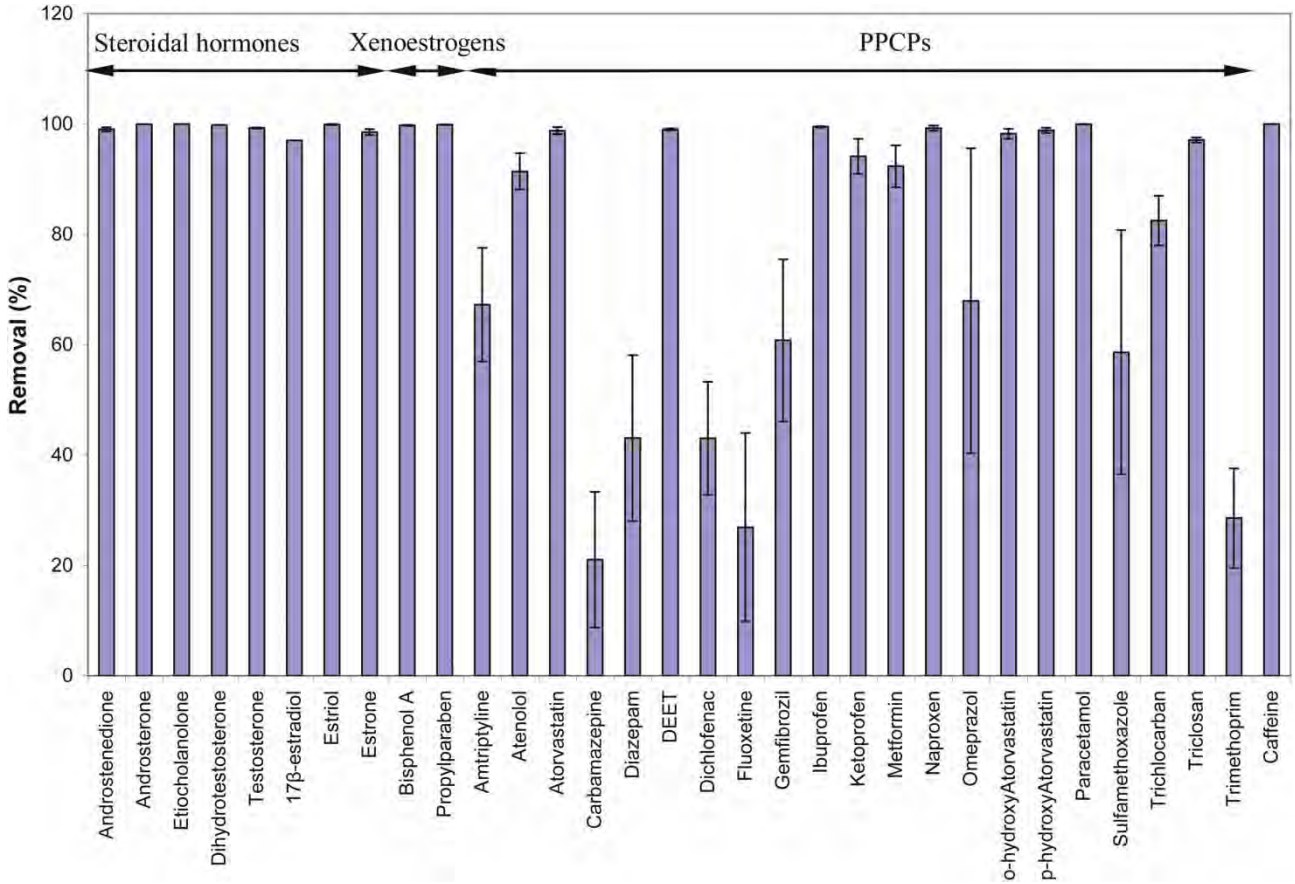

Figure 3 | Removal of trace organic contaminants through the package MBR.

Most of these chemicals were effectively removed by the package MBR. Removal efficiencies of bisphenol A, propylparaben, atenolol, atorvastatin, DEET, ibuprofen, ketoprofen, metformin, naproxen, *o*-hydroxyatorvastatin, *p*-hydroxyatorvastatin, paracetamol, triclosan and caffeine were between 90 and 100%. Previous studies on MBRs reported similar removal efficiencies for bisphenol A, ibuprofen, triclosan and caffeine (Clara *et al.* 2005; Kim *et al.* 2007; Radjenovic *et al.* 2007; Coleman *et al.* 2009; Radjenovic *et al.* 2009). These trace chemical contaminants are typically removed by MBRs via biodegradation and sorption to biomass (Cirja *et al.* 2008). Studies have shown that atenolol, ibuprofen, naproxen, paracetamol and caffeine are readily biodegradable (Abegglen *et al.* 2009; Radjenovic *et al.* 2009) while triclosan can absorb to biomass (Coleman *et al.* 2009). For bisphenol A, both sorption to biomass and biodegradation are significant (Hu *et al.* 2007). The high removal efficiencies noted here can be attributed to the high SRT and MLSS concentration in the MBR (Clara *et al.* 2005; Chen *et al.* 2008; Coleman *et al.* 2009). Amitriptyline, gemfibrozil, omeprazole and sulphamethoxazole were moderately removed by the MBR with removal efficiencies between 59 and 68%. Conversely, carbamazepine, diazepam, diclofenac, fluoxetine and trimethoprim were not effectively removed through the MBR with removal efficiencies of 24–47%. Carbamazepine, diclofenac and trimethoprim have been identified as persistent compounds that are difficult to be removed through MBRs with various removal efficiencies in the literature ranging from 0 to 50%. This is because they are not easily biodegradable and adsorb poorly to biomass (Clara *et al.* 2005; de Wever *et al.* 2007; Kim *et al.* 2007; Radjenovic *et al.* 2007; Radjenovic *et al.* 2009). The trace organic chemical contaminants that are partially removed through MBRs in normal operating conditions are potential indicators for assessing MBR performance as these chemicals are usually sensitive to changes in MBR treatment process performance (Drewes *et al.* 2008).

Despite the high removal efficiencies for most of the chemicals, estrone, caffeine and some PPCPs were detected in the MBR permeate as shown in Figure 4. The concentration of metformin in the MBR

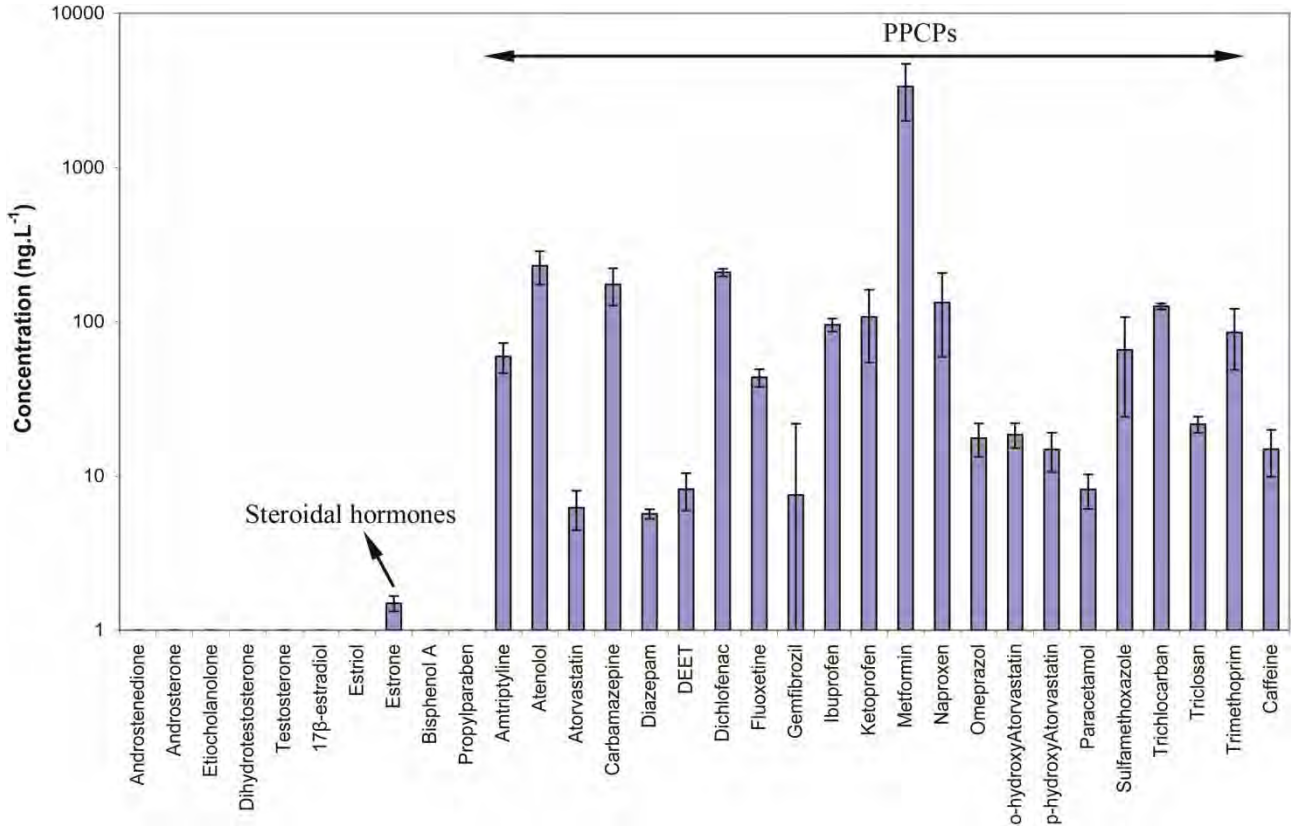

Figure 4 | Concentration of trace organic contaminants in MBR permeate.

permeate was up to 3.3 μg L^{-1}. Atenolol, carbamazepine, diclofenac, ibuprofen, ketoprofen, sulphamethoxazole, triclocarban and trimethoprim were detected in the permeate at concentrations of 66–230 ng L^{-1}. Atorvastatin, caffeine, DEET, omeprazole, *o*-hydroxyatorvastation, *p*-hydroxyatorvastation, paracetamol and triclosan were all detected in the MBR permeate at concentrations in the range of 6.2–21.6 ng L^{-1}. Estrone was the only steroidal hormone detected in the MBR permeate with a concentration of 1.5 ng L^{-1}. These results were 1–6 orders of magnitude lower than Australian guideline values for water recycling (Australian Guidelines for Water Recycling 2008).

CONCLUSIONS

The results of this study showed that the removal of most of the studied trace chemical contaminants through the package MBR was high (above 90%). However, amitriptyline, carbamazepine, diazepam, diclofenac, fluoxetine, gemfibrozil, omeprazole, sulphamethoxazole and trimethoprim were only partially removed through the MBR with removal efficiencies of 24–68%. These compounds are potential indicators for assessing the MBR performance as these chemicals are usually sensitive to changes in MBR treatment process performance. The trace organic chemical contaminants detected in the MBR permeate were 1–6 orders of magnitude lower than guideline values in the Australian Guidelines for Water Recycling. These results enhance our understanding of the levels and removal of a comprehensive list of 48 trace chemical contaminants of concern through MBR systems.

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The application of membrane bioreactors as decentralised systems for removal of endocrine disrupting chemicals and pharmaceuticals

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ABSTRACT

The concentrations of some important endocrine disrupting chemicals and pharmaceuticals after various stages of wastewater treatment were investigated. The endocrine disrupting chemicals included natural and synthetic estrogenic and androgenic steroids. The pharmaceuticals included a series of sulfonamide antibiotics and trimethoprim. The removal efficiency of a membrane bioreactor (MBR) was investigated and compared with a conventional activated sludge (CAS) system. Samples were analysed by liquid chromatography tandem mass spectrometry. Results showed that the MBR and CAS systems effectively removed steroidal estrogens and androgens, but only partially eliminated the target antibiotics from wastewater. The MBR was shown to be more effective than the CAS system which was possibly attributed to the high solid retention time and concentration of biosolids in the MBR. The results highlight the potential wider application of MBRs for the removal of trace chemical contaminants in wastewater and their potential for use as decentralised wastewater treatment systems.

Key words | antibiotics, decentralised system, endocrine disrupting chemicals, liquid chromatography-tandem mass spectrometry, membrane bioreactor

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INTRODUCTION

The presence of micropollutants including endocrine disrupting chemicals (EDCs) and pharmaceuticals in the aquatic environment is of increasing concern due to their biological impacts on aquatic species and ecology. EDCs including natural oestrogens, synthetic steroidal oestrogens, oestrogen mimics and phytoestrogens (plant oestrogens) are known to have interactions with the endrocrine systems of numerous species, and are widely accepted to contribute to the disruption of developmental and reproductive functionality in a range of biological taxa (Tyler *et al.* 1998; Rodgers-Gray *et al.* 2000) even at trace level concentrations of < 1 ng/L (Purdom *et al.* 1994; Jobling *et al.* 2004). Biologically significant levels of EDCs have been found in water bodies (Johnson & Sumpter 2001) and have been linked to adverse effects on wildlife (Tyler *et al.* 1998). Antibiotics are an important class of pharmaceuticals, for which the occurrence and fate in domestic wastewater and treated effluent are currently a subject of rapidly increasing attention to scientists and water quality regulators. Much of this interest is in regard to public health concerns over the presence of residual antibiotics in the treated effluents, toxicological impacts on the aquatic species and organisms, disturbance to microbial ecology in receiving environments and the potential for proliferation of antibiotic resistant pathogens (Hernando *et al.* 2006; Jury *et al.* 2009). Long-term effects of human exposure to most of these EDCs and antibiotics are unknown, but currently hotly debated.

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Decentralised wastewater treatment systems (or package plants) are becoming a preferred option for sewage treatment in areas where connection to a centralised public sewer networks is not possible or is economically unfeasible. Package plants are often designed to have an effective and reliable removal capability, low overall cost, minimal aesthetic impact, simple operation, maintenance and a robust plant design (Stephenson *et al.* 2000; Daude & Stephenson 2003). With such attributes, they can be suitable for the treatment of wastewaters from small residential areas, commercial blocks or industrial enterprises. Commercially available package plants include a range of different unit processes such as sequencing batch reactors, trickling filters, rotating biological contactors, submerged aerated filters and moving bed bioreactors. Recently, submerged MBRs have attracted a significant amount of interest for package plant applications due to their ability to produce high quality effluents in terms of domestic wastewater treatment over conventional CAS systems. The combination of an activated sludge process and microfiltration/ultrafiltration membrane separation in the MBR has the advantages of a small footprint and reduced sludge production (Qin *et al.* 2006). The removal rate of pathogens, organics, nitrogen contaminants, metal ions, pharmaceuticals and personal care products in MBRs are generally similar or higher than in CAS (Clara *et al.* 2005; Joss *et al.* 2005; Bernhard *et al.* 2006; Melin *et al.* 2006). However, available information about the removal of EDCs and antibiotics in small MBRs is very limited. This study aimed to evaluate the performance of an MBR package plant to treat EDCs (12 steroids) and pharmaceuticals (9 sulfonamide antibiotics and metabolites) in comparison with a full scale CAS treatment system.

