Cape Peninsula University of Technology

Removal of natural steroid hormones from municipal MBR effluent with UF/NF/RO membranes

by

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"*In the name of Allah, the Most Gracious the Most Merciful* ". [Al-Quran, 1:1]

"*(Remember), when He covered you with a slumber as a security from Him, and He caused* ***water*** *(rain) to descend on you from the sky, to clean you thereby and to remove from you the evil (suggestions) of Satan and to strengthen your hearts and make your feet firm thereby* ". [Al-Quran, 8: 1]

"He it is Who sends down ***water*** *(rain) from the sky; from it, you drink and from it (grows) the vegetation on which you send your cattle to pasture".* [Al-Quran, 16: 10]

"*And We sent down from the sky* ***water*** *(rain) in (due) measure, and We gave it lodging in the earth, and verily, We are able to take it away*". [Al-Quran, 23: 18]

"*And it is He Who sends the winds as heralds of glad tidings, going before His Mercy (rain), and We send down pure* ***water*** *from the sky*". [Al-Quran, 25: 48]

"*And the two seas (kinds of* ***water****) are not alike, this fresh sweet, and pleasant to drink, and that saltish and bitter. And from them both you eat fresh tender meat (fish) and derive the ornaments that you wear. And you see the ships cleaving (the sea-water as they sail through it), that you may seek of His Bounty, and you may give thanks*". [Al-Quran, 35: 12]

"*See you not, that Allah sends down* ***water*** *(rain) from the sky, and causes it to penetrate the earth, (and then makes it spring up) as water-springs and afterwards thereby produces crops of different colours, and afterwards they wither and you see them turn yellow, then He makes them dry and broken pieces. Verily, in this, is a Reminder for men of understanding*". [Al-Quran 39: 21]

"*And We send down blessed* ***water*** *(rain) from the sky, then We produce therewith gardens and grain (every kind of harvests) that are reaped*". [Al-Quran, 50: 9]

"*Say (O Muhammad): "Tell me! If (all) your* ***water*** *was to be sunk away, who then can supply you with flowing (spring)* ***water***?" [Al-Quran, 67: 30]

"*And have placed therein firm, and tall mountains, and have given you to drink sweet* ***water.****".* [Al-Quran, 77: 27]

DECLARATION

I, **Mujahid Aziz,** declare that the contents of this dissertation/thesis represent my own unaided work and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

M Aziz — December 2019

Signed **Date**

ABSTRACT

In the context of water scarcity, the increasing risks linked with the presence of natural steroid hormones and many emerging anthropogenic micropollutants (MPs) persevering through municipal wastewater treatment works (MWWTWs) are of concern for their endocrine-disrupting activities detected in receiving surface waters. For the last decades, the use of membrane technology has grown considerably in wastewater treatment and has proven to be an effective method for the removal of a wide variety of contaminants from wastewater.

In this study, domestic wastewater treated by a full-scale membrane bioreactor (MBR) at MWWTWs in the City of Cape Town (CoCT), South Africa, was used directly as the influent to a reverse osmosis (RO) pilot-plant for the removal of selected natural steroid hormones; 17β-estradiol (E_2) and Testosterone (T) as a potential indirect water recycling application. Three commercially available ultrafiltration/nanofiltration/reverse osmosis (UF/NF/RO) membranes, namely UA60, NF270 and XLE, were selected for this investigation.

Membrane surface modification was investigated to minimize fouling during the MBR-UF/NF/RO treatment processes. To enhance the resistance to flux decline, a thin film composite reverse osmosis membrane was grafted with polyvinyl alcohol (PVA) through cross-link with glutaraldehyde (GA). To assess the effect of surface modification on the membrane surface, analyses using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and scanning electron microscopy (SEM) were done. Escherichia coli (E. coli) bacterial solution was used as biofouling to evaluate the influence of the surface modification initiated on antifouling properties of the membrane. A model MBR secondary effluent with a bench-scale NF/RO system was used for these fouling tests. The pure water flux decreased somewhat as a result of the morphological structure and chemical property change due to membrane surface modification. The membrane resistance to fouling was better and the biofouling model used exposed the anti-biofouling capacity of the membrane, although a small decrease of salt rejection was detected. The flux decline and flux recovery ratios improved with an increase in PVA concentration. The sterilization ratio increased from 33.8 to 36.8% and the pure water flux decline, reduced from 46.04 to 25.94 % after modification.

The effects of operating conditions on the removal of inorganics by MBR-UF/NF/RO were evaluated. Experimental runs were conducted on a pilot plant in a continuous system, varying the pH, as well as the permeate flux and the percentage recovery in the case of RO membrane. Chemical analysis of different inorganics was conducted to calculate the percentage removal. Results revealed considerable effects of pH control on the removal of the inorganics of interest as well as the carbon oxygen demand (COD).

Adjustment of flux and recovery for the RO membrane was shown to be a factor of consideration for the improvement of inorganics removal in the advanced treatment of domestic secondary effluent. It was shown that the quality of water obtained with the RO membrane could meet quality requirements for reuse application in cooling systems and irrigation among others. The UF and NF reduction of inorganics was shown to be limited to meet the required water standard for some of the reuse applications. The NF membrane was found to produce water suitable for restricted irrigation and cooling systems, while the UF produced permeate that was found appropriate for cooling systems only.

Estrogenicity and androgenicity were assessed using the enzyme-linked immunosorbent assays (ELISA) and the recombinant yeast estrogen receptor binding assays (YES). The influent pH and flux did not have an influence on the rejection of E_2 and T, which was most likely ruled by adsorption, size exclusion and diffusion simultaneously. Size exclusion was seemingly dominant, especially with NF and RO membranes. T with a smaller partitioning coefficient (log Kow) value was most likely adsorbed on the membranes and then passed through it to give a low rejection with all three membranes. RO and NF membrane processes exhibited excellent removal rates (>95%) for E_2 and T. It was revealed that RO showed higher removal percentages when compared with NF and UF. All the E_2 effluent samples with the MBR/UF, MBR/NF and MBR/RO, were higher than the lowest trigger value of 0.4ng/L of the test, but less the than USEPA and WHO of 0.7 ng/L as well as less than the predicted no-effect concentration (PNEC) values for fish (1 ng E_2/L).

Consequently, domestic secondary wastewater treated by full-scale MBR followed with a pilot-plant, NF or RO is acceptable for the effective removal of natural hormones (E_2 and T). Ultimately, a multi-barrier tactic using MBR followed by RO or NF could prove the most effective in pollutant removal followed with a disinfectant at the end.

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DEDICATION

The Almighty, My Creator; Sustainer; Protector…
The Final Prophet (peace and blessings be upon him), the Teacher of Humanity

This thesis is dedicated to

the love of my life

My beautiful wife, **Muneyba**, (brought to me by the mercy of *Allah the Almighty*), whose unconditional love, friendship, support, sacrifice, tolerance and encouragement enabled me to complete this research. Partnered with me on a vibrant journey full of life, continually serving as a support system and the source of my motivation. I am forever thankful, Alhamdulillah.
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our lovely children, **Fatima Tuz Zahra**, **Haameem** and **Fathiyah** who are indeed a treasure from *Above*; our pride and happiness
This doctoral degree is a symbol of what we have accomplished as a family.
This one is for us!

The oppressed of the world, which are always in my supplication, and my heart shall eternally lie.