MATERIALS AND METHODS

Description of the MBR

Samples for this study were collected from a full scale wastewater treatment plant (WWTP) (MidCoast Water, NSW, Australia), which incorporates a CAS process followed by UV disinfection (3800 EP). The MBR package plant (25 EP) has been added as a side-stream, treating a small proportion of the same influent sewage as the CAS system. The sludge retention time (SRT), hydraulic retention times (HRT) and mixed liquor suspended solids (MLSS) of main AS reactor are 20–25 days, 24 hours and 3,800–5,600 mg/L respectively. The MBR is operated with SRT of 40 days, HRT of 30 hours and MLSS of 6,200–7,500 mg/L. The design of the MBR is comprised of two chambers: a biological aeration tank and a chlorination tank (Figure 1). Hollow fibre membrane modules of 0.2 μm pore size are submerged inside the aeration tank to separate the solids and permeate. An electrochlorinator in the chlorination tank (3 hour retention time) is comprised of 12 volt charged platinum/ruthenium plates. The concentration of salt in the MBR permeate provides the electrolyte solution to generate free chlorine. The chlorine in the MBR effluent during the collection events was measured to be less than 0.5 mg/L, significantly lower than the target dose of 1.5–2 mg/L. This was possibly due to the low total dissolved salt (TDS) concentration of 500–600 mg/L in the MBR permeate in comparison with the recommended operating TDS of 3,000–5,000 mg/L for the electrochlorinator. The size and configuration of the pilot scale MBR were selected to represent a future potential decentralised wastewater treatment system. Median values for the MBR effluent quality parameters are presented in Table 1.

Sampling protocol

Samples (3–4 L each) were taken in triplicates ($n = 3$) from the MBR and main CAS plants during three different periods (July, November and January). In each period, grab samples were collected during the hours of expected

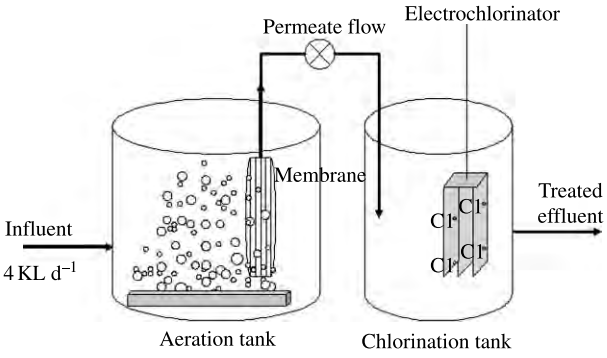

Figure 1 | Schematic diagram of MBR package plant.

Table 1 | Quality of the raw sewage and MBR final effluent

Quality parameters	Median values of incoming influent	Median values of MBR effluent
BOD (mg/L)	280	2
Fecal coli. (cfu/100 mL)	–	9
TSS (mg/L)	196	2
VSS (mg/L)	181	–
Colour (CU)	–	31.5
NH_3 (mg/L)	49	0.15
NO_3^- (mg/L)	1	3.5
Total N (mg/L)	–	16.5
Total P (mg/L)	–	6.8
pH	7.1	7.5

morning peak flows. Collected samples included raw influent ('Raw'), effluent in the MBR chlorination tank ('MBR-Cl'), effluent from the secondary sedimentation tank of the CAS ('CAS') and secondary effluent after UV disinfection ('CAS-UV'). After collection, samples were stored on ice during their transportation to the laboratory. Within 48 hours after collection, samples were extracted for the analysis of EDCs and antibiotics. A diurnal sampling study was also carried out later in Feburary, in which samples from influent, MBR effluent (electrochlorinator shut off) and CAS effluent (before UV disinfection) was collected every 2 hours in triplicate over a 24-hour period. During the diurnal sampling study, samples were extracted on site immediately after collection to minimise any variation in biological degradation, which could occur during storage and transport.

Sample preparation

All samples were filtered through glass fibre filter paper (Milipore 0.7 μm pore size), acidified to pH 3.5 with 1 M H_2SO_4 and then spiked with isotopically labelled standards of steroids and antibiotics as surrogate standards before extracting by SPE. During SPE, each 1 L filtered sample replicate was loaded in hydrophilic-lipophilic balance cartridges (HLB) from Oasis, which had been conditioned with 2 mL MilliQ® water, 2 mL methanol, 2 mL methanol 1% NH_4OH and 2 mL of pH 3.5 MilliQ® water. The flow rate was maintained at less than 10 mL/min at all times. The cartridges were dried under a gentle nitrogen flow for 30 min and then eluted using 2 × 4 mL methanol into borosilicate glass culture tubes. Each sample eluent was centrifugally dried down to approximately 100 μL in a vacuum chamber at 35°C. The eluent was reconstituted back to 1 mL volume with a methanol/MillQ® water (30:70 v/v) solution before analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS).

LC-MS/MS analysis

The concentrations of steroids and antibiotics in the wastewater samples were determined by isotope dilution LC-MS/MS. The analytical instrument includes the Agilent series 1200 LC system coupled with an Applied Biosystems QTrap API 4000 mass spectrometer. LC separation was carried out with a Luna C18, 5 μm, 150 mm × 4.6 mm, 100A column with a security guard cartridge C18, 5 μm, 4 mm × 3 mm, 100A (Biolab). Mobile phases were HPLC grade methanol and MilliQ® water, both containing 0.1% formic acid. Target compounds in this study include five oestrogenic hormones (17-α ethynyloestradiol (EE2), 17-α oestradiol (α-E2), 17-β oestradiol (E2), oestriol (E3), oestrone (E1)); seven androgenic hormones (androstenedione (An), androsterone (A), etiocholanone (E), dihydrotestosterone (DHT), testosterone (Te), testosterone propionate (TP) and trenbolone(Tr)); seven antibiotics (sulfadiazine (SDZ), sulfathiazole (STZ), sulfamerazine (SMR), sulfamethazine (SMT), sulfamethoxazole (SMX), sulfapyridine (SPR), trimethoprim (TRI)) and two metabolites (N_4-acetyl sulfamethazine (aceSMT), N_4-acetyl sulfamethoxazole (aceSMX). Steroids and antibiotics were analysed by two different LC-MS/MS methods using positive mode atmospheric pressure chemical ionisation (APCI +) and positive mode electrospray ionisation (ESI +) respectively. Isotope labelled analogues of the steroids and antibiotics including E-d2, EE2-d4, E2l-d4, E1-d4, DHT-d3, An-d3, T-d2, SDZ-d4, STZ-d4, SMR-d4, SMT-d4, SMX-d4, TRI-d9, aceSMT-d4 and aceSMX-d5 were used as surrogate standards to correct matrix effects, sample processing and instrumental variations. Method detection limits (MDLs) in wastewater influent and effluent matrices were determined based on the S/N ratio of 3. Method quantitation limits (MQLs) were conservatively chosen to

be 3 times MDLs, ranging from 5–30 ng/L for steroids and 1–5 ng/L for antibiotics. Method recoveries in different sample matrices were determined by spiking 10 and 200 ng/L stock solutions of target compounds into the matrix solutions, measured to be between 81–106%. Quantification was performed using the Applied Biosystems Analyst 1.5 software.

RESULTS AND DISCUSSION

The measured concentrations of steroids and antibiotics in wastewater influent and effluent throughout different treatment stages during the 3 grab-sampling periods and diurnal sampling event are presented in Table 2. The compounds TP, Tr, Te, DHT, SDZ, SMR, SMT, STZ and aceSMT are not shown since they were not detected in any samples. Removal efficiencies by MBR and CAS were calculated from the average concentrations using the diurnal samples while the removal efficiencies for other treatment processes were obtained from the measured concentrations of samples in three early periods, also presented in Table 2.

The oestrogenic steroids E1, E2, α-E2, E3 and EE2 were found in raw sewage at concentrations up to 491 ng/L. The oestrogens E1, E2, α-E2, E3 are naturally excreted by humans in the forms of either inactive glucuronide or sulphate conjugates; however, these conjugates can be rapidly cleaved into their active steroidal parent compounds by enzymes. While E1 was detected in all collected influent samples, other oestrogens found only intermittently. The less frequent presence of E2 and α-E2 compared to E1 in raw sewage was possibly due to the rapid transformation of E2 to E1 (Ternes *et al.* 1999; Servos *et al.* 2005). Other previous studies have also reported that E2 was not often detected in domestic wastewater (Lee *et al.* 2005; Tan *et al.* 2008) or else found in the low ng/L range (Hu *et al.* 2007). EE2, the main active ingredient of the contraceptive pill, was only found in raw sewage in one sampling event at a concentration of 125 ng/L, possibly reflecting the non-continuous excretion pattern of this pharmaceutical by people in this very small wastewater catchment. The levels of E3 found in this study (up to 491 ng/L) are comparable with several previous studies (Choi *et al.* 2007). Some androgenic steroids like TP, Tr, and DHT were below analytical detection limits in all samples. The synthetic hormonal growth promoters for beef cattle, TP and Tr were not expected to occur in domestic wastewater and thus their measured absence was not surprising. The testosterone metabolite DHT was also not observed and a detailed literature search showed no previous studies reporting the presence of DHT in wastewaters. By contrast, A and E were detected in all raw samples at very high concentrations (up to 2.02 μg/L and 3.52 μg/L respectively). The presence of androgens in much higher concentrations than oestrogens in wastewater was anticipated because of their higher excretion rate by humans (Choi *et al.* 2007). Other androgens including An and Te were also detected in raw samples, but less frequently and in lower concentrations. This was possibly due to the fact that An is often excreted in urine as A and E while Te is excreted unchanged but can be quickly metabolised into 5α- and β-androstanediol by enzymes (Uralets & Gillette 1999). No steroids were detected in the effluent of either CAS or MBR except for a single case in July in which E2 was not detected in the influent but found at 20 ng/L in AS effluent samples. It is possible that due to its high lipophilic property, E2 was trapped within lipids in the influent (Carballa *et al.* 2004) and released back into the aqueous phase during biological treatment. The variation in grab samples may also be attributed to this observation since the concentration of E2 is close to method detection limits. However, no E2 was detected after the CAS effluent samples were disinfected by UV radiation. A previous study by Zhang & Zhou (2008) reported that E1 and E2 are prone to UV photodegradation and the complete elimination of these EDCs in wastewater effluent was achieved after 30 min illumination in UV reactor.

For antibiotics, it can be seen from Table 2 that TRI, SMX and SPR were detected at all stages during the wastewater treatment processes while SDZ, STZ, SMR and SMT were not found in any samples. The concentrations for TRI and SMX in wastewater influent (498–853 ng/L and 201–1,740 ng/L respectively) were quite similar to those reported in a previous Australian study (Watkinson *et al.* 2007). The common occurrences of TRI and SMX in domestic wastewater was somewhat expected due to their extensive use in Australia (Khan & Ongerth 2005).

Table 2 | Average concentrations (ng/L) of steroids and antibiotics in domestic wastewater treatment and their removal (percentage) by MBR and CAS (non-detected compounds are presented as < (less than) MQL)

Concentration (ng/L)		Steroids									Antibiotics			
Events	**Types**	**E3**	**An**	**E**	**A**	**E1**	**E2**	**α-E2**	**EE2**	**Te**	**TRI**	**SMX**	**SPR**	**Ace SMX**
Jul ($n = 3$)	Raw	<20	68	4,710	1,150	350	33	48	<20	<5	583	1,740	4,260	445
	MBR-Cl	<15	<15	<10	<10	<10	<10	<10	<10	<5	339	542	739	35
	CAS	<15	<15	<10	<10	<10	<10	<10	<10	<5	349	763	794	66
	UV	<15	<15	<10	<10	<10	<10	<10	<10	<5	316	279	377	47
Nov ($n = 3$)	Raw	359	57	2,260	932	324	25	35	125	<5	858	735	48	390
	MBR-Cl	<15	<15	<10	<10	<10	<10	<10	<10	<5	47	300	253	27
	CAS	<15	<15	<10	<10	<10	<10	<10	<10	<5	438	912	395	5
	UV	<15	<15	<10	<10	<10	<10	<10	<10	<5	331	755	382	8
Jan ($n = 3$)	Raw	<20	64	3,423	2,017	251	<10	<10	<20	20	546	201	1,307	37
	MBR-Cl	<15	<15	<10	<10	<10	<10	<10	<10	<5	9	198	375	8
	CAS	<15	<15	<10	<10	<10	20	<10	<10	<5	414	1,020	972	3
	UV	<15	<15	<10	<10	<10	<10	<10	<10	<5	309	1,280	1,080	8
Diurnal ($n = 36$)	Raw	491	64	3,518	1,799	264	17	37	<10	19	498	1,061	922	831
	MBR	<15	<15	<10	<10	<10	<10	<10	<10	<5	27	265	225	19
	CAS	<15	<15	<10	<10	15	<10	<10	<10	<5	564	357	524	5
Morning peak flow ($n = 3$)	Raw	339	35	3,640	1,743	231	39	43	<20	13	250	1,240	895	1,505
	MBR	<15	<15	<10	<10	<10	<10	<10	<10	<5	24	196	215	8
	CAS	<15	<15	<10	<10	<10	<10	<10	<10	<5	506	371	572	4
Removals (%)		**E3**	**An**	**E**	**A**	**E1**	**E2**	**α-E2**	**EE2**	**Te**	**TRI**	**SMX**	**SPR**	**Ace SMX**
MBR		>97	>77	>99	>99	>97	>41	>73	>92	>74	95	75	75	98
MBR-Cl		>96	>82	>99	>99	>97	>69	>79	>92	>75	42 to 98	1 to 69	−427 to 71	78 to 93
CAS		>97	>77	>99	>99	94	>41	>73	>92	>74	−13 to 49	−407 to 66	−696 to 43	78 to 99
CAS-UV		>96	>82	>99	>99	>97	>69	>79	>92	> 75	24 to 61	−537 to 84	−696 to 71	78 to 98

Although SPR is not prescribed for direct human treatment, high concentrations of SPR found in wastewater influent and effluent samples (up to 4,260 ng/L) could be attributed to the high consumption of sulfasalazine, an anti-inflammatory drug in Australia (Khan & Ongerth 2005). After being administered, up to 60% of sulfasalazine was metabolised and excreted in the form of SPR (Dougados 1998). The absences of SDZ, STZ, SMR and SMT were expected since these antibiotics are only used for treating infections in animals in Australia. From previous studies, STZ was reported to occur in Australian domestic wastewater, but at concentrations of 2 ng/L or lower (Watkinson *et al.* 2007). However, that result was for a much larger wastewater catchment with a significantly increased chance of agricultural run-off to the system. Consistent with the identification of parent drugs, the sulfamethoxazole metabolite (aceSMX) was observed in the municipal wastewater samples, while the sulfamethazine metabolite (aceSMT) was not.

The analytical results of diurnal samples showed that the concentrations of the steroids and antibiotics in influent samples varied significantly while those in effluent samples were relatively stable during a day (results not presented here). The first phenomenon reflects the temporal variation in water use leading to variable degrees of dilution while the later implies the mixing effect within treatment systems of the long HRT (more than 1 day). For most naturally excreted steroids, average concentrations in influents from the diurnal samples ($n = 36$) were relatively similar to the concentrations in influents of the samples collected during the morning peak flow period on a single day, but quite varying for antibiotics (Table 2). This is expected since the significant load of naturally excreted steroids entering WWTP would be from the full number of domestic households in the morning (i.e. morning peak flow), while load of antibiotics and synthetic steroids entering the wastewater stream were heavily influenced by the variable drug consumption pattern within the small community.

Removal efficiency of steroids by MBR and CAS were from 41% to > 99%, resulting in the concentrations below MQL in effluents of the two biological treatment processes. This result is consistent with previous studies (Braga *et al.* 2005; Leusch *et al.* 2006; Coleman *et al.* 2008), which have showed secondary treatment to be an effective means for removing androgenic and estrogenic steroids from wastewater with removal efficiencies from 85% to up to > 99%. The very high removal of steroids in the MBR demonstrates the comparable performance of the pilot scale MBR with respect to the full scale CAS for effectively eliminating steroidal compounds in wastewater.