RESEARCH OUTPUTS

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Kasongo G; Steenberg C; Morris B; Kapenda G; Jacobs N and **Aziz M;** 2019; Surface grafting of polyvinyl alcohol (PVA) cross-linked with glutaraldehyde (GA) to improve resistance to fouling of aromatic polyamide thin-film composite reverse osmosis membranes using municipal membrane bioreactor effluent, IWA*: Water Practice, and Technology*, 14 (3), 614-626 [ISSN 1751-321X / DOI.org/10.2166/wpt.2019.047]

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LIST OF SYMBOLS

A	Area (m^2)
EC	Conductivity (µS)
J	Water flux ($L.m^{-2}$ hr^{-1})
Mw	Molecular weight (g/mol)
P	Pressure (bar)
R	Salt rejection (%)
T	Temperature (°C)
t	Time (hours)
Δt	Time interval (min)
V	Volume (L)

Subscripts

f	Feed
p	Permeate
wf	Time dependant
wi	Initial water
wt	Time dependant
Ow	Octanal/water

LIST OF ACRONYMS and ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
AA	Acrylic acid
AC-HBPAE	Acrylated hyperbranched poly (amino ester)
AEMA	Amino ethyl methacrylate
AFM	Atomic force microscopy
ATR-FTR	Attenuated total reflectance–Fourier transform infrared
ANOVA	Analysis of Variance
AS	Activated sludge
BOD	Biological oxygen demand
BSA	Bovine serum albumin
CA	Cellulose acetate
CAS	Conventional activated sludge process
CBMA	Carboxybetaine methacrylate
CIP	cleaning in place
CF	Correction factor
COD	Chemical oxygen demand
DI	Deionized
CPRG	chlorophenol red galactopyranoside
DPR	Direct Potable Reuse
DNA	Deoxyribonucleic acid
DTAB	Dodecyl trimethyl ammonium bromide
EEQ	Estrogenic equivalent
ERα	Estrogen receptor alpha
ERA	Environmental risk assessment
EC	Electrical conductivity
EDC	Endocrine-disrupting compound
EfOM	Effluent organic matter
EMP	Emerging micro-pollutants
E_2	17β-estradiol
EC	Emerging contaminant
EC_{50}	Effect concentration for 50% of test organisms
EDC	Endocrine-disrupting contaminant

FDR	Flux decline ratio
FRR	Water recovery ratio
GA	Glutaraldehyde
GWRC	Global Water Research Coalition
hAR	Human androgen receptor
hER	Human estrogen receptor
HF	Hollow fiber
HRT	Hydraulic retention time
K_{ow}	octanol-water coefficient
LD_{50}	Lethal dose for 50% of test organisms
LOEC	Lowest observable effect concentration
LOD	Level of detection
LOQ	Level of quantification
LPRO	Low-pressure reverse osmosis membrane
MeOH	Methanol
MBR	Membrane Bioreactor
MPs	Micropollutants
MSWWTP	Municipal Sewage Waste Water Treatment Plant
NF	Nanofiltration
NOEC	No observable effect concentration
NIPAm	N-isopropyl acrylamide
ng	Nanograms
NOM	Natural organic matter
OD	Oxidation ditch
P	Pressure
PA	Polyacrylic acid
PCW	Ponds and constructed wetlands
PPCP	Personal care products and pharmaceuticals
PMS	Premenstrual syndrome
PCOS	Polycystic ovary syndrome
PPE	Personal Protective Equipment
PVA	Polyvinyl alcohol
QSAR	Quantitative structure-activity relationship
SWRS	Singapore Water Reclamation Study

RO	Reverse osmosis
R	Rejection
RCBA	Recombinant Yeast Cell Assay
RNA	Ribonucleic acid
PNEC	Predicted no effect concentration
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SEM	Scanning Electron Microscopy
SPE	Solid-phase extraction
SRT	Solid retention time
SS	Suspended solids
T	Testosterone
TDS	Total dissolved solids
T/BF	Trickling biological filter
TFC	Thin-film composite
PIPR	Planned Indirect Potable Reuse
UOSA	Upper Occoquan Sewage Authority
UIPR	Unplanned Indirect Potable Reuse
USEPA	United States Environmental Protection Agency
TFN	Thin-film Nano-composite
UF	Ultra-filtration
VTG	Vitellogenin
WHO	World Health Organization
XPS	X-ray photoelectron spectroscopy
XLE	Extra-low energy
WCWSS	Western Cape Water Supply System

Chapter 1

INTRODUCTION

1 INTRODUCTION

1.1 Background

Growing fears around water resources are becoming an imperative topic as a severe paucity of water has been seen all over the globe. Water is a highly treasured resource. Statements have been made that in the future war might not only be fought over resources like oil, gold, money or personal freedom but also over water (http://www.serageldin.com/Water.htm):

> "If the wars of this century were fought over oil, the wars of the next century will be fought over water –unless we change our approach to managing this precious and vital resource (Serageldin, n.d.)"

This life-threatening statement confirms that the accessibility and accordingly the reuse of water will become more and more important in the future, especially in regions where accessibility of freshwater is rare. Worldwide, only 0.3% of the water on mother earth is available as fresh water in meres and waterways. Irrigation uses 70% throughout the globe and the remainder is used for domestic and industrial applications. In First World, high-income nations, industrial usage can be as high as 59%. Reuse of water is therefore especially important for industrial purposes and should become a focus in the future (Qin et al. 2005; Lee et al. 2008; Kappel et al. 2014a; Falizi et al. 2018; Farrokh Shad et al. 2019).

Growing population size and prospects for water use based on lifestyle changes have an impact on water quality, resulting in increasing demand on water supplies. The stresses pertain to amplified quantity and quality of water. Quantity demands may only be met by re-use and quality demands by advanced treatment, both cases indicating a potential role for membrane technologies (Howell 2004). Bohn et al. (2009) writes that one of the greatest prevalent difficulties troubling people throughout the globe is available of clean water and sanitation. Difficulties with water are expected to grow worse in the coming decades, with water scarcity occurring worldwide, even in regions currently considered water-rich. Solving these difficulties calls for a great amount of research to be conducted to identify effective new methods of cleansing water at lower cost and with less energy, while at the same time minimizing the use of chemicals and their impact on the environment.

Cartagena et al. (2013) and Bunani et al. (2018) concur; that the need for maintainable water treatment processes has become vital with increasing freshwater scarcity. The reuse application of treated wastewater for agricultural, industrial and urban usages will increase water resources in water-scarce regions. To accomplish this, various actions can be applied to obtain a better quality of water.

According to Comerton et al. (2005), treated municipal wastewater is discharged to the environment and measured as waste, but should instead, be viewed as a resource from which high-quality water for reclaim can be produced. Comerton et al. (2008), Dolnicar and Schäfer (2009) and Kappel et al. (2014b) say that water recycled from municipal wastewater could be obtained by a membrane bioreactor (MBR) for combined biological and membrane treatment. Microfiltration (MF) or ultrafiltration (UF) membranes are applied to separate biological sludge solids from the treated water stage and bring high quality and solids-free product. However, MBR permeate can still contain viruses and dissolved organic contaminants that must be removed before the water can be reclaimed. Dense membrane processes such as nanofiltration (NF) or reverse osmosis (RO) can achieve this.

Ghayeni et al. (1998), states that municipal wastewater, which comprises between 80 – 90% of consumed water in most cities, is one of the greatest consistent sources of water, since its volume varies little, throughout the year. The reuse of municipal wastewater requires treatment to an acceptable quality level, which gratifies regulatory guidelines and meets the water quality standards of users. Recycling and reuse of treated sewerage water is a common practice applied in various forms in the world to augment water supply sources for agricultural, industrial or domestic use. The plants at California (USA) and Bedok (Singapore) are two popular examples of typical systems where the water is recycled and blended in with fresh water earmarked for domestic consumption. Both these plants utilize membrane filtration as part of the treatment train. According to Leusch et al. (2008), depending on the treatment processes (and related combination of membrane systems), the final quality of the treated water obviously determines the level and acceptability of utilization for domestic purposes.

The existence and occurrence of organic micro-pollutants such as endocrine-disrupting compounds (EDCs), pharmaceutically active compounds, pesticides and other organic micro-pollutants are becoming a concern in drinking water, wastewater, and water reuse applications employing membrane treatment due to potential adverse health effects associated with these compounds (Bohn et al. 2009; Truter et al. 2016).

Yoon et al. (2004, 2006) comments that numerous studies have examined the removal of emerging micropollutants (EDC/PPCPs) by RO, NF, and UF membranes using a dead-end stirred-cell filtration system and cross-flow filtration system. Reverse Osmosis (RO) is one of the techniques, in membrane technology that has been explored as a potential process to purify recycled water to consumable level. The market for RO is expanding to meet the increasing requirement for use of seawater and wastewater resources. Both desalination and water reuse are relatively small sources at present. However, groundwater is effectively a non-renewable resource, so it is inevitable that the contribution of groundwater to total abstraction diminishes. Desalination and water reuse are therefore predicted to move into a period of significant sustained growth to compensate diminishing supply and increased demand (Bohn et al. 2009).

Now and in the future, the ever-growing demand for drinking water will lead many countries to implement indirect water reuse programs, where wastewater effluent becomes part of drinking water sources. Pollution of those sources with micropollutants (MPs), such as endocrine-disrupting compounds (EDCs) and pharmaceutical and personal care products (PPCPs) is a fact known globally. The presence of these emerging micropollutant contaminants is of great concern in drinking water treatment plants, resulting in recycled wastewater effluents and wastewater contaminated surface water usage (Yangali-Quintanilla 2010; Silva et al. 2017b; Abbas et al. 2019)

The impact of domestic and industrial wastewater discharges, either treated or untreated, in surface water allocated to produce drinking water is an increasing concern due to the introduction of various emerging MPs in the water cycle that may result in alarming health and environmental consequences. These discharged MPs are either moderately removed or not at all during wastewater treatment and afterwards during conventional drinking water treatment. As a result, MPs have been detected in many surface waters in China, Greece, Italy, Netherlands and USA, to name a few. (Ternes et al. 1999; Braga et al. 2005; Kim et al. 2007a; Snyder et al. 2007; Benotti et al. 2009; Coleman et al. 2009; Yangali-Quintanilla 2010; Trinh et al. 2016; Silva et al. 2017c; Kase et al. 2018; Abbas et al. 2019).

Tisdale et al. (2019) stated that 17β-estradiol and testosterone are steroid reproductive (sex) hormones. Estradiol is the most potent of the estrogens, best known for its roles in the development of female sexual characteristics and in female reproductive physiology.

A possible answer to the unforeseen consequences that those MPs may cause lies in the study of their removal through membrane treatment. It is presently of great scientific interest for the future, as water sources become scared and the demand for recycling increase. Reverse Osmosis (RO) has been demonstrated to be an appropriate technology for removing many MPs, but nanofiltration and ultrafiltration also constitute a good option although they are believed to have certain limitations. (Nghiem et al., 2004; Heo et al., 2013; Linares et al., 2011; Garcia et al., 2013).

Apart from the many small and medium scale brackish water and seawater desalination systems supplying water for domestic use in South African, the Western Cape Province has several membrane bioreactor (MBR) filtration plants integrated with sewerage treatment systems (Malmesbury, Bellville, Khayelitsha and Stellenbosch MSWWTWs). In the City of Cape Town (CoCT) alone, plants treat several hundreds of megalitres per day of domestic sewerage. This treated water is a potential source of reuse. In 2018, the CoCT suffered a third consecutive year of serious drought and was faced with the threat of “Day Zero”, when it was feared that the taps could run dry (Booysen et al. 2019). Due to the major shortage of water in the Western Cape, recycling of water by direct or indirect methods is urgently needed.

Many MBR-RO full-scale plants are operational world-wide for reuse application, but no peer review literature is available. There is more than enough peer review literature on laboratory-scale MBR and NF/RO with synthetic feeds. If a real feed is used, it is contaminated with known MPs.

The purpose of this study is to develop a database for full-scale MBR with UF/NF/RO that may provide an understanding for design, prediction and improvement for future work and in the process may fill the literature void.

1.2 Aim and Objectives

The aim of this research is to investigate the development and performance of a full-scale MBR with tertiary UF, NF and RO processes for the treatment of municipal sewage wastewater with respect to the removal efficiencies of estrogenic and androgenic activity, caused by natural steroidal hormones. The view is to contribute to the database and provide an understanding of the design of such processes for the purpose of potential water reuse.

The objectives of this research are to:

- Investigate the antifouling performance of PVA cross-linked with glutaraldehyde grafted membrane using a simulated MBR secondary effluent in a laboratory-scale RO treatment unit

- Design-build and commission a UF/NF/RO pilot-plant adjacent to a full-scale UF-MBR system, at a Municipal Waste Water Treatment Works (MWWTWs), in the City of Cape Town (CoCT)

- Evaluate three types of spiral-wound membranes (UF, NF and RO) based on their different characteristic properties, to measure the best quality of effluent with the removal of targeted inorganics

- Investigate the removal efficiencies of the estrogen, 17β-estradiol (E_2) and the androgen testosterone (T), using in-vitro biological analytical methods; enzyme-linked immunosorbent assays (ELISAs) and recombinant yeast estrogen screening (YES), for quantification in the water.

1.3 Delineation

This study focuses on the removal of 17β-estradiol and testosterone and targeted Inorganics with a full-scale membrane bioreactor (MBR) plant followed with a UF/NF/RO pilot plant. Concentrations of (E_2 and T) in the influent and effluents, were screened by ELISA and YES bioassays. All others are delineated.

1.4 Thesis outline

This thesis contains seven chapters.

Chapter 1 provides a detailed background of the study, aim and objectives, delineation as well as the thesis outline.

Chapter 2 presents an introductory but detailed theoretical background of literature and theory in relation to the different topics linked to this research project. Important concepts, definitions and methods used throughout the thesis are discussed.

Chapter 3 gives details of the procedures, equipment, apparatus as well as the pilot plant detailed used for data generation.

Chapter 4 presents and discusses the different results obtained from experimental runs from surface grafting of PVA on TFC RO membranes. quantitative analysis is included.

Chapter 5 presents and discusses different results obtained from experimental runs from the UF/NF/RO treatment processes. Targeted inorganic analysis is included.

Chapter 6 presents and discusses different results obtained from experimental runs from the UF/NF/RO treatment processes. Including screening assay details.

Chapter 7 presents the conclusion and recommendations.

Chapter 2

LITERATURE REVIEW

2 LITERATURE REVIEW

2.1 Source, occurrence and threat of natural steroid hormones

The occurrence of many substances in water bodies that can harm human and animal health has caused increasing fear. Among these substances are developing anthropogenic micropollutants (MPs), present in both industrial and domestic wastewater in unnoticeable amounts, with concentration fluctuating in scale from $\mu g.L^{-1}$ and $ng.L^{-1}$(Silva et al., 2017, Abbas et al., 2019). Numerous natural and synthetic compounds have been shown to modify endocrine activity in vertebrates. Compounds acting in this way are collectively referred to as endocrine-disrupting chemicals (EDCs) (Blake et al., 2010). The discharge of endocrine-disrupting chemicals (EDCs), pharmaceuticals, and personal care products (PPCPs) into the aquatic environment is currently of great concern. These chemicals enter the sewerage system through disposal or excretion and are not completely removed during wastewater treatment. Numerous studies have detected EDCs, such as natural steroidal hormones(Braga et al. 2005; Kim et al. 2007b; Coleman et al. 2009; Kase et al. 2018) and PPCPs (Ternes et al. 1999; Kim et al. 2007b; Coleman et al. 2009; Archer et al. 2017b) in environmental samples. Studies have shown antagonistic effects on aquatic wildlife (Ellis et al. 2003; Vethaak et al. 2005) that have been linked to the presence of EDCs and PPCPs.

These endocrine disrupters (ED), which are more than 70 000 chemicals believed to have ED potential (Gillesby and Zacharewski 1998) are agents that interfere with the synthesis, secretion, conveyance, binding and exclusion of natural hormones in the body. These natural hormones are accountable for maintenance, reproduction, expansion and behaviour of organisms (Larissa L S Silva et al., 2017; Routledge and Sumpter, 1996; Hu et al., 2013; Bhandari et al., 2015; Makene and Pool 2019). Among the sources of these substances are natural steroid hormones, industrial chemicals, pharmaceuticals and many others (Silva et al. 2017a).