Concerning antibiotics, SMX was removed up to 66% by CAS treatment. Similar removal of this substance during biological treatment has previously been reported (Carballa *et al.* 2004). Negative removal was also observed for SMX and SPR after CAS processes and UV treatment in Nov and Jan sampling events, possibly due to retransformation of N4-acetyl metabolites back to parent compounds during treatment process as previously described (Gobel *et al.* 2005). Several previous studies have reported negative eliminations of SMX and SPR from influents to final effluent (Karthikeyan & Meyer 2006; Gobel *et al.* 2007). Therefore, the actual removal of SMX and SPR should be determined based on the removal of total sulfonamide antibiotics and their metabolites if analytical results of the metabolites are available. The removals of (SMX + aceSMX), SPR and TRI in CAS were up to 81%, 43% and 49% respectively, indicating the incomplete elimination of these antibiotics by CAS. A previous investigation by Gobel *et al.* (2007) reported that removal rates for these antibiotics in CAS significantly vary from negative elimination of − 107% to 76%. UV disinfection did not show any significant effect on the elimination of these antibiotics from the final effluent. This insignificant removal by UV radiation is consistent with the fact that these antibiotics do not strongly absorb radiation at 254 nm; typical UV disinfection dosages are not sufficient for photochemical oxidation; and high concentrations of organic materials in wastewater often reduce the effective dose for UV photolysis (Adams *et al.* 2002).

The analytical results in this study demonstrated that the pilot scale MBR removed 75%–95% of antibiotics from final effluent, similar to the removal rate achieved by an MBR reported in a previous study (Gobel *et al.* 2007). The electrochlorinator added to MBR to disinfect permeate showed no noticeable improvement in antibiotics' removals, possibly due to the low free chlorine dose (less than 0.5 mg/L). This observation may imply a potential problem with disinfection and the addition of salt to MBR

membrane permeate could be necessary to increase the free chlorine production by electrochlorinator and maintain sufficient disinfection dose. Some previous studies have shown that the treatment for these antibiotics in surface water by chlorination is only effective at chlorine dose of higher than 1 mg/L and with 24 hr contact time (Adams *et al.* 2002; Chamberlain & Adams 2006; Gibs *et al.* 2007). Despite the incomplete elimination, the MBR performance was observed to be better than the CAS system for removing antibiotics from wastewater. The high sludge retention time and high concentration of biosolids in the MBR are thought to be the reasons for the better performance since these factors can have an effect on certain wastewater and sludge characteristics as well as biodiversity of microbial flora present in the reactor (Gobel *et al.* 2007).

The results have demonstrated that MBR is comparable with -or more effective than- CAS in term of eliminating endocrine disrupting chemicals and antibiotics in wastewater. Therefore, the potential application of an MBR system, in a similar scale to that investigated in this study, for decentralised treatment of sewage is highly encouraging. In addition, it is anticipated that the quality of the pilot scale MBR effluent may be suitable as a unit process for yet-to-be-identified non-potable reuse applications, providing more sustainable alternatives to water management practices.

CONCLUSIONS

The steroid oestrogens E1, E2, a-E2, E3 and EE2 were detected in raw sewage at concentrations consistent with previous studies. These compounds were commonly found in sewage due to human excretion. The steroid androgens A and E were found at very high concentrations in raw sewage whereas An and Te were detected at lower concentrations. The higher concentrations of androgens compared to oestrogens were attributed to the higher excretion rates for androgens compared to oestrogens by humans. The antibiotics TRI, SMX and SPR were detected at all stages of the treatment system while SDZ, STZ, SMR and SMT were not found in any sample. Excellent removal rates were observed for all oestrogens and androgens in the MBR and CAS systems, reflected by their concentrations below MQLs in both effluents. Removal rates of 75–95% for the antibiotics were observed for the MBR system and were much higher than those of the CAS system. This was attributed to the high sludge retention time and concentration of biosolids in the MBR. The findings of this study highlight the potential of MBR systems for the removal of trace chemical contaminants and are highly encouraging from the point of view of implementing these systems as 'package plant' units for the decentralised treatment of effluent and the potential application of the treatment effluent for alternative water management practices such as water reuse.

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FATE OF ENDOCRINE DISRUPTING CHEMICALS DURING WASTEWATER TREATMENT BY A MEMBRANE BIOREACTOR

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ABSTRACT

This study provides a comprehensive insight into the levels and fate of 14 endocrine disrupting chemicals (EDCs) through a package membrane bioreactor (MBR) plant treating municipal wastewater in New South Wales, Australia. Results showed that the MBR consistently achieved very high removal of the EDCs within the range of 94.6 to >99.9%. A mass-balance demonstrated that sorption to biomass was the main removal mechanism for 17β-estradiol, while biodegradation was responsible for the removal of the remaining 9 detected EDCs. This knowledge can be used to optimise the performance of MBRs in removing EDCs to achieve the best possible effluent quality for water reuse applications.

INTRODUCTION

Throughout the last decade, membrane bioreactors (MBRs) have emerged as an important technology for water recycling as they are capable of transforming wastewater to high quality effluent suitable for various reuse applications (Yang et al., 2009). Recently, interest in the ability of MBRs to eliminate trace organic contaminants such as endocrine disrupting chemicals (EDCs) has increased – particularly for decentralised systems in regional water reclamation schemes (e.g. direct or indirect potable reuse) (Le-Minh et al., 2010; Trinh et al., 2011a). The removal mechanisms for EDCs through MBRs are complex and include biodegradation/transformation, sorption to biomass, volatilisation and physical retention by the membrane (Stevens-Garmon et al., 2011; Trinh et al., 2011a). Given that the molecular weight cut off for ultra-filtration MBR membranes is about 100-200 kDa, they are not expected to retain EDCs, unless EDCs adsorb to larger particles (de Wever et al., 2007). In addition, the low Henry's constant for the targeted EDCs in this study ($H < 10^{-5}$) suggests that volatilisation is an insignificant removal mechanism for these compounds (Stevens-Garmon et al., 2011; US EPA, 2011). Therefore, biodegradation/transformation and sorption to biomass are the two most important removal pathways for these EDCs. Biodegradation/transformation are grouped together since it is often difficult to distinguish between processes of chemically or biologically mediated transformation or degradation processes. This is largely due to current analytical limitations for the analysis of metabolites and other transformation products (Trinh et al., 2011a).

In most of the previous studies (Le-Minh et al., 2010; Trinh et al., 2011b), analysis of EDCs has been conducted in aqueous phase solely, therefore the removal by transformation/biodegradation or by adsorption to biomass cannot be distinguished. To better understand the fate and removal mechanisms of EDCs through MBRs, both aqueous and solid phases of the MBR need to be investigated.

The aim of this study was to investigate the fate and removal of 14 EDCs through a full-scale, package MBR plant treating municipal wastewater in New South Wales, Australia. Both aqueous (influent and effluent) and biomass samples were analysed. A full mass balance was calculated to estimate the contribution of biodegradation/transformation and sorption to biomass to the overall removal of the EDCs by the MBR. The 14 target EDCs in this study include 12 steroidal hormones (17α-estradiol, 17β-estradiol, estrone, estriol, 17α-ethynylestradiol, mestranol, levonergestrel, androstenedione, etiocholanolone, androsterone, dihydrotestosterone, testosterone) and 2 other widely used oestrogenic chemicals (bisphenol A, propylparaben).

METHODOLOGY

Description of the package MBR

Samples were collected from a full-scale package MBR plant (800 equivalent persons) located in Wolumla, Bega Valley, New South Wales, Australia. The treatment process comprised of a fine screen (3 mm), a bioreactor tank, two parallel-submerged membrane modules and a medium pressure ultra-violet (UV) disinfection unit. The sludge retention time (SRT) of the bioreactor was 10-15 days, the hydraulic retention time (HRT) was 1 day and the mixed liquor suspended solids (MLSS) concentration was 7.5-8.5 g/L. The bioreactor tank was operated with a 10 minute cyclic on/off aeration pattern (dissolved oxygen (DO) set-point of 1 mg/L). The submerged membrane modules comprised of hollow fibre membranes (Koch Puron) which have an effective pore size of 0.1–0.2 µm. The final effluent was used for irrigation. The mean water quality values in the raw sewage and MBR permeate are presented in Table 1.

Table 1: Quality of Raw Sewage and MBR Permeate (mean values reported, n=6)

Quality parameters	Raw sewage	MBR permeate
DOC (mg/L)	147	14.9
NH_3 (mg/L)	22.4	0.1
Total N (mg/L)	71.6	1.9
Total P (mg/L)	Unavailable	2.7
pH	7.0	7.7

Sample collection and preparation

Daily composite aqueous samples of raw sewage (0.5 L), MBR permeate (1 L) and grab samples of mixed liquor (0.5 L) were taken in triplicate over a 6-day-period in September 2010 (giving a total of 18 raw sewage samples, 18 MBR permeate samples and 18 mixed liquor samples). The sample preparation procedure for biomass and aqueous samples is reported in a previous publication (Trinh et al., 2011a).

Liquid chromatography tandem mass spectrometry (LC/MS-MS) analysis

The concentrations of bisphenol A and propylparaben in the samples were analysed by LC-MS/MS using negative mode electrospray ionisation (ESI-) following an adaptation of a previously published method (Vanderford and Snyder, 2006; Trinh et al., 2011c). D6-bisphenol A was used as an internal standard for quantification of both bisphenol A and propylparaben.

Gas chromatography tandem mass spectrometry (GC/MS-MS) analysis

After analysis by LC-MS/MS, the same samples were processed by GC-MS/MS for steroidal hormones using a previously published method (Trinh et al., 2011d).

Mass balance calculation

The concentrations of EDCs in raw sewage, MBR permeate and biomass were used together with the aqueous and biomass flow data to establish a mass balance for the fate of each EDC. These mass balances were calculated based on Equation 1:

Influent load = effluent load + biomass load + biodegradation load (Equation 1)

RESULTS

Concentrations of the EDCs in raw sewage, MBR permeate and biomass

The concentrations of the EDCs in raw sewage, MBR permeate and biomass are presented in Table 2. The main components of the contraceptive pill (17α-ethynylestradiol, mestranol and levonorgestrel) and the natural estrogen 17α-estradiol were not detected in raw sewage. Natural estrogens detected in raw sewage include 17β-estradiol and its metabolised products estrone and estriol. The androgen, testosterone and its metabolised products dihydrotestosterone, androstenedione, androsterone, etiocholanolone, were also detected. The levels of androgens were higher than those of estrogens which may be due to the higher excretion rates of androgens compared to estrogens in humans (Le-Minh et al., 2010). Generally, the levels of steroidal hormones within the raw sewage were comparable to values reported in previous research conducted on raw sewage in Australia (Coleman et al., 2009; Coleman et al., 2010; Le-Minh et al., 2010); with the exception of testosterone and dihydrotestosterone, which were found to be one to two orders of magnitude higher in the current study. This may be due to the higher sensitivity of the GC/MS-MS method used here compared to other studies which used a less sensitive LC-MS/MS method (Coleman et al., 2009, 2010; Le-Minh, 2010).

Table 2: Mean Concentrations and Standard Deviations of EDCs in Raw Sewage, MBR Permeate and Biomass (samples taken in triplicate over a 6-day-period)

EDCs	Raw sewage	MBR permeate	Biomass
	(ng/L)	(ng/L)	(ng/g dried biomass)
17α-Estradiol	<1	<0.5	<3.0
17β-Estradiol	30.1 (±9.0)	<0.7	40.3 (±5.5)
Estrone	110 (±33)	1.5 (±0.2)	18.3 (±4.2)
Estriol	1.29×10^3 (±680)	<1.5	<9.0
17α-Ethynylestradiol	<1.2	<0.6	<3.5
Mestranol	<1.2	<0.6	<3.5
Levonorgestrel	<7.0	<3.5	<20.0
Testosterone	55.7 (±68)	<3.0	<17.5
Dihydrotestosterone	455 (±54)	<7.5	<43.0
Androstenedione	117 (±42)	<2.8	<16.0
Androsterone	1.74×10^3 (±250)	<0.7	<4.0
Etiocholanolone	4.34×10^3 (±380)	<3.2	<18.5
Bisphenol A	493 (±340)	<10	82.2 (±28)
Propylparaben	690 (±150)	<0.5	<3

The other EDCs detected in raw sewage included bisphenol A and propylparaben. Bisphenol A is used to produce polycarbonate plastic and epoxy resins (Staples et al., 1998) and propylparaben is a preservative typically found in many water-based

cosmetics, such as creams, lotions and some bath products. The level of bisphenol A and propylparaben detected was of the same level of magnitude as values reported in previous studies (Lee et al., 2005; Regueiro et al., 2009).

17β-Estradiol, estrone and bisphenol A were detected in biomass at average concentrations of 40.3 ng/L, 18.3 ng/L and 82.2 ng/L respectively while other EDCs were undetected. Estrone was the only EDC detected in MBR permeate at a mean concentration of 1.5 ng/L.

Fate of the EDCs through MBR process

The fate of the EDCs during the MBR treatment process is presented in Figure 1. The output loads (MBR permeate, biodegradation/transformation and sorption to biomass) are expressed as proportions relative to the influent load. It is noted that the fractions of EDCs sorbed to the biomass and then biodegraded was considered as being removed via biodegradation/transformation. The fractions of EDCs removed by sorption to biomass were the fractions remaining in the waste biomass. If the the EDCs were not detected in MBR permeate or biomass, the limit of detection values were used to calculate the mass balance and the results were presented as less than (<).

Only 17β-estradiol, estrone and bisphenol A were detected in the biomass. The mass balance calculation result for 17β-estradiol (Figure 1) shows that sorption to biomass was the main removal mechanism for this compound which contributed to 76.4%, while biodegradation contributed to >21.3% of the overall removal. This high percentage of sorption to biomass is expected since 17β-estradiol is a very hydrophobic compound with log $D_{pH=8}$= 4.14 (Scifinder Scholar, 2011). Previous studies have hypothesised that sorption to biomass is an important removal mechanism for trace contaminants with log D > 3.2 (Wells, 2006; Tadkaew et al., 2011).

Estrone and bisphenol A are also hydrophobic compounds with log $D_{pH=8}$= 3.62 and log $D_{pH=8}$= 3.64, respectively (Scifinder Scholar, 2011). Figure 1 shows that sorption to biomass and biodegradation contributed to 9.5% and 88.5% respectively, of the overall removal of these compounds. A previous study reported a similar concentration of estrone in the MBR biomass (Hu et al., 2007); however, this previous study reported a large concentration variation of bisphenol A in MBR biomass ranging from 0.01-34,350 ng/g of dried biomass (Hu et al., 2007).

The remaining EDCs were not detected in biomass or MBR permeate. The results in Figure 1 show that sorption to biomass was insignificant and biodegradation was the dominant removal mechanism for estriol, propylparaben and androstenedione. The low sorption of these EDCs to biomass is in agreement with the hypothesis from a previous study by Tadkaew et al. (2011) since log $D_{pH=8}$ of these EDCs are < 3.2.
Dihydrotestosterone, androsterone and etiocholanolone were not significantly removed by sorption to biomass although they have log $D_{pH=8}$ values of 3.93. As these EDCs were not detected in both biomass and MBR permeate, no real conclusion with respect to the partitioning of these compounds can be made. However, these compounds are highly biodegradable, so the fractions that sorbed to biomass may be degraded quickly and therefore not be detected in the waste biomass.

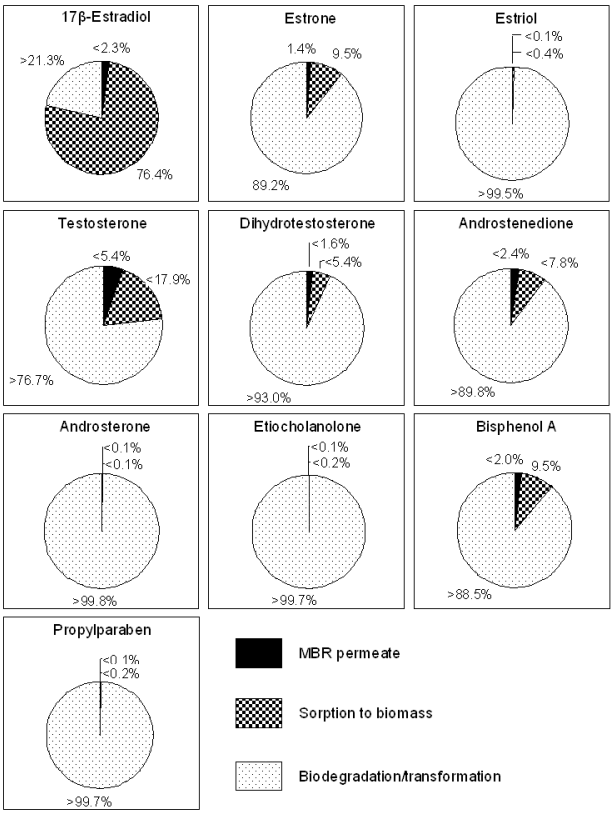

Figure 1: Relative Removal Mechanisms of EDCs through MBR

The MBR consistently achieved high rates of removal of the EDCs ranging from 94.6 to > 99.9%. These excellent removal efficiencies are consistent with previous studies on MBRs (Coleman et al., 2009; Le-Minh et al., 2010; Tadkaew et al., 2010; Cases et al., 2011; Tadkaew et al., 2011; Trinh et al., 2011a).

CONCLUSION

This study investigates the fate and levels of 14 EDCs through a package MBR treating municipal wastewater in New South Wales, Australia. The results show that the MBR treatment was highly effective in removing all of the detected EDCs, with removal efficiencies of 94.6 - >99.9%. Estrone was the only EDC detected in the MBR permeate at a mean concentration of 1.5 ng/L. A mass-balance shows that sorption to biomass was the main

removal mechanism for 17β-estradiol while biodegradation was the dominant removal mechanism for the other detected EDCs. The outcomes of this research could be used to optimise the performance of MBRs in removing EDCs to achieve the best possible effluent quality for water reuse applications.

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VALIDATION OF A FULL-SCALE MEMBRANE BIOREACTOR FOR WATER RECYCLING: CHARACTERISING PROCESS VARIABILITY

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ABSTRACT

This study characterised the removal of five indigenous microbial indicators through a full-scale membrane bioreactor. Samples were taken from the raw sewage, mixed liquor and permeate and assayed for F-specific RNA bacteriophage, sulphite reducing clostridia, enterococci, *E. coli* and total coliforms. Log_{10} removal values for vegetative bacteria were in the range of 5.0 – 5.9 log_{10} units, and for clostridia, they were marginally lower at 4.9 log_{10} units. The removal of FRNA bacteriophage was in excess of 4.9 log_{10} units. This study also used probabilistic tools to characterise process variability. The data presented in this paper can be used to better inform the risk management of membrane bioreactors that are used in water recycling schemes.

INTRODUCTION

Implementation of water recycling processes such as membrane bioreactor (MBRs) must be validated to demonstrate that the process can produce water of the required microbial quality (Department of Health Victoria 2010; NRMMC/NHMRC 2006). Validation is most often based on characterising the removal of pathogens or their surrogates (e.g. fecal coliforms, bacteriophage and spores).

MBRs are well known for their ability to remove microbial hazards found in sewage and removal values are well documented; however, most of the published data has been derived from pilot- or laboratory-scale investigations. Without complementary research at the full-scale level, it can only be assumed that removal values noted in pilot studies reflect the performance of larger-scale systems. Furthermore, few assessments effectively characterise the temporal variability in MBR performance, which is highly useful information for assessing the impacts of treated effluent on human health – especially for pathogens because their health effects are associate with single dose exposures (Haas and Trussell 1998).

Accordingly, this paper used probabilistic tools to characterise the temporal variability of a full-scale MBR's ability to eliminate microbial indicators. Information was collected about the capacity of the overall MBR process in removing microbial indicators, as well as the contribution of unit processes (e.g. the bioreactor alone). Furthermore, this study compared grab and time-proportional composite sampling methods to determine which technique is best for characterising process variability.

METHODOLOGY

MBR description and sampling

Grab samples (taken 8:00 am) and time-proportionate composite samples (taken every 2 h, between 8:00 – 18:00 h) were collected from a full-scale, decentralised MBR plant (800 equivalent persons) located in New South Wales. Samples were collected daily from the raw sewage, mixed liquor (ML) and permeate over a two week period.

The treatment process is comprised of a fine screen (3 mm), a bioreactor tank, two parallel-submerged membrane modules and a medium pressure ultra-violet disinfection unit. The sludge retention time of the bioreactor was 10-15 days, the hydraulic retention time was 24 h and the mixed liquor suspended solids (MLSS) concentration was 6.8−8.1 $g.L^{-1}$. The bioreactor tank was aerated in 10 min on/off cycles (set point of 1 $mg.L^{-1}$ DO). The submerged membrane modules are comprised of hollow fibre membranes (Koch Puron) which have a pore size of 0.1–0.2 µm. 100% of the final effluent is used for irrigation.

Microbiological analysis

Five indigenous microbial indicators were monitored onsite, which included total coliforms, *Escherichia coli* (*E. coli*), enterococci, Sulphite Reducing Clostridia (SRC), and F-RNA bacteriophage (F-RNA phage). Brilliance agar (Oxoid CM1046) was used to enumerate both *E. coli* and Total coliforms, which were incubated at 37°C for 24 h. SRC were enumerated using the Tryptose Sulphite Cycloserine Agar for Clostridium

perfringens (Oxoid CM0587), and incubated anaerobically at 35°C for 24h. FRNA bacteriophage were quantified using the double agar layer technique as per the method of Noble et al. (2004), using *E. coli* F-amp (ATCC # 700891) as the host and MS2 bacteriophage as the positive control. SRC and FRNA bacteriophage were included because they are widely used as surrogates for measuring the inactivation protozoa and enteric human viruses respectively. All bacterial indicators measured within the permeate were quantified using membrane filtration (Method 9215D, APHA 1992), whereby a desired volume of sample (typically 5, 50 and 100 ml) was filtered through a 47mm diameter, 0.45 µm gridded filter membrane (Millipore, S-Pak, type HA). The filter membrane was then transferred onto the surface of a well dried plate of selective agar.

Monte Carlo simulation

Evaluation of treatment variability was achieved by summarising the densities of microbial indicators using cumulative probability distribution analysis and Monte Carlo simulation, using the methods described by (Khan 2010). Microbial indicators were fitted to a lognormal probability distribution function (PDF) using @Risk software (Palisade Corporation, version 5.5). PDF fitting was undertaken in preparation for a Monte Carlo simulation to estimate indicator removal along the MBR treatment train. Monte Carlo simulations were also performed using @Risk software with Latin Hypercube sampling (using 10,000 iterations). This approach provides quantitative evaluation of exposure to hazards from water recycling schemes, and is viewed as 'best practice' in quantitative microbial risk assessment (QMRA) (Haas et al. 1999; Olivieri et al. 1999).

RESULTS AND DISCUSSION

Probability distribution analysis of all microbial indicators within the raw sewage showed that composite samples provided the same information on sewage variability as did the 8:00 am grab samples (e.g. Figure 1). The lack of difference between these two sampling methods was probably due to the high numbers of indicators (10^5 – 10^8 organisms 100 ml^{-1} sewage), and thus higher dilution effects (greater than that provided by diurnal changes in sewage strength) was needed to influence the collective densities of indicators within the composite samples.

Densities of *E. coli* and total coliforms within the ML mirrored that of the sewage which suggests that no inactivation can be expected to occur by the bioreactor itself (see Table 1). In contrast, the numbers of FRNA phage within the ML was significantly lower than the sewage. The decline in ML FRNA phage was parallel with earlier studies, which have shown that ML alone, or activated sludge treatment more generally, can remove bacteriophage by a factor of 0.5–1.0 log_{10} units via predation (Shang et al. 2005; Wu et al. 2010). SRC appeared to accumulate within the ML, which can largely be attributed to the ability of its spores to strongly adhere to biomass and resist predation (Wen et al. 2009).

In contrast to the sewage, probability distribution analysis of all bacterial indicators within the permeate showed that grab sampling provided more information on permeate variability than did composite sampling. Differences between the two sampling methods were most pronounced within the lower quartile region (see Figure 1). Conversely, concentrations of FRNA phage within the permeate were stable, as numbers were constantly below the detection limit of 1.0 log_{10} pfu 100 ml^{-1}. The failure to detected phage was probably due to a combination of factors: (i) phage numbers may have been too low to challenge the membrane and ii) the low sensitivity of the phage detection method, which was constrained by the low sample volume (i.e. 5 ml).

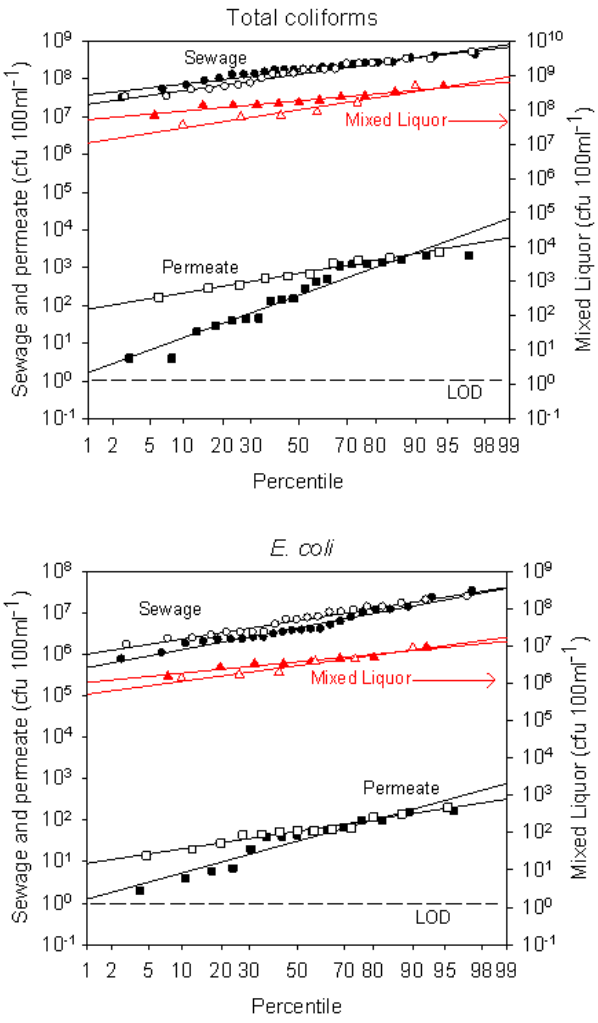

Figure 1: Example of lognormal cumulative probability distribution plots of microbial indicators. Open symbols denote time-proportional composite sampling. Closed symbols denote grab sampling taken between the hours of 8:00 – 8:30 am.

Table 1: Lognormal PDF coefficients parameters (log_{10} cfu or pfu 100 ml^{-1}).

Microbial indicator	Sewage				Mixed Liquor				Permeate				log_{10} removal			
	Grab		Composite		Grab		Composite		Grab		Composite		Grab		Composite	
	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ
FRNA Phage	4.65	0.49	4.90	0.57	4.07	0.31	3.10	0.58	<1	0	<1	0	>4.65	0.00	>4.90	0.00
SRC	5.87	0.45	5.88	0.45	7.21	0.72	6.43	0.66	0.97	0.68	1.27	0.33	4.90	0.82	4.61	0.55
E. coli	6.69	0.51	6.80	0.40	6.63	0.18	6.78	0.39	1.60	0.48	1.74	0.24	5.09	0.70	5.06	0.46
Total coliforms	8.21	0.27	8.20	0.37	8.43	0.26	8.21	0.38	2.30	0.99	2.86	0.44	5.91	1.02	5.34	0.58
enterococci	5.76	0.18	5.95	0.21	5.95	0.45	NA	NA	0.72	0.90	0.75	0.45	5.04	0.91	5.20	0.45

Monte Carlo simulations were performed using lognormal PDFs generated from the sewage and permeate data sets to estimate the overall log_{10} reduction values (LRV) (see Table 1). The mean LRVs of all microbial indicators are comparable to those reported in earlier pilot- and full-scale studies (e.g. Ottoson et al. 2006; Zhang and Farahbakhsh 2007 and Pettigrew et al 2010). The LRV of SRC was marginally lower than all vegetative bacterial indicators and may be viewed as a useful worst-case performance benchmark. Removal of FRNA phage reached >4.9 log_{10} units, however a reliable estimate of their removal was not obtained because they were not detected within permeate.

Numerically, the mean LRVs of all microbial indicators were similar for grab and composite samples (Table 1); however probability distribution plots presented in Figure 2 showed that grab sampling captured more information about the variability in MBR log removal performance. This was most likely because the removal of microorganisms by MBRs are largely a function membrane permeability, which is influenced by a wide range of operational parameters (e.g. membrane backwashing, automatic cleaning cycles, air scour, etc); all of which varied significantly between each grab sampling event. The composite samples on the other hand provided information on average MBR treatment performance.

CONCLUSIONS

This study provided information about the short-term variability of a decentralised MBR's ability to eliminate microbial indicators from municipal wastewater. Grab sampling captured more information about the variability in MBR treatment performance, which was most likely a result of cyclic changes in MBR operational parameters. In light of our findings, it is reasonable to argue that grab sampling is an acceptable strategy for assessing the variability in the load and removal of microbial indicators when validating decentralised MBRs. It is unclear whether the same findings apply to larger centralised facilities, which generally produce more stable effluent, and if the frequency distribution patterns seen here using microbial indicators mirror that of human pathogens.

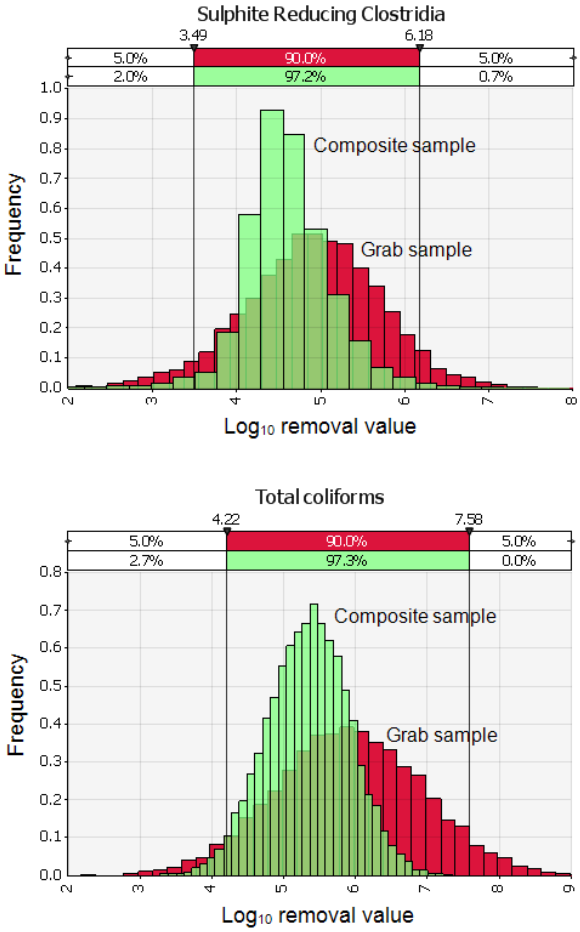

Figure 2: Example of estimated log_{10} removal PDFs for sulphite reducing clostridia and total coliforms, comparing composite and grab sampling techniques.