The damaging effects on humans and animals, such as endocrine system irregularities, cancer, a decrease of sperm number and endometriosis, caused by natural steroids in water bodies have been observed by various authors (Silva et al., 2017; Gillesby and Zacharewski, 1998; Aneck-Hahn, Bornman and De Jager, 2009). Several authors have confirmed decreased testosterone level and heightened anxiety (Hu et al., 2013; Bhandari et al., 2015; Silva et al., 2017). Some scholars have also suggested a synergy between EDCs in the environment and human sperm number, and breast, testicular and prostate cancers (Racz and Goel 2010). MPs can perform

concurrently with other substances, exacerbating the undesirable effects (Luo et al. 2014). Of all endocrine disruptors (ED), environmental estrogens are the most investigated (Jobling et al. 1995). Minimizing exposures to EDs is a significant sustainability challenge. The high volume EDs that sufficiently evade water treatment processes to threaten the environment and human health are among the most difficult MPs to manage (Mills et al., 2015; Kinney et al., 2006; Onundi et al., 2017; Swartz et al. 2006).

Steroidal hormones are a collection of biologically dynamic compounds that are synthesized from cholesterol and shares a cyclopentane-o-perhydrophenanthrene ring (Ying et al., 2002). The natural steroid estrogens, estrone (E_1), 17β-estradiol (E_2) and Estriol (E_3) as well as the synthetic, 17α-Ethinylestradiol, (EE_2), are the most extensively studied. This is because of their high estrogenicity at low concentrations and their presence in several matrixes, like drinking, ground and surface water as well as effluents from wastewater treatment plants (WWTP) (Racz and Goel, 2010; Bhandari et al., 2015; Liu, Kanjo and Mizutani, 2009; Zhang and Li, 2014). E_1, E_2 and E_3 are primarily female hormones. They are important for maintaining the health of the reproductive tissues, breasts, skin and brain, while EE_2 is a synthetic steroid, used as a contraceptive and hormone replacement therapy (Racz and Goel 2010). All humans, as well as animals, excrete steroid hormones (Desbrow et al., 1998; Hanselman et.al., 2003; Zheng, Yates and Bradford, 2008) in different amounts, depending on age, state of health, diet, or pregnancy (Zheng et al., 2008; Lintelmann et al., 2003; Makene and Pool 2019). The most compelling estrogen, 17β-estradiol (E_2), and its precursor, testosterone (T), play critical roles in mammalian reproductive procedures. Evidence specifies that these steroids are present in a bioactive form in the excretions of several mammals (Elliott, et al., 2017). The natural androgen, T and the natural estrogen, E_2, will end up in the environment through sewage discharge and animal waste disposal (Kim et al. 2007a).

In the CoCT rivers treated sewage runoff and natural surface water mixture are used straight for irrigation of agricultural zones. The Western Cape Province, South Africa, has very little or no rain in summer and high of rainfall in winter. During the summer months, most of the water in these rivers is treated sewage effluent (Swart and Pool 2007). Thus, estrogens and androgens are not totally removed by MWWTPs, vindicating the project of this research to find additional effective processes to remove these intractable contaminants. Given that conventional treatment processes are inefficient, the scientific community has set out in search of novel processes and operating conditions which might increase treatment efficiency of wastewater (Dewil et al. 2017; Myburgh and Aziz 2019). NF/RO membrane filtration processes have also been used to yield high-quality water from non-traditional sources such as brackish water, seawater or secondary

treated wastewater (Khawaji et al. 2008; Khan et al. 2014a; Aziz and Kasongo 2019; Hacıfazlıoğlu et al. 2019a). Pilot and full-scale NF/RO applications have confirmed the capacity to remove a large range of MPs by various studies. (Bellona et al. 2008; Al-Rifai et al. 2011; Nguyen et al. 2013).

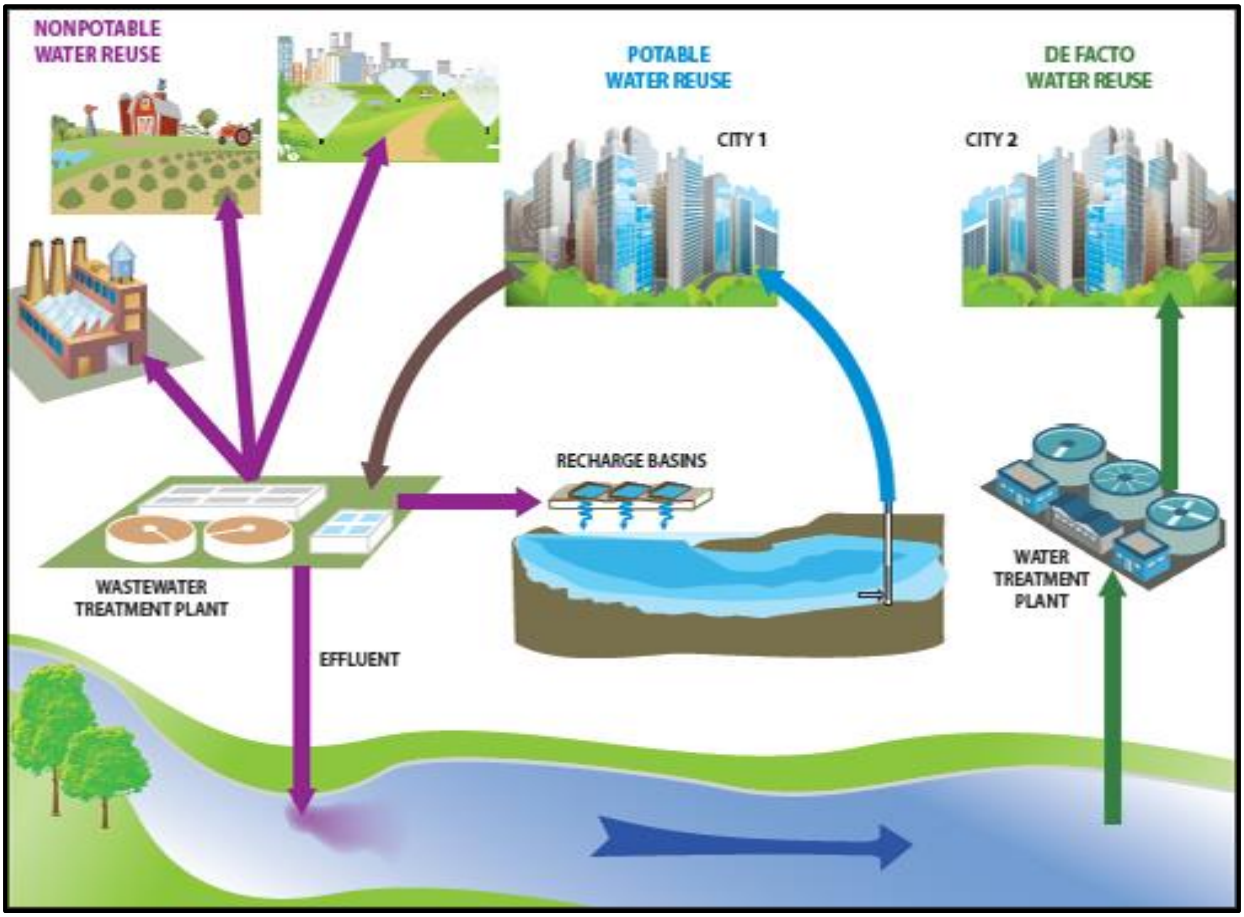

Figure 2. 1: Water recycling and reuse is the process of collecting, treating and using wastewater, particularly from municipalities, industry, and agriculture (dailymarketinsight.com)

Studies have shown a critical involvement of the androgen receptor in abnormal sexual growth. The occurrence of trace organic impurities with destructive properties on the human androgen receptor (hAR) has been described as emanating from paper-mill effluents and as a result of intensive farming (Jenkins et al., 2003; Lemaire et al., 2004). Chemical and immunological approaches are frequently used to spot steroid hormones in food, medical practice, and environmental samples. Owing to the excessive variability of chemicals with hormone-like activity, these methods have the disadvantage that they only measure the compound of interest and are not able to determine the biological activity of unknown compounds and their metabolites. Biological (receptor based transcription activation) assays can be used to detect all compounds

having an affinity for a given receptor (García-Reyero et al., 2001; Mueller, 2002). The receptor gene bioassays are different from the receptor binding assays, in that they include transactivation step, which enables them to distinguish between receptor agonists and receptor antagonists. This property strongly supports identification of both known and unknown compounds (Sonneveld et al., 2006; Bovee and Helsdingen, 2007).