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IMPACTS OF 2,4-DINITROPHENOL SHOCK ON MEMBRANE BIOREACTOR PERFORMANCE

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ABSTRACT

A 2,4 dinitrophenol (DNP) shock was introduced as a single dose to the membrane bioreactor (MBR) to have a concentration in a mixed liquor of 200 $mg.L^{-1}$. DNP shock caused a significant reduction in chemical oxygen demand (COD) removal and a considerable increase in capillary suction time (CST) and transmembrane pressure (TMP). The impacts were not fully recovered 72 h after the shock. Under DNP shock conditions, the removal of moderately and very hydrophobic trace chemicals was not affected. It is possible that DNP shock inhibited the biodegradation process in the aqueous phase of the reactor but within the biomass cell there may be still biodegradation occuring. The moderately hydrophobic and very hydrophobic chemicals can adsorb to the biomass and thus these chemicals were still biodegraded. For hydrophilic chemicals, the removal of chemicals with moderate or low biodegradability was significantly reduced while the removal of easily biodegradable chemicals was only slightly affected.

INTRODUCTION

2,4 dinitrophenol (DNP) has been used commercially for a number of purposes such as a dye, a wood preservative and a pesticide (Rich and Yates, 1955). In addition, DNP is also referred to as an "uncoupler" because at low $ng.L^{-1}$ concentrations, it has been shown to considerably reduce sludge yield in activated sludge (AS), but does not significantly affect chemical oxygen demand (COD) removal rate or settleability of activated sludge (Chen et al., 2008, Rich and Yates, 1955). Thus, it is suggested to use DNP at low $ng.L^{-1}$ concentrations to reduce the sludge production of wastewater treatment plants (Chen et al., 2008, Mayhew and Stephenson, 1998). As DNP has been used widely in various applications, the chance that this chemical entering sewage system accidentally or even intentionally increases (Rich and Yates, 1955). DNP is an electron inhibitor and is very toxic. It can persist in the environment due to the presence of nitrite groups on the phenolic parent compound which can deter enzyme attack (Bruhn et al., 1987). This DNP shock experiment is selected as a representative as a shock caused by toxic chemicals.

MATERIALS AND METHODS

Based on results of previous studies on AS (Kelly et al., 2004, Henriques et al., 2007), 200 $mg.L^{-1}$ DNP was selected as a shock dose for this experiment with an estimation that there will be some visible impacts on the membrane bioreactor (MBR) performance. DNP (analytical standard) was purchased from Sigma Aldrich.

The experiment was conducted in the two identical pilot MBRs. One of the MBRs was operated at steady-state operational conditions as a control and another MBRs was subjected to DNP shock. The two MBRs were seeded with biomass from an existing MBR at a municipal sewage treatment plant (STP) in NSW. The settled raw sewage from the STP was filled in the influent tank of the MBR system daily after being passed through a 1 mm fine screen. Raw sewage from the influent tank flowed to the MBRs by gravity. There was a cistern valve to control influent flow for each reactor. Each MBR had an aerobic chamber and a membrane chamber. The aerobic chamber was intermittently aerated with 15 min cycle on/off to stimulate nitrification and denitrification. The membrane chamber was aerated continuously. The peristaltic pump continuously sucked water through the hollow fibre membrane and the effluent was stored in the effluent tank. The MBRs were operated at hydraulic retention time (HRT) of 1 d, solid retention time (SRT) of 30 d and a flux of 10 $L.m^{-2}.h^{-1}$. There were mixers in the influent tank and in each MBR to assure well mixing environment in these tanks. A computer was connected to the MBRs for controlling effluent pump flow and measuring transmembrane pressure (TMP) using Labview 2012 software. During the experiment, If TMP reaches 50 kPa (0.5 bar), membrane modules were taken out of reactors and put in buckets filled with tap water for backwashing. The backwash was carried out at twice the normal flow for a period of 20 min.

DNP was introduced as a single dose to the MBR to have a concentration in mixed liquor of 200 $mg.L^{-1}$. Raw sewage samples were taken in triplicate every day after filling the influent tank. Effluent samples from the control and the shock reactor were taken before introducing DNP (at 0 time point) and after introducing DNP at 1 h, 2h, 3h, 24h, 48h and 72h. Mixed liquor samples were taken before introducing DNP (at 0 time point) and after introducing DNP 3h, 24h, 48h and 72 h. Raw sewage and effluent samples were analysed for pH, COD and trace chemicals of interest. Mixed liquor samples were analysed for mixed liquor suspended

solids (MLSS), mixed liquor volatile suspended solids (MLVSS), capillary suction time (CST) and trace chemicals of interest. In addition, TMP is also monitored during the experiment.

COD was measured by The HACH method 8000 (reactor digestion method). pH was measured by the 5-Star portable pH meter from Thermo Scientific Orion. MLSS and MLVSS was measured followed the Standard Method for Examination of Water and Wastewater (APHA, 1995). CST was measured by a Triton Type 319 multipurpose CST. 44 trace chemicals including steroidal hormones, xenoestrongens, pesticides, pharmaceuticals and personal care products were analysed using previous gas chromatography-tandem mass spectrometry (GC-MS/MS) (Trinh et al., 2011) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Trinh et al., 2012, Vanderford and Snyder, 2006) methods

RESULTS AND DISCUSSION

Key operational parameters

Results of key operation parameters including pH, COD, MLSS, MLVSS, CST and TMP during DNP shock experiment are presented in this section. Results show that pH of MBR effluent from the shock reactor was around 6.3 to 6.8 which is similar to that of the control experiment.

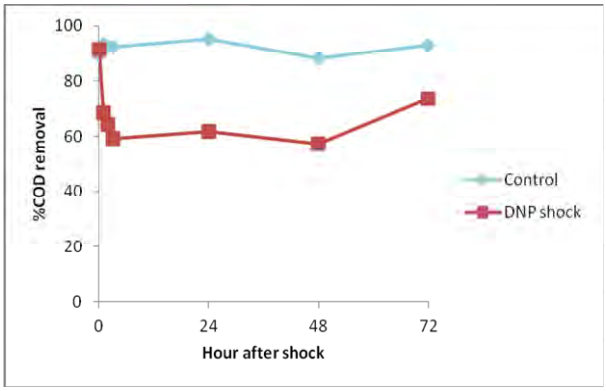

Figure 1: COD removal efficiency of the control and the DNP shock reactors.

COD removal efficiency of the control and the DNP shock reactor is presented in Figure 1. Results show that after introducing DNP, COD removal efficiency in the shock reactor immediately decreased from 92% to 52% after 1 h and reduced further to 64% and 59% after 2h and 3h respectively. The COD removal efficiency was remained at this level for 48 h and then slightly improved to 74% but still not fully recovered after 72 h. Previous studies found that at low concentration, as an uncoupler, DNP has been found to stimulate the electron transfer and respiration rate (Mitchell and Moyle, 1967, Henriques et al., 2005). However, at high concentration, the respiration stimulation effects changed to respiration inhibition (Henriques et al., 2005). At high concentration, uncouplers like DNP can harm bacterial by inhibiting bacterial metabolic process including interfering amino acid and nutrient transportation into bacteria cells (Brummett and Ordal, 1977, Decker and Lang, 1977, Bakker and Randalll, 1984, Henriques et al., 2005, Nicholas and Ordal, 1978) and hindering protein translocation into cytoplasmic membrane (Enequist et al., 1981, Geller, 1991, Henriques et al., 2005). The stress protein was found to be induced in bacteria in response to DNP shock (Bott et al., 2001). This stress protein induction was hypothesised as a cause in biochemical oxygen demand (BOD) removal efficiency reduction during biological treatment processes because of temporary redirection of energy away from growth and towards protein biosynthesis (Love and Bott, 2002). In addition, in DNP shock condition, significant potassium (K+) efflux was induced as a physical bacterial stress response mechanism. This response resulted in deflocculation of biomass (Love and Bott, 2002, Bott and Love, 2002).

Literature reported a large variation in DNP concentrations that inhibited COD removal efficiency of AS. A study on batch AS reactor fed with synthetic wastewater found that at 20 $mg.L^{-1}$ DNP, COD removal of an AS reduced from 90% to 53% (Chen et al., 2006) while another study on SBR fed with domestic wastewater reported no affect on COD removal efficiency at DNP concentration up to 107 $mg.L^{-1}$ (Henriques et al., 2007). It is possible that the domestic wastewater fed to the sequencing batch reactor (SBR) may contain a certain level of DNP and the SBR has a certain population of DNP-degrading bacteria so it can tolerate the DNP better than the previous study fed with synthetic wastewater (Jo and Silverstein, 1998). This experiment here was conducted in a MBR fed with real municipal wastewater and real biomass from an existing MBR. So the MBR may have a certain population of DNP-degradation bacteria. However, the shock dose of 200 $mg.L^{-1}$ introduced in this experiment may exceed the toleration of the biomass in the MBR, so the biodegradation process was seriously affected resulting in a significant reduction in COD removal efficiency.

MLSS and MLVSS concentration of mixed liquor of the control and the DNP shock reactors are presented in Figure 2. Results show that MLSS and MLVSS concentration in the shock reactor was lower than that of the control. This result is expected as DNP has shown to inhibit the growth of activated sludge (Chen et al., 2006, Henriques et al., 2007). The MLSS and MLVSS results show the same trends with previous study, however, the magnitude of the biomass reduction in the shock reactor is smaller than that of these studies (Chen et al., 2006, Henriques et al., 2007, Kelly et al., 2004). This may be due to the much higher MLSS concentration in MBRs in the current study compared to the MLSS concentrations in AS in previous study. Literature has reported that reactors with higher MLSS and MLVSS

concentrations provide better tolerance to DNP shock (Hess et al., 1993, Jo and Silverstein, 1998).

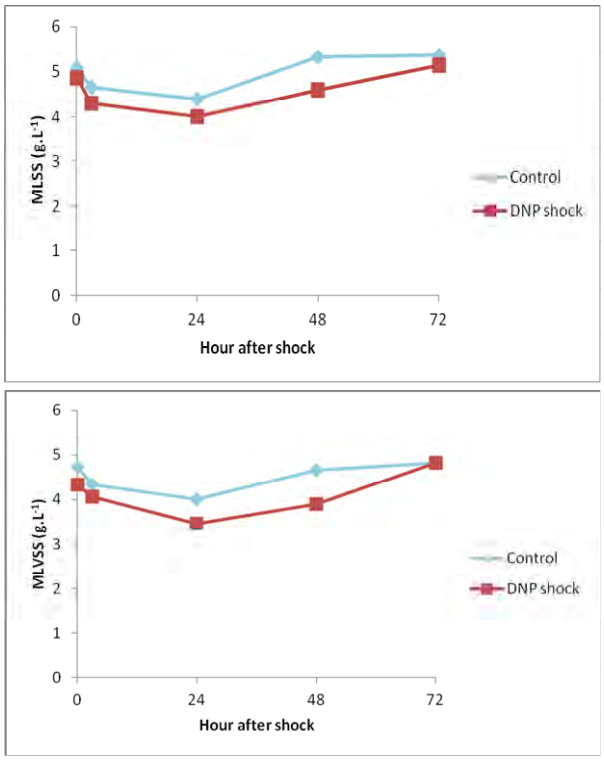

Figure 2: MLSS, MLVSS concentrations of the control and the DNP shock reactors.

CST of mixed liquor from the control and the DNP shock reactors is presented in Figure 3. Results show that CST of the DNP shock reactors was significantly higher than that of the control, which implies that filterability of the mixed liquor from the DNP shock reactor was notably reduced after introducing DNP and still not fully recovered 72 h after the shock. This result is in agreement with previous study which found that in the presence of DNP concentration higher than 5 $mg.L^{-1}$, the sludge dewatering and settling ability was reduced (Chen et al., 2006, Henriques et al., 2007). This result is consistent with the TMP result, after introducing DNP, TMP of the DNP shock reactor rapidly rose and reached 25 kpa while TMP of the control remained around 11 kpa. TMP of the DNP shock reactor stayed 14-15 kpa higher than that of the control until the end of the experiment.

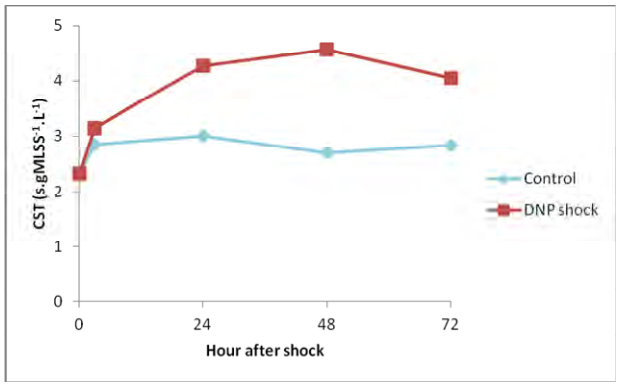

Figure 3: CST of the control and the DNP shock reactors.

Trace chemicals

Removal of trace chemicals by MBRs under control and DNP shock conditions is presented in this section. Among 44 analysed chemicals, 17α-estradiol, 17α-ethynylestradiol, mestranol, levonorgestrel, nonylphenol, 4-tert-octylphenol, diazepam, dilatin, enalapril, hydroxyzine, omeprazole, simvastatin, simvastatin hydroxy acid, atrazine, linuron, chlorpyrifos, diazinon, methotraxate were not detected in raw sewage samples. Some trace chemicals (dihydrotestosterone, androstenedione, amitriptyline, risperidone, triamterene) were only detected in a few raw sewage samples. The trace chemical results were divided in 3 groups including hydrophilic chemicals (log DpH8 < 2), moderately hydrophobic chemicals (2 ≤ log DpH8 ≤ 3. 2), very hydrophobic chemicals (log DpH8 > 3.2) (Tadkaew et al., 2011).

Figure 4 presents removal efficiency of hydrophilic trace chemicals by the control and the DNP shock reactors. Results show that DNP shock caused a negative impact on removal of most of hydrophilic trace chemicals by the MBR. Overall removal of sulfamethoxazole, ibuprofen, ketoprofen, gemfibrozil and naproxen was significantly reduced from above 80% to below 40% after the DNP shock and still remained at this level or just slightly improved 3 d after the shock. However, the removal of caffeine and paracetamol in the shock reactor only reduced slightly by the DNP shock. This may be because paracetamol and caffeine are very easily biodegradable compounds so they can withstand the shock. The easily biodegradable characteristic of paracetamol was demonstrated by it high biodegradation constant K_{biol} = 106 - 240 $L.gMLSS^{-1}.d^{-1}$ (Joss et al., 2006) compared to other hydrophilic chemicals in Table 6.1, which has Kbiol from 0.2 to 38 $L.gMLSS^{-1}.d^{-1}$ (Urase and Kikuta, 2005, Joss et al., 2006, Abegglen et al., 2009, Fernandez-Fontaina et al., 2013). K_{biol} of caffeine was not able to be found but a study on biodegradability of this compound show that caffeine is a very easy degradable compound (Lin et al., 2010).