Chemical analysis alone is insufficient to monitor endocrine-disrupting chemicals present in water due to their complex blend and widespread range of impurities. In-vitro bioassays connected to hormonal activity, which includes androgenic, progestagenic, glucocorticoid, thyroid and estrogenic activity. These assays can be used to measure endocrine activity in environmental waters such as wastewater effluent and surface water (Ain et al., 2014; Scott et al., 2014; Thomas et al., 2002) with less focus on cleaner water sources, such as advanced treated or drinking water (Conley et al. 2017). In addition, most chemical analytical procedures are not enough to recognize all the environmental chemicals and to predict a grouping of toxicity and bioavailability. It is also very expensive compared to bioassays.

According to Aneck-Hahn et al. (2009), it is better to screen samples, using moderately low-cost bioassays for signs of toxic properties and then to prioritise them for chemical analyses or more rigorous investigations. Most of the literature emphasises detecting agonistic activity in the water, but some environmental pollutants can act as antagonists, which, if present in a screen sample, can decrease the agonistic response in-vitro. It is important to evaluate both agonism and antagonism in the environmental samples (Ihara et al. 2014). Many endocrine-disrupting chemicals are present in the water environment at low concentrations, which stresses the necessity of sensitive methods to detect both endocrine agonists and antagonists at environmentally relevant levels. The estrogenicity of a compound is determined by its ability to bind to the estrogen receptor, thereby mimicking or blocking the activity of natural estrogens (Leusch et al. 2017). The human estrogen receptor contains a gap in the ligand-binding domain which is almost double the size required for E_2, thus providing space for a variety of other molecules to bind with the estrogen receptor. Total estrogenicity of a sample is commonly measured in order. To avoid comprehensive chemical analysis in complex screening samples, which also include wastewater samples, total estrogenicity of a sample is measured first (Racz and Goel 2010). There is thus a strong need for rapid, simple, and cost-effective methods for quantitative analysis of estrogenic hormones, such as enzyme-linked immunosorbent assay (ELISA). ELISA kits are commercially available for the quantification of estrogenic and androgenic hormones. Large numbers of samples can be analysed simultaneously and machines

that do the readings are relatively cheap and available in a portable format that can be used in field studies (Swart and Pool 2007).

In vitro bioassays are central to the ecotoxicological assessment of water and wastewater quality due to their toxicity determination caused by complex samples which are regarded as a specific mode of action (Escher et al. 2014, 2018; Leusch et al. 2017). Bioassays are normally used in monitoring operations and are adequately progressive to be integrated into water and wastewater guidelines (Escher et al. 2018; Altenburger et al. 2019; Neale and Escher 2019).

The yeast estrogen screen (YES) and the ER-CALUX are bioassays that use reporter genes to quantify complete estrogen activity of a sample. Several assays have been developed for this purpose, using both mammalian and yeast cells. Transcription activation assays based on mammalian, or more particular human, cell lines have been shown to be more sensitive than yeast-based assays (Racz and Goel 2010). Metabolic conversion can either activate or inactivate some compounds (Hoogenboom LAP et al. 2001), whereas the relatively low metabolic capacity of yeast, ensures that the test reflects the activity of the original compound. The advantage of yeast-based assays is that they are inexpensive and easy to apply. (Graumann et al. 1999; Dhooge et al. 2006). Yeast cell assays are really vigorous and endure extracts from murky sample matrices such as sediments, urine, and feed (Rehmann, Schramm and Kettrup, 1999; Michelini et al., 2005; Bovee and Helsdingen, 2007).

2.2 Water shortage and challengers

Water is available through groundwater, river water and water from canals or irrigation ponds. The Western Cape's water supply is from 36 dams which have a storage capacity of 1841.7 million cubic meters. Cape Town, dam levels were at their lowest for almost a decade, due to drought in 2016 and 2017. The average dam levels for those two years were 35.6 and 21.1 %, respectively (Basson et al. 2018).

In the present planning situation it is predicted that water demand in regional water resource network will exceed supply by 2020. Known as the Western Cape Water Supply System (WCWSS), this network supplies greater Cape Town and the province's west coast. The province's water resources are also becoming increasingly vulnerable to climate variability. Climate models show that the Western Cape will become warmer and drier, leading to reduced water availability, while experiencing more strong rainfall events (Siebrits and Fundikwa 2017). The Breede-Gouritz and the Berg-Olifants are two major water management areas (WMAs) within the Western Cape Province. Irrigation to support agriculture is the major water use in these two WMAs, followed by urban water uses. Given its impact on the agricultural sector, this growing scarcity will have a negative effect on the country's economy. In 2015/16, the impact of the worst South African drought since 1904 was felt countrywide. Droughts are caused by the cyclical El Niño weather pattern, and the country has always had variable rainfall. However, the effects of climate change mean the country will continue to experience increasing water scarcity and rainfall variability (Siebrits and Fundikwa 2017).

South Africa is a semi-arid country due to geographic location where the amount of rain is low at an average of 5mL per annum and evaporation rates are high with an average of 17mL per annum. This causes a depletion of the availability of fresh water. Potable water is of the highest quality and is frequently used for landscape irrigation, toilet and urinal flushing and industrial applications. This practice is not practical in the context of water shortage (Adewumi et al. 2010).

The drought had a negative impact on the economy of the province, where businesses shutdown, financial losses and retrenchments followed. Water restrictions pressurised businesses and residents to reduce their demand for municipal potable supply. The drought has also resulted in municipalities procuring emergency augmentation supplies and re-assessing their longer-term plans for drought-resilient supply options. (Basson et al. 2018)

According to Adewumi et al., (2010), the possibility of wastewater recycling for non-drinking water necessities is a feasible substitute in overcoming the trials of the present and imminent water shortages in South Africa. A wide range of effective wastewater treatment technologies exist, and successful wastewater reuse applications have been observed in many countries like Singapore, USA, Israel, Australia, Spain, Germany, United Kingdom and many more. Wastewater originates from different sources ranging from industrial to municipal.

The World Economic Forum (WEF) ranked South Africa as the third-highest risk of doing business in 2017 due to the water predicaments. South Africa is ranked as the 30th driest country in the world with risky climate and rainfall variations. Municipalities are interested in drinking water reuse of their municipal wastewater. In the City of Cape Town, there is a potential market of around R2 billion. Over the next 10 years, R70.4 billion of investment in national water infrastructure will be needed to ensure potable water supply (Basson et al. 2018).

In South Africa, municipal wastewater reuse can be promoted due to the high volume of its population.

2.3 Municipal wastewater treatment processes

Melvin and Leusch (2016) have specified five treatment technologies for MSWW treatment. These technologies include; conventional activated sludge (CAS), oxidation ditches (OD), ponds and constructed wetlands (PCW), trickling biological filters (TBF) and membrane bioreactors (MBR).

2.3.1 Problem-related to the discharge of municipal wastewater effluent

Robinson et al. (2016) state that when secondary MSWW effluent is discharged into surface water and waterways, the risk of triggering pervasive ecological responses includes the negative effects on the physiology, development, and survival of certain ecologically significant water plants; it also disrupts the ecological balance among vascular plants and algae and has a direct effect on the mortality rates of fish and other invertebrates.

2.3.2 Reverse osmosis (RO) and nanofiltration (NF) membranes

According to Cartagena et al. (2013), nanofiltration (NF) and reverse osmosis (RO) are the most promising technologies for the exclusion of emerging micro-pollutants (MPs). (Lee et al. 2008; Dolar et al. 2012) concur that the reverse osmosis (RO) process can be used to remove organic

and inorganic impurities present in secondary municipal effluent for reuse applications. The nanofiltration and reverse osmosis membranes remove most of the MPs that occur in the MBR effluent. NF membranes need less energy than the RO membranes, although many energy-saving RO membranes are available. These RO membranes produce high flow rate rejections at low pressures (Cartagena et al. 2013).

According to Porter (1989) and Kucera (2011), the phenomenon of osmosis, which is the diffusion of a liquid through a semi-permeable membrane, was discovered in 1748. The liquid diffuses from a channel with a low solute concentration to a channel with a higher solute concentration until an equilibrium is reached. When pressure is applied, water flows through a membrane from high to the low solute concentration side and the separation of water from the solution is attained.