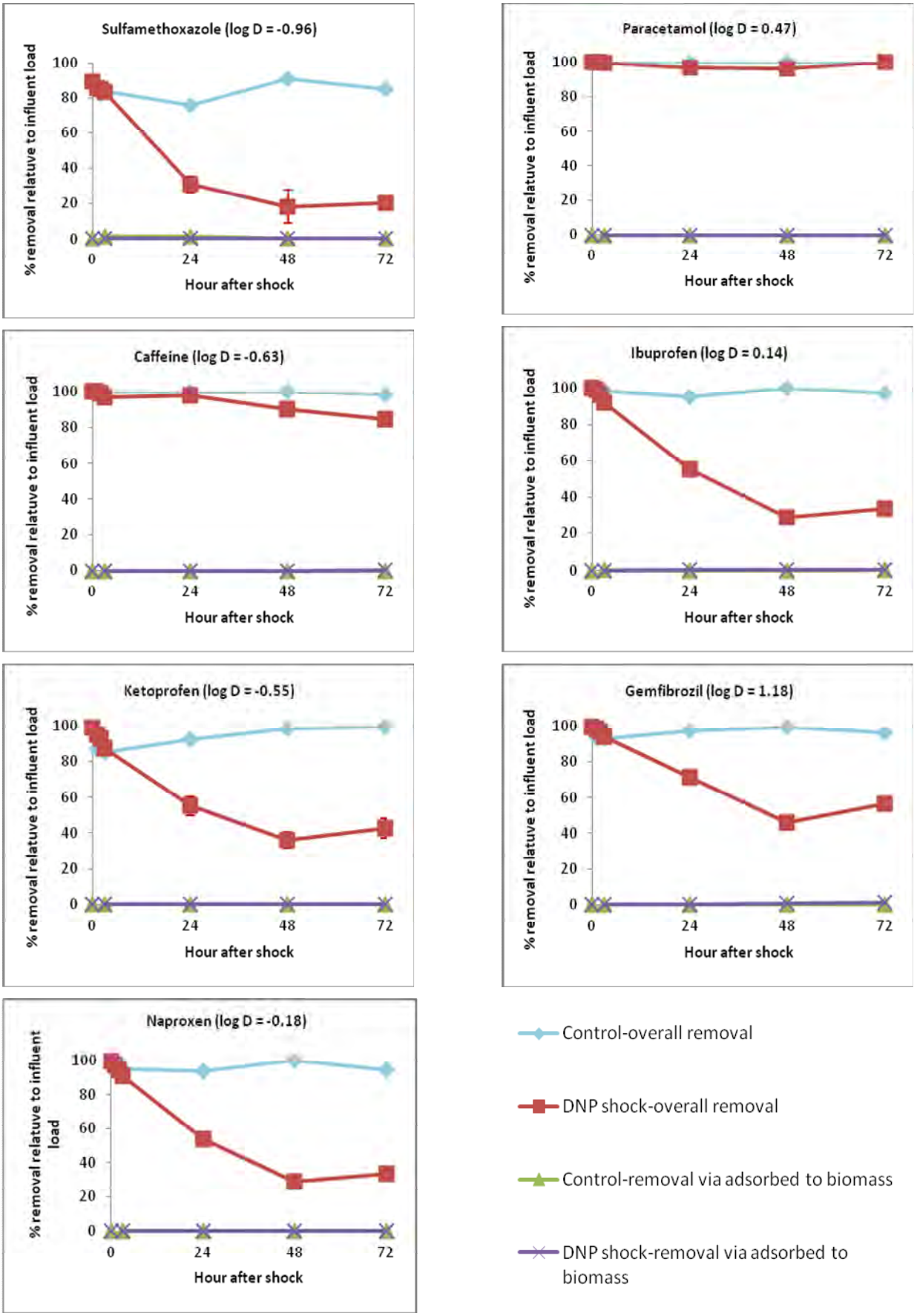

Figure 4 Removal of hydrophilic chemicals (log D_{pH8} < 2) by the control and the DNP shock reactor

Table 1 Biodegradation constant (K_{biol}) of hydrophilic chemicals.

Chemical	K_{biol} ($l.gMLSS^{-1}.d^{-1}$)	Reference
Sulfamethoxazole	0.13-0.39	(Fernandez-Fontaina et al., 2013)
	0.18-0.22	(Abegglen et al., 2009)
	0.16-0.22	(Abegglen et al., 2009)
Caffeine	n.a but study concluded that caffeine is very easy biodegradable.	(Lin et al., 2010)
Ketoprofen	0.03	(Urase and Kikuta, 2005)
Naproxen	0.65-5.45	(Fernandez-Fontaina et al., 2013)
	0.06-0.96	(Abegglen et al., 2009)
	0.4-0.8	(Joss et al., 2006)
Ibuprofen	7.8-49.3	(Fernandez-Fontaina et al., 2013)
	>3	(Abegglen et al., 2009)
	1.31-1.35	(Abegglen et al., 2009)
	9-22	(Joss et al., 2006)
Paracetamol	106-240	(Joss et al., 2006)
Gemfibrozil	0.06	(Urase and Kikuta, 2005)

Results from Figure 4 confirm that removal via adsorption to biomass was an insignificant removal mechanism for these hydrophilic chemicals and this was unchanged under DNP shock condition (< 2%). Biodegradation/transformation was the main removal mechanism for these hydrophilic chemicals and this removal mechanism was inhibited under DNP shock conditions since the biological degradation process of the reactor was inhibited.

Figure 5 presents the removal efficiency of moderately hydrophobic chemicals by the control and the DNP shock reactors. Results show that the overall removal of the moderately hydrophobic chemicals oestriol, propylparaben and testosterone was high (above 90%) and not affected by DNP shock. Removal via adsorption to biomass was an insignificant removal mechanism for these chemicals. It is noted that the concentration of testosterone in biomass was under limit of reporting so the limit of reporting value was used to calculate the mass balance, so the percentage of testosterone removed via adsorption to biomass was < 5%.

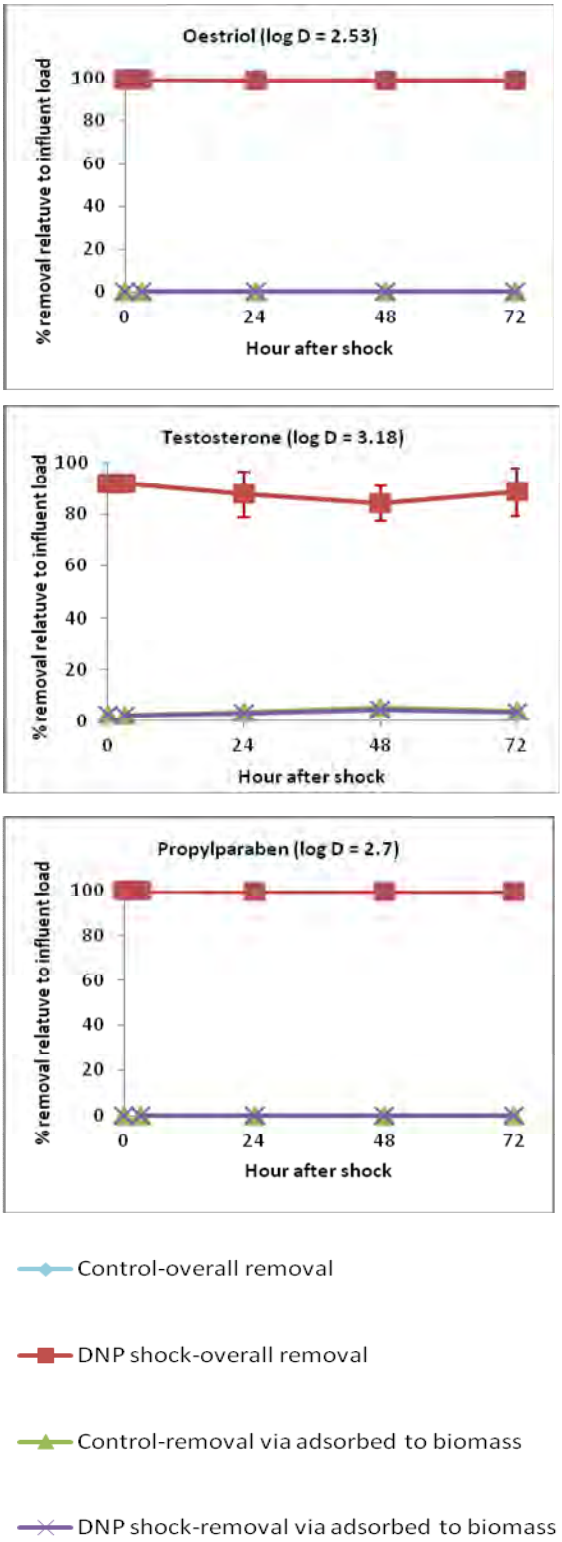

Figure 5 Removal of moderately hydrophobic chemicals ($2 \leq \log D_{pH8} \leq 3.2$) by the control and the DNP shock reactors.

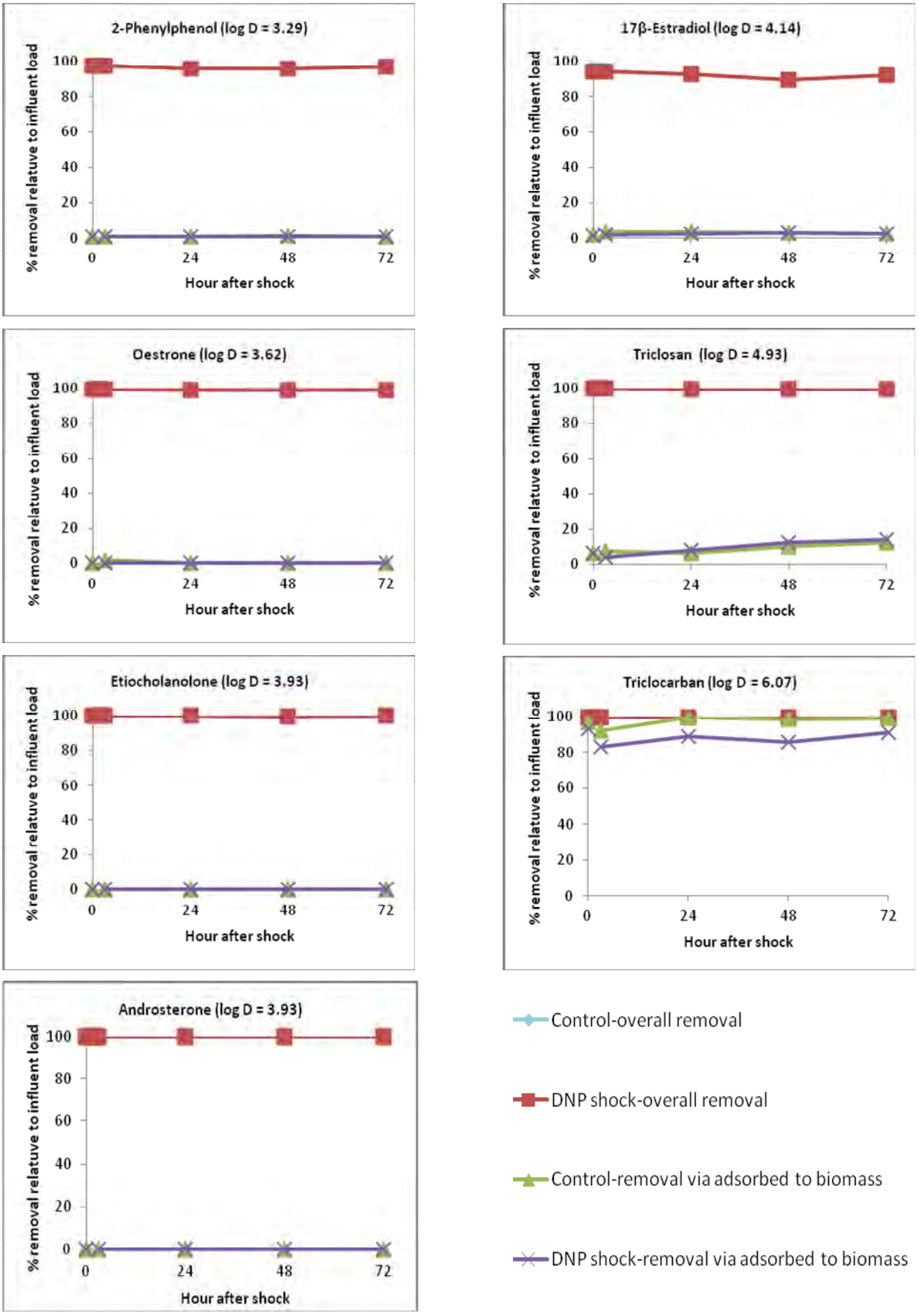

Figure 6 Removal of very hydrophobic chemicals (log D_{pH8} > 3.2) by the control and the DNP shock reactors.

Figure 6 presents the removal efficiency of very hydrophobic chemicals by the control and the DNP shock reactors. Similar to moderately hydrophobic chemicals, results show that the removal efficiency of very hydrophobic chemicals was not affected by DNP shock conditions. The overall removal of 2-phenylphenol, oestrone, etiocholanolone, androsterone, 17β-estradiol, triclosan and triclocarban was always high above 90% during the experiment. Removal via adsorption to biomass contributed up to 14% to the overall removal of triclosan while it was less than 5% for other chemicals. For triclocarban, percentage removal via adsorption to biomass was 90% and 100% of the overall removal in the shock reactor and control reactor respectively. This variation was within the variation of percentage removal via adsorption to biomass of triclocarban between the MBRs during reproducibility experiment (25%).

The results show that under DNP shock conditions, the removal very hydrophobic chemicals was not affected. It is possible that DNP shock inhibited the biodegradation process in the aqueous phase of the reactor but within the biomass cell there may be still biodegradation occurring. The moderately hydrophobic and very hydrophobic chemicals can adsorb to the biomass and thus these chemicals were still biodegraded. For hydrophilic chemicals, the removal of chemicals with moderate or low biodegradability was significantly reduced while the removal of easily biodegradable chemicals was just slightly affected.

CONCLUSION

A DNP shock at a concentration of 200 $mg.L^{-1}$ was simulated in an MBR. Results show that DNP shock condition caused a significant reduction in COD removal and a considerable increase in CST and TMP. The COD removal, CST, TMP and removal of hydrophilic chemicals were not fully recovered 72 h after the shock. Under DNP shock conditions, the removal of moderately and very hydrophobic chemicals was not affected. It is possible that DNP shock inhibited the biodegradation process in the aqueous phase of the reactor but within the biomass cell there may be still biodegradation activity. The moderately hydrophobic and very hydrophobic chemicals can adsorb to the biomass and thus these chemicals were still biodegraded. For hydrophilic chemicals, the removal of chemicals with moderate or low biodegradability was significantly reduced while the removal of easily biodegradable chemicals was slightly affected. Removal via adsorption to biomass was the main removal mechanism for triclocarban. Both removal via adsorption to biomass and biodegradation/transformation were significant removal mechanisms for triclosan. For the other chemicals, biodegradation/transformation was the dominant removal mechanism.

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Chapter 7

Impacts of hazardous events on performance of membrane bioreactors

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ABSTRACT

Membrane bioreactors (MBRs) can provide effective treatment for many wastewater contaminants including chemicals and microorganisms. Operational performances for the removal of these contaminants are most typically characterised under what are considered to be normal operating conditions. However, all MBR systems are continuously subjected to the risk of deviations in operating conditions during what have been termed 'hazardous events'. Hazardous events may include such things as sudden changes in source water composition, extreme weather events, human error and mechanical malfunctions. 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. This chapter describes potential hazardous events and their impact on MBR operation. Expected consequences are identified, along with techniques for assessing the likelihoods for some types of hazardous events. Finally, recommendations are made for the management of hazardous events through engineered redundancy and multiple barrier treatment systems.

7.1 INTRODUCTION – HAZARDOUS EVENTS IN RISK ASSESSMENT

The operational performance of any wastewater treatment system can be viewed from two distinct perspectives. The first, and most commonly considered, is the inherently variable treatment performance that may be achieved when the system is operating within a defined set of 'normal' operational conditions. The less commonly considered perspective regards the consideration of how the system may perform in the event of a disruption to normal operating conditions. In the field of risk assessment, a departure from normal operational conditions is commonly termed a 'hazardous event'.

Hazardous events that may affect the operation of wastewater treatment systems can include sudden changes in source water composition, extreme weather events, human error and mechanical malfunctions.

Since hazardous events may occur from time to time, and may have significant impacts on short-term operational performance, the characterisation of the likelihoods and consequences of these events is necessary in order to fully characterise the long-term performance of the system. Indeed, hazardous event scenarios are commonly the scenarios that present the greatest levels of risk related to final water quality. Therefore, characterisation of these events is required to properly characterise risks including those posed to the environment and to human health.

The vast majority of observed waterborne disease outbreaks in developed countries during the last few decades have been associated with hazardous events, such as unusual weather patterns, plumbing errors or treatment failures (Hrudey & Hrudey, 2007; Rizak & Hrudey, 2007). Consequently, the assessment of hazardous event scenarios has become an integral component of drinking water quality management in many countries. This approach is encapsulated within the Australian Drinking Water Guidelines (NWQMS, 2011) and the World Health Organization Guidelines for Drinking Water Quality (WHO, 2011).