Table 2.1: Characteristics of composite polyamide RO membranes. (Kucera 2015).

Property	**Value for PA Membranes**
Membrane Type	Homogenous asymmetric, thin-film composite
Pore size	~0.67 nm
Salt rejection (%)	~98+
Silica rejection (%)	~96+
pH range	2-12
Feed pressure (brackish membrane)	8.6 - 27.6 bar
Temperature tolerance	Up to 45°C
Surface charge	Negative (anionic)
Chlorine tolerance	< 0.02 ppm
Biological growth	Causes membrane fouling
Fouling tolerance	Fair
Surface roughness	Rough

2.3.3 Fouling

Xu et al. (2010) say that membrane fouling is a serious challenge when operating membranes in water and wastewater treatment plants, irrespective of the enhancement in membrane technologies. Dissolved solids present in the secondary effluent can adsorb, accrue or precipitate within or on the membrane surfaces, resulting in membrane fouling.

Declining membrane performance and lifespan over an extended time period is a foremost challenge in the treatment of wastewater due to fouling. Therefore, various approaches have been established to alter membrane surface characteristics to improve membrane performance

and minimize fouling. Previous studies have found that membrane surface characteristics and interaction between membrane surfaces and foulants causes to fouling the mechanism (for example Kang and Cao 2012).

The quality of the influent is a deciding factor that will show the extent of membrane fouling with RO (Khan et al. 2014b). Fouling during wastewater treatment is more intricate than fouling that occurs in the treatment of seawater, due to the complex compounds in the feed (Tang et al., 2016). During RO fouling, a cake layer is formed by colloids and interactions between organics and adhesion and growth of bacteria onto the surface of the membrane and this is due to scaling by inorganics. These mechanisms may take place concurrently as well (Liu et al., 2015; Tang et al., 2014)

Pandey et al., (2012) indicate the presence of a large portion of colloidal, soluble impurities, as well as suspended solids in UF. RO fouling is due to treated effluent and more specifically, effluent organic matter (EfOM). When biologically treated secondary effluent is treated with RO, foulants such as low-molecular-weight dissolved organic components, soluble salts, metal oxides, hydroxides and biological agents form the bulk of the membrane fouling.

2.3.4 Remediation of membrane fouling

According to Sun et al. (2016), the aim of membrane modification is to handle the fouling effects when polyamide (PA) thin-film composite (TFC) RO membranes are used for the removal of impurities. Membrane modification is classified into two groups namely: the modification of the membrane matrix (or bulk modification), which include blending and copolymerizing; and secondly membrane surface modification, which introduces polar groups or grafting hydrophilic monomers to the membrane surface.

Nguyen et al. (2013) confirm that membrane surface modification can efficiently inhibit the growth of microorganisms as it provides bacteriostatic properties to the membrane. Saqib and Aljundi (2016) indicate that membrane surface modification can be done through surface coating or surface grafting. However, it is pointed out that the weakness of surface coating is that the fragile interaction between the membrane and the coated layer ends up sweeping away that layer. Modification by surface grafting is one of the favoured methods to alter membrane surfaces and minimize fouling. This involves covalent bonding among grafted chains with associated membranes.

2.4 Conventional activated sludge (CAS)

A representative process for municipal wastewater entails primary, secondary and tertiary treatments. The consequential effluent is low in turbidity and can be disinfected before discharge.

Radjenović et al., (2008) divide a conventional activated sludge (CAS) treatment process into the following stages:

- Pre-treatment which includes screening and gridding;
- The physical process of settling in a primary clarifier using a primary sedimentation unit;
- Secondary biological treatment with an anaerobic and aerobic process;
- The effluent of the biological treatment is then sent to secondary clarifiers;

Peinemann and Nunes, (2010) demonstrate the CAS system in the diagram in Figure 2.1, below:

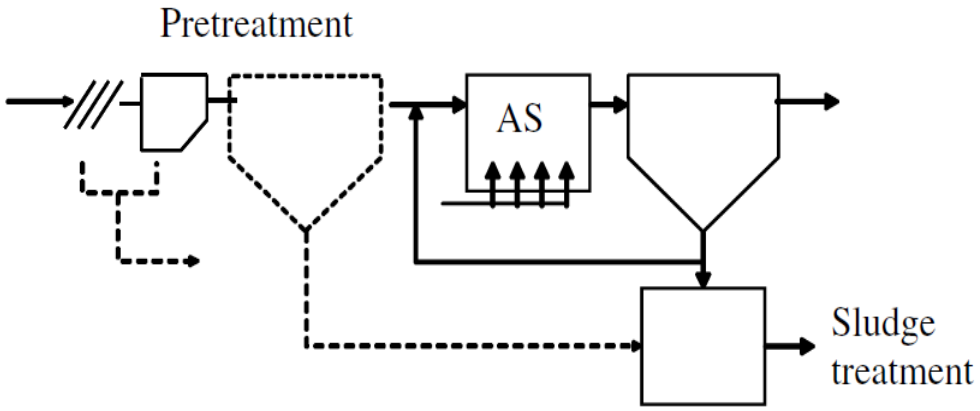

Figure 2.2: Conventional activated sludge system (adapted from Peinemann and Nunes, 2010)

According to Çeçen and Aktas (2011) conventional activated sludge process stabilizes the wastewater aerobically or anaerobically where the growth of an activated mass of microorganisms is used. This process removes organic matter, most nutrients, nitrogen, phosphorus, as well as trace concentrations of inorganics.

During this process, the total dissolved solids are not removed, and the water is not conducive to recycling. When tertiary effluent from a conventional treatment process is supplied to an RO system, it normally fouls. This fouling entails colloidal, biological, scaling and organic fouling (Bartels et al. 2008).

2.5 Membrane bioreactor (MBR)

Judd, (2015) says that a membrane bioreactor (MBR) is a hybrid system of biological and filtration treatment, where micro or ultrafiltration membranes are used. MBR has received much attention as it produces clarified and largely disinfected effluent. Lazarova and Bahri (2004) indicated that MBR can be used as a final treatment process in water reuse if followed by disinfection of the effluent.

Radjenović et al. (2008) stated that for the past 25 years the MBR, one of the advanced technologies, has gained more attention over the CAS process due to high quality treated effluent. MBR is a possible solution for safe water reclamation.

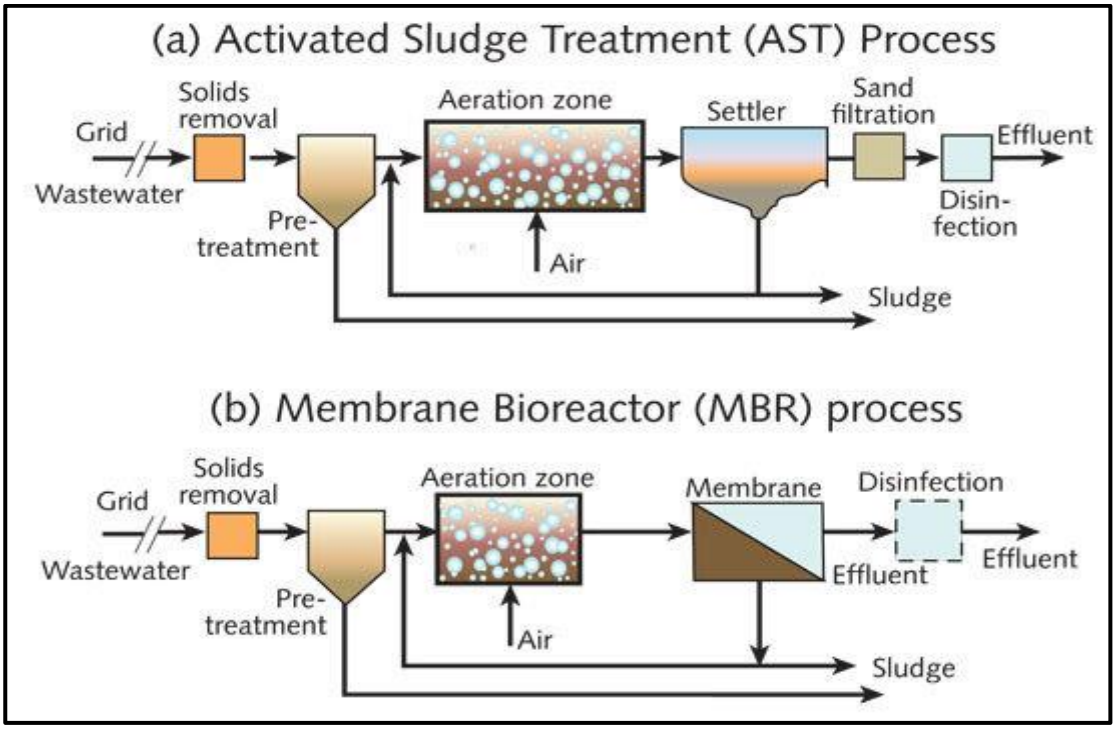

Figure 2.3: Conventional wastewater treatment stages and an alternative membrane-based process scheme. (a) Conventional activated sludge treatment (CAS) and (b) Membrane bioreactor treatment (MBR), (Pandey et al. 2012)