Following this trend in drinking water management, the Australian Guidelines for Water Recycling (NRMMC & EPHC, 2006) have adopted a consistent approach for the qualitative incorporation of hazardous event analysis in overall system performance assessment. In this context, potential hazardous events are identified and each is allocated a qualitative measure for both its perceived 'likelihood' (Table 7.1) and its 'consequence' or impact (Table 7.2).

Table 7.1 Qualitative measures of likelihood.

Level	Descriptor	Example description
A	Rare	May occur only in exceptional circumstances. May occur once in 100 years
B	Unlikely	Could occur within 20 years or in unusual circumstances
C	Possible	Might occur or should be expected to occur within a 5- to 10-year period
D	Likely	Will probably occur within a 1- to 5-year period
E	Almost certain	Is expected to occur with a probability of multiple occurrences within a year

Source: NRMMC and EPHC (2006).

Table 7.2 Qualitative measures of consequence or impact.

Level	Descriptor	Example description
1	Insignificant	Insignificant impact or not detectable
2	Minor	Health – Minor impact for small population
		Environment – Potentially harmful to local ecosystem with local impacts contained to site
3	Moderate	Health – Minor impact for large population
		Environment – Potentially harmful to regional ecosystem with local impacts primarily contained to on-site.
4	Major	Health – Major impact for small population
		Environment – Potentially lethal to local ecosystem; predominantly local, but potential for off-site impacts
5	Catastrophic	Health – Major impacts for large population
		Environment – Potentially lethal to regional ecosystem or threatened species; widespread on-site and off-site impacts

Source: NRMMC and EPHC (2006).

Once a suitable qualitative measure of likelihood and consequences has been allocated to each identified (potential) hazardous event, a qualitative risk estimation or 'risk rating' can be applied according to the risk matrix presented in Table 7.3. The specific characterisation (e.g., low, moderate, high, very high) of risks relating to various combinations of likelihood and consequence measures may be adapted for particular systems and applications. The example given in Table 7.3 is that used in the Australian Guidelines for Water Recycling (NRMMC & EPHC, 2006) and is very similar to those presented in the Australian Drinking Water Guidelines (NWQMS, 2011) and the World Health Organization Guidelines for Drinking Water Quality (WHO, 2011).

Table 7.3 Qualitative risk estimation.

	Consequences				
Likelihood	**1-Insignificant**	**2-Minor**	**3-Moderate**	**4-Major**	**5-Catastrophic**
A Rare	Low	Low	Low	High	High
B Unlikely	Low	Low	Moderate	High	Very high
C Possible	Low	Moderate	High	Very high	Very high
D Likely	Low	Moderate	High	Very high	Very high
E Almost Certain	Low	Moderate	High	Very high	Very high

Source: NRMMC and EPHC (2006).

This risk assessment process provides a basis for managing risks and applying *preventive measures*. In the context of wastewater and recycled water management, preventative measures most commonly refer to actions, activities and processes used to prevent significant hazards from being present in final effluents or to reduce the hazards to acceptable levels. Risk should be assessed at two levels:

- Maximum (unmitigated) risk, which is risk in the absence of preventive measures – assessment of maximum risk is useful for identifying high-priority risks, determining where attention should be focused and preparing for emergencies.
- Residual risk, which is risk after consideration of existing and proposed preventive measures – assessment of residual risk provides an indication of the safety and sustainability of the system or the need for additional preventive measures.

The following sections are intended to provide insights to the potential impacts of hazardous events on the ongoing performance of membrane bioreactors. It is proposed that this information will be significant value to system managers, people responsible for system performance assessment and validation, health and environmental regulators and, ultimately, to the designers and manufacturers of future, more resilient systems.

7.2 CHARACTERISATION OF POTENTIAL HAZARDOUS EVENTS AND THEIR IMPACT ON MBR OPERATION

In order to characterise hazardous events relevant to MBR operation, it is first necessary to describe the elements of an MBR process in relation to hazard analysis terminology. The primary hazard within the MBR process is presented by the components of the mixed liquor solution of an activated sludge system. In particular, pathogenic microorganisms within the activated sludge constitute a human health hazard, while bulk parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD),

and total suspended solids (TSS) present environmental risks. The concentration of pathogenic microorganisms in activated sludge has been observed to be similar to sewage for indicator species prone to biological predation, such as *E. Coli.* However, indicators that exhibit resistance to biological degradation and are of greater diameter than the membrane pore size, such as sulphite reducing clostridia, have been shown to accumulate within the activated sludge (Marti *et al.* 2011; van den Akker *et al.* 2012). As a result the concentration factor for resistant pathogens and indicators is expected to be proportional to the MBR solid retention time (SRT).

Due to the health and environmental hazard associated with the components of the mixed liquor, hazardous event scenarios are expected to include any deviation from normal MBR operation, which would lead directly, or indirectly to 'loss of containment' of the activated sludge. Loss of containment in MBR is expected to result from membrane/module integrity failure, overflow from the bio- or membrane reactor or decrease in the treatment efficiency of the activated sludge system. A range of threats could be defined within the various treatment steps of the MBR plant (Collection, Pre-treatment, Activated Sludge Process, Membrane and Post Treatment).

7.2.1 Deviation from normal operation

7.2.1.1 Collection

Collection of MBR influent may occur downstream of primary settling or pre-screening at a municipal wastewater treatment facility or following an equalisation tank in smaller decentralised systems. Nominal feed quality will be subject to diurnal, seasonal and regional variations. Shock loadings have been also widely reported to occur within the sewage collection, generally due to upstream intermittent discharge from industry, heavy rainfall event or via ingress into aged and damaged sewer mains.

Shock loads resulting from seawater ingress (Severn, 2003), unregulated upstream discharge of industrial wastes and high loadings of non-dissolved material during storm weather flow were reported to affect nominal operation of MBRs (van Bentem *et al.* 2007). Maintenance cleaning of upstream unit operations, without appropriate isolation, can also result in shock loading of downstream processes with high concentrations of suspended solids and grease causing clogging of pre-treatment equipment and membrane units (Lazarove *et al.* 2008).

7.2.1.2 Pre-treatment

Arguably one of the most important aspects of operation of MBR, pre-treatment of sewage with fine screening (1–3 mm) with the possible addition of micro sieving (down to 250 μm), grit and grease removal is essential to preserve the integrity of downstream membranes. Bypass of screens due to seal and screen failure or even deliberate screen removal has been reported and can increase the likelihood of membrane damage by foreign materials (metal shavings, fibrous rag material, leaves, etc.).

Failure of fine screening caused accumulation of solids and grit in the membrane compartment leading to increased membrane cartridge damage and replacement rate; up to 50% of the inventory reported by Nishimori *et al.* (2010). Self-cleaning micro sieve systems can also pose a source of abrasive contaminants through loss of brush fibres during operation (van Bentem *et al.* 2010).

7.2.1.3 Activated sludge process

Threats to activated sludge include loss of aeration and circulation due to port clogging, mechanical fault or power loss and overdose of membrane cleaning chemicals (Judd, 2011). Disturbances, particularly to

influent quality, can result in foaming, leading to potential loss of containment via overflow of the aeration tanks. Simulations of hazardous events on activated sludge in MBR have revealed decreased capacity for removal of bulk parameters such as BOD, COD and total nitrogen, however, simulations of microbial quality of the permeate was not possible with the model utilised (Friedler *et al.* 2008).

During operation, biological treatment processes may be exposed to changing environmental conditions such as variations in the flow rate, concentration, and quality of the raw wastewater entering the process. In general, any rapidly occurring or immediate change in the chemical or physical environment might be classified as a system 'shock'.

Organic shock loads have been described in terms of quantitative shock loads and qualitative shock loads (Gaudy & Engelbrecht, 1961). *Quantitative shock load* implies a rapid increase in organic loading by rising high concentration of substrate to which the sludge is acclimated or to which it needs no acclimation (Gaudy & Engelbrecht, 1961). However, waste streams do not often have constant chemical composition of the organic constituents. A qualitative change in the chemical composition of the substrate (with constant TOC concentration) may constitute a serious type of system shock. This is termed a *qualitative shock load* (Gaudy & Engelbrecht, 1961). It implies that the composition of the carbon source has changed from that to which the sludge is normally acclimated while it does not imply that the change is toxic. For example, the substrate may change from a predominantly carbohydrate waste to a proteaceous or a fatty waste, from simple sugars to polymers, or from sucrose to lactose.

An important variation on quantitative shock loads is 'starvation shock'. Most treatment systems are designed to manage some variability in flow regimes. However, in extreme conditions, some treatment plants exhibit feed starvation periods during which no appreciable wastewater feeds the systems. This discrepancy between the conceptual design and the practical situation may lead to process upsets and unsatisfactory system performance (Beler Baykal *et al.* 1990).

Toxic shock involves an influx of organics or inorganic constituents and radicals, which wholly or partially inhibit or damage the existing metabolic pathways or disrupt the established physiological condition of the microbial population (Gaudy & Engelbrecht, 1961). Rapid changes in pH of the waste are also considered to be in this class of shock loading although they are more easily controlled and may be of less significance than other toxicity shock loads.

Waste streams with high ammonia concentration are very commonly produced by human handling (Campos *et al.* 2002). Sudden increase in ammonia concentration in biological treatment process can be due to increase ammonia concentration in raw sewage or inhibition of nitrification in the biological treatment process (Hart *et al.* 2003). Similarly, pH changes in biological treatment processes can be due to pH variation in raw sewage or due to failure of denitrification process within the biological treatment units.

Temporary interruptions to aeration of MBR systems would be expected to have a detrimental impact on the aerobic metabolic degradation of chemical contaminants and potentially lead to change within the microbial community. Loss of aeration may also lead to loss of suspension of the MLSS, potentially causing damage to MBR membranes.

7.2.1.4 Membrane filtration

Crucial threats at the membrane filtration stage regarding the containment of activated sludge can be encompassed within the integrity failure of the membrane or the module itself (seals, gaskets, connections). Through fault tree analysis based on the top event of cryptosporidium release, threats were scoped for an ultrafiltration plant (Beauchamp *et al.* 2010) and can be equally applicable to the membrane filtration step of a MBR.

In addition to the case of membranes exhibiting manufacturing defects, solid particles and foreign bodies within the bioreactor can breach or damage the membrane. Moreover, inappropriate high dosing of cleaning chemicals, and pressure shock (due to air from integrity testing or water from hydraulic shock of a pump start up) are expected to increase the likelihood of membrane integrity failure (Beauchamp *et al.* 2010). Integrity failure can be induced via sparks from welding in the vicinity of membranes (Ayala *et al.* 2011) and high pressure hosing during maintenance cleans (Le-Clech *et al.* 2005).

Failure of the module integrity results in short circuit of the membrane by constituents present in the mixed liquor. Module weak points include seals couplings and membrane-frame/pot interface. Module failure likelihood is increased as a result of the seal being of poor quality or inevitable wear out due to an insufficient replacement regime. Coupling failure of MBR cassette has been previously attributed to the strong mechanical forces in the module header due to the air-cycling fouling mitigation system (van Bentem *et al.* 2007).

The cleaning regime frequently imposed on membranes to remove fouling and recover hydraulic performance result in gradual changes in the physical and chemical membrane properties (especially decrease of mechanical strength) (Hajibabania *et al.* 2012). A decrease in mechanical strength of the hollow fibre membrane is expected to significantly increase the likelihood of membrane integrity failure.

7.2.1.5 Post treatment

MBR permeate is sometimes disinfected and/or stored shortly before discharge. The major post treatment threat can be defined as the bacterial regrowth in permeate lines or storage reservoirs, which have been reported to cause detectable levels of total coliforms in the permeate of MBRs (Zhang & Farahbakhsh, 2007).

7.3 EXPECTED CONSEQUENCES OF KEY HAZARDOUS EVENTS TYPES

Very little research has been reported to specifically examine the consequences of hazardous events to MBR performance. However, many insights can be obtained from previous studies of conventional activated sludge systems since the biological characteristics of the two types of systems are similar. The following sections discuss the expected impacts of hazardous events on the removal of chemical and microbial constituents, with observations derived from studies on both MBR and conventional activated sludge systems.

7.3.1 Impact on the removal of bulk organic matter and nutrients

Consequences of hazardous event conditions on conventional activated sludge and MBR treatment performance are summarised in Table 7.4.

Results of quantitative organic shock load studies to activated sludge treatment systems show that reactors which were operated stable at influent COD concentrations above 100–500 mg/L can withstand influent shock concentrations of up to 1500 mg/L COD, even when the shock durations varied from hours to weeks (Gaudy & Engelbrecht, 1961; Saleh & Gaudy, 1978; Normand & Perdrieux, 1981). However, at influent shock concentrations around 3000 mg/L COD, the change may exceed the maximum assimilation capacity of the biomass, leading to an increased deterioration of effluent quality caused by loss of biological solids (Saleh & Gaudy, 1978; Manickam & Gaudy, 1985). A 3000 mg/L COD shock load to an AS system was reported to cause a rapid growth in biomass, a noticeable change

in colour of the mixed liquor, a decrease in floc size, an increase in filamentous forms and a reduction in the number of protozoa (Saleh & Gaudy, 1978). Disruption in COD removal capacity and the change in colour of an AS system were observed to be correlated with changes in the biochemical composition of the sludge (Manickam & Gaudy, 1985). In general, high organic concentration in influent wastewater is known to inhibit nitrification as it supports the growth of heterotrophic bacteria, which compete with autotrophic nitrifying bacteria for oxygen, nutrients and space.

Table 7.4 Consequence of hazardous event conditions on AS and MBR treatment performance based on select studies.

Event type	System	Monitored parameters	Consequence on removal	References
Organic shock	AS operated stably at influent COD of 100–500 mg/L	COD	Influent COD increased to $\leq$1500 mg/L: no impact	1, 2, 3
			Influent COD increased to $\geq$3000 mg/L: biomass grown rapidly, floc size decreased, filametous forms increased and number of protozoa reduced, loss of biomass causing deterioration of effluent quality	2, 4
Starvation shock	AS system subjected to 10 d starvation period	Biomass characteristics	After shock 8 d, biomass concentration and respiration activity decreased sharply due to degradation of proteins polysaccharides contents in biomass	5
	AS system subjected to 21 d starvation period	pH, SS, VSS, CODd, DOC, biomass characteristics	Biomass concentration, bacteria cell size and respiration activity decreased sharply during first 4 d, disappearance of some typical microbial groups in AS. CODd and DOC in liquid phase increased sharply between day 4 and 9 due to release of organic material from death microorganisms.	6
	MBR system (hollow fibre, 0.4 μm) subjected to 5 d starvation period	COD, TOC, TSS, TKN, phosphate, biomass characteristics	After 5 d starvation, removal efficiencies of COD, TOC, TSS, TKN, phosphate reduced significantly and they recovered fully after 6 days of normal operation. Biomass concentration and activity reduced significantly and took a month to recover	7
Salinity shock	AS system subjected to NaCl up to 45 g /L	COD, biomass characteristics	COD removal and biomass settleability reduced	8
	As systems subjected to NaCl from 0 to 60 g/L	COD, biomass characteristics	NaCl $\leq$ 10 g/L: DOC removal slightly increased NaCl $>$ 10 g/L: DOC removal reduced NaCL $\geq$ 15 g/L: morphological changes in microbial population NaCl $\geq$ 30 g/L: effluent turbidity increased	9

Source: 1. Gaudy and Engelbrecht (1961); 2. Saleh and Gaudy (1978); 3. Normand and Perdrieux (1981); 4. Manickam and Gaudy (1985); 5. Urbain *et al.* (1993); 6. Coello Oviedo *et al.* (2003); 7. Yogalakshmi *et al.* (2007); 8. Dan *et al.* (2003); 9. Ng *et al.* (2005).