The biological degradation of organic matter present in wastewater with membrane filtration is an integrated technology that surpasses the shortcomings of the CAS treatment such as limited operational solids retention time (SRT) and sludge settling characteristics. Results comparing biological performances of the full-scale conventional activated sludge and pilot-scale hollow-

fiber (HF) and flat-sheet (FS) membrane bioreactor are shown in Table 2.2 (adapted from Radjenović et al. 2008).

Table 2. 2: Comparison of biological performances of CAS, HF and FS-MBR (Radjenović et al. 2008)

		Average concentration (mg/L)	
Parameter	**CAS effluent**	**HF MBR**	**FS MBR**
TSS	20	1.5	2
NH_4	30	0.4	0.8
BOD	88	40.5	31
COD	15	4	4

Table 2.3: water quality requirement: a case of irrigation (agriculture, landscape, turf grass) (adapted from Pedrero et al., 2010).

	Degree of restriction use			Unit
Salinity	**Low**	**Moderate**	**severe**	**dS/m**
EC	≤0.7	0.7–3.0	≥3.0	mg/l
TDS	450	450-2000	2000	
Sodium				
Surface irrigation	≤3	3–9	≥9	mg/L
Sprinkler irrigation	≤70		>70	mg/L
Chloride				
Surface irrigation	≤140	140–350	≥350	mg/L
Sprinkler irrigation	mg/l ≤100		>100	mg/L
Nitrogen (total)	≤5	5-30	>30	mg/L
Bicarbonate	≤90	90-500	>500	mg/L
Residual Chlorine	≤1	1-5	>5	mg/L
pH	Range from 6.5-8.			

According to Sabrina et al. (2013), before membrane filtration, the wastewater biological treatment converts the particulates into end products. It is important to ensure that the secondary wastewater treatment provides water quality good enough for reuse applications. Failure to properly treat and manage wastewater could result in adverse health effects (Pedrero et al. 2010).

Hoinkis et al. (2012) stated that the exclusion of impurities from MBR secondary effluent is only effective to a certain extent, so it only applied as a pre-treatment process. If higher water quality water is wanted, a successive added membrane tertiary treatment step should follow for the purpose of water reuse.

MBR technology combines suspended biomass, similar to the CAS process, with immersed microfiltration or ultrafiltration membranes that replace gravity sedimentation and clarify the wastewater effluent (Sert et al. 2017). Applications of MBR in developing countries include the direct treatment of raw sewage, specifically in megacities. Valuable resources are extracted from sewage, such as clean water and nutrients namely, nitrogen (N) and phosphate (P), as well as energy. The small footprint, flexible design, and automated operation of MBR make it ideal for restricted, decentralized sewage treatment in the developing world. One of the growing applications of MBR is waste pre-treatment prior to RO, which, when followed by UV disinfection, can produce water for indirect or direct potable use (Sert et al. 2017; Tay et al. 2018).

Findings of Xu et al. (2006) indicated the exclusion of emerging trace organic contaminants with RO performs similar to NF and ULPRO membranes (MWCO of 200 Da and less) while operating at lower feed pressure. For tight high-pressure membranes, the membrane surface charge is more important for rejection than the MWCO, although a minimal MWCO is necessary.

According to Adham et al. (2001) and Snyder et al. (2007), conserving natural resources used for drinking water supply wastewater reclamation is acquiring more attention globally. MBR technology has proven to be a viable pre-treatment step due to the superior effluent water quality before the RO as a final polishing step in reducing the salinity of reclaimed water.

Porter (1989), further states that the major removal mechanism in membrane filtration is straining, or size exclusion, so the process can theoretically achieve perfect exclusion of particles regardless of operational parameters such as influent pressure and concentration. Reverse osmosis, however, involves a diffusive mechanism so that separation efficiency is dependent on solute concentration, pressure and water flux rate.

Cartagena et al. (2013) and Sert et al. (2016) state that the MBR system is one of the most suitable technologies for secondary wastewater treatment. The advantages of MBR technology over CAS plants are better effluent quality, good disinfection, smaller footprint and reactor volume, less sludge production and the possibility of operating at higher biomass concentrations.

According to Tam et al. (2007), a major obstacle to the efficient application of MBRs in current or next-generation reuse systems is membrane fouling, particularly when it leads to flux losses that cleaning cannot restore.

2.6 The reclamation of municipal sewage wastewater

According to the Singapore Water Reclamation Study (NEWater Expert Panel 2002), when discussing the reuse of treated effluent for potable purposes, the following definitions are useful to distinguish between "indirect" and "direct" potable reuse and between "planned" and "unplanned" potable reuse. According to this study, Planned Indirect Potable Reuse (PIPR) is the treatment and supply of water for drinking from natural source water (dam, river, lake or aquifer) that is purposely and partly fed by the discharge of treated wastewater effluent. This type of potable reuse is becoming more common as other viable water sources become scarcer because of population growth and watershed urbanisation. Examples of this application are the Water Factory 21, Orange County Water District, Southern California and Upper Occoquan Sewage Authority (UOSA), Virginia, all situated in the U.S.

Unplanned Indirect Potable Reuse (UIPR) occurs when a water supply is abstracted for potable purposes from a natural source (surface or groundwater) that is fed in part by the disposal of wastewater effluent (treated or not). The potable reuse of the wastewater effluent is not an intentional part of the effluent disposal plan. This type of potable reuse occurs whenever an upstream water user discharges wastewater effluent into a water source (dam, river, lake or aquifer) that serves as a water supply for a downstream user. Large communities unintentionally have been practising unplanned indirect potable reuse. Some examples are the Rhine and Thames rivers in Europe, Mississippi River in the U.S., Yangtze River in China, and Mekong River in Indo-China. Direct Potable Reuse (DPR) is the instantaneous adding of reclaimed water to the potable water distribution system. This practice has been applied in Windhoek, Namibia (NEWater Expert Panel 2002).

Le-Minh et al. (2010) agree that municipal water recycling for industrial, agricultural, and non-potable municipal uses is an increasingly important component of water resources management practices in many parts of the world. In some countries, such as the USA, Singapore, Mexico and Belgium, treated effluents are intentionally used to supplement drinking water supplies.

Municipal wastewaters are mostly treated by conventional activated sludge or membrane bioreactor processes. The MBR process is simply an integrated treatment system of microfiltration (MF) or ultrafiltration (UF) membranes with a biological treatment reactor. Secondary treatment effluents such as MBR effluents often include high concentrations of dissolved matter, pesticides, pathogens heavy metals and micropollutants (Hacıfazlıoğlu et al. 2018).

This makes a further treatment necessity to reclaim water for reuse. There is still a void of information about tertiary processes and their EDC exclusion capacity from environmental matrixes at trace concentration in MWWTPs (Silva *et al.*, 2017).