Studies have shown that biomass concentrations have decreased sharply during the first four days of a starvation shock and then reduced more slowly after that (Urbain *et al.* 1993; Coello Oviedo *et al.* 2003). In addition, the bacteria cell size was also found to be reduced, which was described as one of the adaptive responses to starvation conditions (Kjelleberg *et al.* 1987; Urbain *et al.* 1993; Coello Oviedo *et al.* 2003). These responses were related to the degradation of both proteins and polysaccharides contents of the sludge and led to a decrease in respiratory activity of the microorganisms. After 3–4 days under starvation conditions, the biomass drastically lost its ability to biodegrade exogenous nutrients reactions (Urbain *et al.* 1993). Starvation shocks also resulted in disappearance of some of the typical microbial groups usually found in an activated sludge, and appearance of other opportunistic microorganisms (Coello Oviedo *et al.* 2003).

The removal efficiencies of COD, TOC, total suspended solid (TSS), total Kjeldahl nitrogen (TKN) and phosphate by a MBR were reduced significantly under a feed starvation shock load of 5 d (Yogalakshmi *et al.* 2007). In addition, a large fraction of biomass wash off and a reduction in microbial activity inside the reactor was observed. The removal of organics and nutrients was recovered back to steady state conditions after six days of normal operation. However, it took nearly a month of continuous operation to regain the amount of biomass lost during feed starvation shock load (Yogalakshmi *et al.* 2007).

High salt concentrations in a biological reactor have been reported to reduce organic removal efficiencies and biomass settleability (Dan *et al.* 2003; Ng *et al.* 2005). This is because salty conditions produce high osmotic pressure on bacteria cells, which can inhibit bacterial growth and floc formation (Dan *et al.* 2003). Additionally, high salt concentration conditions also reduce gravity separation due to lower density difference between water and biomass (Ng *et al.* 2005).

Failure modes leading to physical membrane damage tend to be gradual rather than sudden and are easily identified by long-term changes in flux or operating pressures. Accordingly, their relevance as 'hazardous events' leading to sudden deterioration in water quality appears low. Nonetheless, there is some evidence to suggest that events such as chemical membrane cleaning and accidental exposure to excessive chlorine concentrations may physically harm some types of water treatment membranes leading to reduced performance (Simon *et al.* 2009; Beyer *et al.* 2010).

7.3.2 Impact on the removal of microorganisms and microbial indicators

Information on the impact of hazardous events on the removal of pathogenic microorganisms by MBRs is scarce. Research has traditionally focused on studying the behaviour of microbial indicators (model organism) under a range of event conditions. Most of this information has been derived from lab- and pilot-scale studies, whereby key operating parameters can be easily adjusted and challenged under controlled conditions. The impact of key operational events on the microbial removal efficiency of MBRs are summarised in Table 7.5.

What is clear from Table 7.5 is that the most important mechanisms responsible for removing microorganisms are membrane rejection and biodegradation. Generally, pathogen removal improves as membrane fouling layers develop, and thus events that lead to the removal or disturbance of fouling layers (e.g., membrane cleaning, backwashing and change in permeate flux) can adversely influence removal. The extent of membrane fouling is commonly quantified by the monitoring changes in the permeate flux or the transmembrane pressure (TMP).

MBRs are well known for their ability to remove a wide range of model indicator organisms (e.g., bacteria, phage and spores) and what is clear from the literature is that each organism behaves differently. Notably, the removal of membrane fouling influences the rejection of phage more so than bacteria; simply because phage are much smaller than the pore size of membranes. As a result, phage removal is typically less consistent and is more subject to the type of membrane and its pore size (microfiltration vs. ultrafiltration) and to changes in

operation, such as membrane TMP, permeate flux and spikes in initial feed concentrations. Therefore, bacteriophage appear to be a superior model organism for understanding the impacts of hazardous event conditions on the microbial removal efficiency of MBRs.

Table 7.5 Consequence of operational and event conditions on the removal of microbial indicators based on select studies.

Event type	Membrane	Model organisms	consequence on LRV	References
Chemical backwash	Zenon ZW-500C-SMC	Somatic coliphage	Small decrease (from 3.0 to 2.5)	10
		F-specific coliphage	No significant impact	
Formation of fouling	Memcor 0.2 μm	Indig. somatic coliphage	Increase from 1.2 (clean) to 2.0 (fouled)	11*
			No impact	
Increase in flux for clean membrane			Decrease from 2.2 (50 $Lm^{-2}h^{-1}$) to 1.7 (85 $Lm^{-2}h^{-1}$)	
Increase in flux for fouled membrane			2.3 (25 $Lm^{-2}h^{-1}$), 2.7 (50 $Lm^{-2}h^{-1}$) and 2.3 (85 $Lm^{-2}h^{-1}$)	
Longer filtration/relaxation cycle	Six MBR systems	Seeded MS-2 phage	Increase from 2.9 (1 min cycles) to 3.4 (8–18 min cycles)	12
Relaxation period/ air scouring			Small decrease by 0.25 LRV	
Change in pore size (0.03–0.1 μm)			Increase from 1.5 (0.1 μm) to 4.5 (0.03 μm)	
		Indig. coliphage	No impact, due to particle association	
Change in pore size (0.03–0.2 μm)	Nine MBR systems	Coliform bacteria	No impact	13
		Indig. coliphage	No impact	
Increase in MLSS conc.(3 to 9 g/L)	Hollow fibre, 0.4 μm	Indig. somatic coliphage	No impact on LRV, but change in biological action	14
Formation of fouling			Increase from 0.6 (clean) to 1.5 (fouled)	
Chemical backwash			Decrease by 0.5 (*attributable to biomass only)*	
Change in SRT (10 to 50 d)			Weak increase by 0.05 (*attributable to biomass only)*	
Change in HRT (8 to 13 hr)			Increase from 1.5 to 1.9 (*attributable to biomass only)*	
Filtration of supernatant	Flat sheet, 0.4 μm	T-even-like indig. phage	LRV across membrane only: 0.5	15

(*Continued*)

Table 7.5 Consequence of operational and event conditions on the removal of microbial indicators based on select studies (*Continued*).

Event type	Membrane	Model organisms	consequence on LRV	References
Operation with mixed liquor			Increase to 4	
Power failure to air scour and influent pump			Increase from 0.4 to 1.0, possibly due to increased fouling	
Clean membrane (filtration of supernatant)	Hollow fibre, 0.4 μm	MS-2 phage	0.3–0.4	16
High flux operation			Decrease	
Operation with mixed liquor			Increase to 1.0 (after 9 hr) to 2.0 (21 d)	
Change in MLSS conc. (6–10 g/L)			No impact	
Formation of fouling	Flat sheet, 0.4 μm	Indig. somatic coliphage	No significant impact	17
		Indig. FRNA phage	Increase from 4.5 to 4.8	
		Bacterial indicators (spores, *E.coli*)	No impact	
Membrane rinsing	Hollow fibre, 0.22 μm	T4 coliphage	Decrease from 5.8 to 3.1	18
Chemical cleaning			Decrease from 5.8 to 1.7	
Chemical cleaning	Hollow fibre, 0.22 and 0.1 μm	Coliphage f2	Decrease from 3.9 to 0.8	19

Source: 10. Zhang and Farahbakhsh (2007); 11. Farahbakhsh and Smith (2004); 12.Hirani *et al.* (2010), 13. Hirani *et al.* (2012); 14. Wu *et al.* (2010); 15. Ueda and Horan (2000), 16. Shang *et al.* (2005); 17 Marti *et al.* (2011); 18. Lv *et al.* (2006); 19. Zheng and Liu (2006).
**Direct filtration of sewage, no MBR.*

Not all phage species behave the same. Different species feature varying retention mechanisms, owing to differences in surface properties. For example, F-specific phage have a higher tendency to adsorb to membrane surfaces and suspended biomass more so than somatic phage, exhibiting a more even removal pattern during maintenance cleaning events (Zhang & Farahbakhsh, 2007). The removal patterns of native and laboratory-grown phage strains can also differ (Hirani *et al.* 2010). Selection of the right model organisms (i.e., one that shares a similar fate to target pathogen) is therefore crucial when characterising the impacts of hazardous events on MBR performance.

Research characterising the removal of model organisms by MBRs also suggests that the suspended biomass (mixed liquor) can play a very important role in the elimination of pathogens via adsorption and predation Table 7.5. The contribution of biomass, however, is dependent on inter-related parameters including the concentration of mixed liquor suspended solids, the sludge retention time and the food to mass ratio; and thus operational events that lead to changes in these parameters may influence pathogen removal. At this time, the relative impact of the fouling layer on the rejection capability of the membrane has still not been clearly demonstrated. The role of the irrecoverable fouling layer formed over years of continuous operation is expected to be responsible for the build-up of a protective layer suitable for adsorption for viruses. However, Table 7.5

indicates that the various types of cleaning used in MBR maintenance generally result in lower pathogen rejection.

7.4 ASSESSING LIKELIHOODS OF MBR HAZARDOUS EVENTS

Techniques for quantitatively assessing the likelihoods of specific hazardous events could be investigated including the use of historical data such as weather patterns and frequencies of power failures or mechanical malfunctions. An alternative approach is by the use of available mechanical reliability measures such as critical component analysis methodology (Shultz & Parr, 1982; Olivieri *et al.* 1996; Eisenberg *et al.* 1998, 2001).

A critical component analysis can be carried out by creating a list of all components in a facility and then categorising the components by treatment unit, component and subcomponent. Data are collected for all planned and unplanned maintenance events and then used to compute performance statistics for treatment units and for individual components in the treatment system. The performance statistics describe the expected time between failures for treatment units, the overall mean time between failures of components, and the fraction of time that a unit or component was operating, either including or excluding preventative maintenance.

This type of analysis provides a foundation from which an assessment of the inherent reliability of a treatment system may be made. For example, if it can be demonstrated that a treatment facility is operational nearly 100 per cent of the time on a long-term basis, plant performance data may be used to evaluate the probability that the effluent will meet a specified set of criteria. Otherwise, it may be necessary to investigate if and how component failures impact treatment plant effluent quality.

The established engineering parameters Mean Time Between Failures (MTBF, a function of reliability) and Mean Time to Repair (MTTR, a function of availability) may be used to calculate the operational availability (A_o, the probability that an item is in an operable state at any time) as shown in Equation 1.

Equation 1: Determination of operational availability from MTBF and MTTR

$$A_o = \frac{\text{MTBF}}{\text{MTBF} + \text{MTTR}}$$

Reliability of machinery can be derived through parametric models to serve as population models for failure times arising from a wide range of products and failure mechanisms. Weibull statistics provide a life distribution model, which has been useful in many engineering applications to derive failure rates (Carrasco *et al.* 2008; Davis *et al.* 2008; Erumban, 2008). The two-parameter Weibull distribution function has been used to derive a reliability function R(t) given by the cumulative form (Equation 2).

Equation 2: Reliability function R(t) from the cumulative form of the Weibull distribution

$$R(t) = \int_t^\infty f(x)\mathrm{d}x = e^{-(x/\beta)^\alpha} \quad t \geq 0,\ \alpha > 0,\ \beta > 0$$

where α is the Weibull shape parameter, β is the scale parameter, and t is the time of operation. **AQ8**

The scale parameter β has the same units as t and the shape parameter α is a dimensionless quantity. When $\alpha = 1$, representing a constant failure rate, the reliability model is simplified to the form presented in Equation 3.

Equation 3: Reliability function R(t) for a constant failure rate ($\alpha = 1$)

$$R(t) = e^{-\lambda \cdot t} \quad \text{with the failure rate } (\lambda), \qquad \lambda(t) = \frac{1}{\beta} = \frac{1}{\text{MTBF}}$$

Process reliability for an MBR system may be engineered through reliability assessments made using Weibull distribution databases for all mechanical components (Moore *et al.* 2008). Historical MTTR for each component can be tracked and updated through corrective maintenance work orders. The MTBF and MTTR values analysed may also form part of an asset replacement strategy.

7.5 MANAGEMENT OF HAZARDOUS EVENTS THROUGH ENGINEERED REDUNDANCY AND MULTIPLE BARRIER TREATMENT SYSTEMS

It is generally not possible to guarantee the prevention of many types of hazardous events. Accordingly, systems must be designed with a degree of robustness to manage impacts to ongoing operation as well as risks to human health and the environment when hazardous events occur. Important concepts for managing hazardous events are the incorporation of multiple barriers in the design and the establishment of a monitoring program that is suitable to constantly assess proper system performance. The selection of multiple barriers and a monitoring program will depend on the context in which an MBR is employed. Meeting effluent discharge standards will require a different management approach to potential hazardous events as compared to practices where MBR effluents are used for non-potable or potable reuse applications given the higher degree of potential exposure to public health.

Multiple barriers in water treatment and reclamation are aimed at ensuring that performance goals are met by (1) expanding the variety of contaminants a process train can effectively address by providing engineered redundancy (i.e., robustness) and (2) by improving the extent of consistent performance of a unit process to attenuate a contaminant (i.e., reliability) (National Research Council, 2012).

Even when true redundancy is not provided, multiple barriers can reduce the consequences of hazardous events when they do occur. The independence of multiple barriers is a key aspect of system reliability and safety (Drewes & Khan, 2011). For example, to mitigate the risk from pathogen exposure, all MBRs usually employ a disinfection step either using a chlorine-based disinfectant or UV irradiation, in addition to the MF or UF membrane that serves as a barrier to pathogens.

The extent of system performance and water quality monitoring will depend on project-specific water quality objectives and the potential impact from hazardous events. An idealized monitoring program would measure critical process parameters and microbial and chemical contaminants in real time in the finished product water. However, real-time monitoring comes at significant capital and maintenance expenses and needs to be balanced against the estimated likelihood of certain hazardous events.

Monitoring requirements usually become more stringent (e.g., more frequent and broader in scope) as the potential for human contact with the reclaimed water increases (e.g., non-restricted irrigation of public parks; indirect potable reuse). Monitoring programs to assure that water quality requirements are met most commonly include effluent turbidity and residual chlorine. Operational parameters that are measured in real-time include flow measurements, transmembrane pressure, bioreactor tank levels,

dissolved oxygen concentration of the bioreactor, as well as status of pumps and critical valves (i.e., on/off). These parameters are recorded in the Supervisory Control and Data Acquisition System (SCADA) of the treatment facility and usually linked to certain threshold levels. An exceedance of these threshold levels that might be caused by a hazardous event will result in shut-down of the system to mitigate the negative impact of that event.

7.6 CONCLUSIONS AND FUTURE OUTLOOK

The possibility or frequency of hazardous events plays a significant role in defining the overall risks to health and the environment from wastewater treatment by MBRs. Potential hazardous events are diverse and even prediction of a comprehensive suite of events that may disrupt and MBR performance is difficult. However, important examples include rapid and/or significant changes in influent water quality impacting the biological integrity and physical damage, which may impact membrane integrity. Hazardous events may lead to drastic loss of treatment performance by impeding microbial degradation processes or by impeding the retention of particulate substances by membranes.

Formalised risk assessment procedures, aimed at rating potential hazardous events in terms of their likelihood and consequences are well suited for assessing MBR system vulnerabilities. Existing risk management approaches including the multiple barrier approach and a focus on monitoring the performance of operational parameters can be effective means for managing these vulnerabilities for the protection of health and the environment.

As important as the proper assessment and management of system failures and risks may be, surprisingly little attention has been paid to this topic for MBRs. More comprehensive future risk management will benefit from focused investigation of a wider range of potential failure modes, their consequences particularly in terms of their impacts to final water quality, and statistical descriptions of their likelihood. These factors will enable informed assessment of risks and better direct efforts towards more effective risk management.

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