Some studies have determined that an integrated system of MBR-NF/RO could be considered as a good alternative for recapture and reuse of treated wastewater for irrigation (Sert et al. 2017; Cinperi et al. 2019). Water reclamation (reuse) refers to the treatment of used water or wastewater to the quality suitable for water recovery by Reverse Osmosis (RO) process. The secondary effluent from an MBR treatment can be used directly as feed water to the RO treatment process (Qin et al. 2006; Chen and Lin 2016; Tay et al. 2018). RO processes are endorsed for rejection of various compounds whose molecular weight is up to 200 g/mol (Sui et al. 2010). Therefore, it has been reported that rejections of micropollutants are up to 99 % with the use of RO membranes (Linares *et al.*, 2011; Garcia *et al.*, 2013). In some cases, traces of MPs remained in the effluent, which might require a final step of removal. (Nghiem *et al.*, 2004; Heo *et al.*, 2013).

2.6.1 Micro (MF) and Ultra-Filtration (UF) membranes

The fouling in CAS treatment can be minimized or prevented, if the West Basin model (in the USA) demonstrated by Hydranautics (membrane manufacturer) is followed. After the municipal primary wastewater, secondary feed is produced. This is followed by tertiary treatment which consists of microfiltration (MF) or ultrafiltration (UF) hollow fibre membranes and produces RO feed water of turbidity of 0.03NTU (Bartels et al. 2008).

A feasibility study was conducted by Qin et al. (2005) for the reclamation of secondary treated sewage effluent from mainly industrial sources using MF or UF/RO process. It concluded that the the quality of the treated water from the UF-RO membrane process is comparable with the NEWater system for 18 out of 22 specific parameters. Qin et al. (2005) also conducted a pilot plant study for the reclamation of secondary treated sewage effluent using an MF/RO system. The product water generated is comparable with the quality of NEWater.

2.6.2 Reverse Osmosis (RO) and Nano Filtration (NF) membranes

Arceivala and Asolekar (2007) confirmed that RO membranes have successfully treated and provide water which exceeds reuse quality requirements. Numerous large-scale commercial membrane plants are now being used to reclaim municipal wastewater. Examples of these plants are the 50 000m^3/day West Basin, California; the Kranji 40 000m^3/day and the 32 000m^3/day Bedok plants in Singapore; the 270 000m^3/day plant in Orange County in California and the big 380 000m^3/day plants in Sulaybia, Kuwait. With these examples, Bartels et al. (2008)

demonstrate the acceptance that this RO based technology has gained over the years for water reclamation. This study further mentions that conventional polyamide and low fouling membranes have been used successfully at all the above-mentioned plants.

In the past decade, the use of MBR in municipal wastewater treatment has grown widely. This is due primarily to more stringent effluent water quality requirements, space constraints, lower operator involvement, modular expansion characteristics and consistent effluent water quality capabilities. MBR provides a viable and cost-effective alternative to conventional treatment within a considerably reduced footprint. Pathogens, viruses and other constituents of concern, which are not typically reduced to desirable levels by CAS processes, are reduced to regulation standards by MBR treatment (Bunani et al. 2015).

MBR technology combines CAS treatment with low-pressure membrane filtration, thus removing the need for a clarifier or polishing filter. The membrane separation process provides a physical barrier to contain microorganisms and assures consistent high-quality reuse water. MBR technology is also ideally suited for an array of municipal and industrial wastewater applications such as irrigation, aquifer replenishment, wetlands development, industrial process water, boilers and cooling systems(Arceivala and Asolekar 2007).

The reuse of wastewater can assist in maintaining environmental quality and relieving the unrelenting pressure on conventional and natural fresh water sources. Cleaner secondary effluent will be flushed downstream. This will minimise trace elements in the water and will have a lesser negative effect on the environment such as wastelands, etc. (Larsson et al. 1999). According to Etchepare and van der Hoek (2015), reuse of water to supplement limited supplies has been a longstanding water strategy. The South African Water Act of 1956, which also applied in Namibia, makes a return of well-treated effluents to source a condition for abstraction. This approach ensures the extension of limited supplies and leads to indirect reuse. Apart from this indirect reuse through a return to the source, extensive reuse through irrigation, industrial use, and even potable supply is practised in Southern Africa.

2.7 Membrane surface modification

The demand for membrane technology in the field of wastewater treatment, medicine, pharmaceuticals, gas separation, and desalination has become significant worldwide. The properties of polymers are of primary importance in many industrial applications. Much attention has been paid to membrane modification as the performance of membranes is strongly influenced by their structures. Furthermore, the membrane surface can be contaminated, leading to deterioration in membrane performance. Thus, many studies have been devoted to the surface modification as a way of modifying the intrinsic properties of membranes and imparting improved anti-fouling, easy-cleaning, lower flux decline and higher rejection properties ((Khulbe et al., 2004; Wang et al., 2016).

According to Kang et al., (2011), membrane modification ranges from physical to chemical treatment. Both can be applied for the improvement of surface morphology and properties.

The treatments are classified as follows:

- Surface adsorption
- Surface coating
- Hydrophilization treatment
- Surface grafting

2.7.1 Surface modification by graft polymerization (chemical reaction)

Grafting involves the attachment of polymeric chains on the surface of a polymeric membrane. Although this method is used for any polymeric materials, it is mostly used for the surface modification of polyamide thin-film composite membranes or porous polypropylene membranes (Xu et al. 2009).

The membrane surface can also be modified by chemical reaction. Hydrophilic materials are grafted on the surface of the membrane to improve the hydrophilicity of the surface of the membrane thus reducing adsorption of foulants to the surface of the membrane. The hydration layer produced by hydrophilic material is accountable for the enhanced properties of the membrane surface (Shahkaramipour et al. 2017).

Freger et al., (2002) state that polymers attached to the surface of a membrane grow by propagation and factors such as modification time, polymer concentration and initiator concentration affect the coverage of the surface by polymers.

Modifying the surface via chemical bond formation also improves membrane chlorine resistances and anti-biofouling properties. High chlorine-resistance polymers such as poly N,N-dimethylaminoethyl methacrylate and polyvinyl alcohol can be used for the treatment of membranes (Wei et al. 2010).

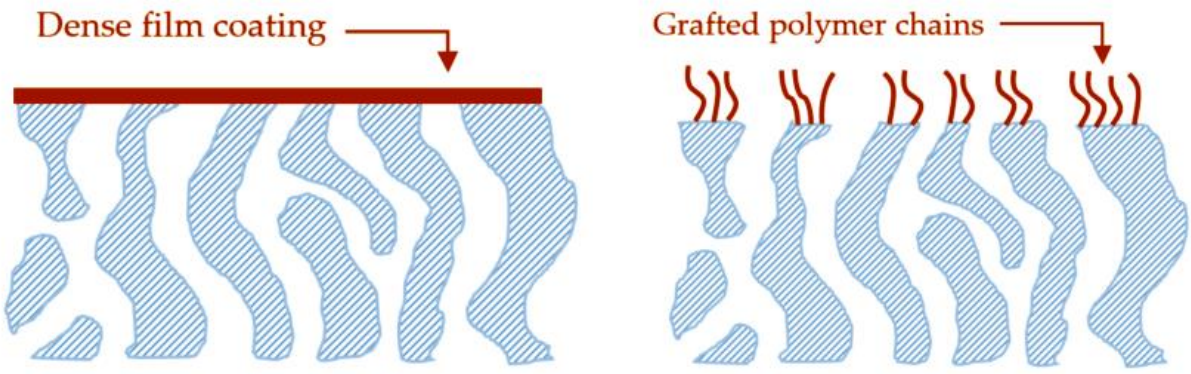

Figure 2.4: Diagram showing the difference between grafting chains onto the surface of the membrane and coating onto the surface of the membrane (Shahkaramipour et al. 2017)

2.7.2 Modifying agents used in this study

According to Rana and Matsuura, (2010), different studies have reported the use of dimethyl aminoethyl (meth) acrylate (DMAEMA) for the graft polymerization of membrane surfaces where fouling tests were conducted using Escherichia coli bacterial model foulant as well as bovine serum albumin (BSA) and lysozyme solutions. The authors pointed out a considerable improvement was observed with surface-modified membranes when fouling was eradicated. Polyvinyl alcohol has been used in the modification of NF and RO membrane surfaces for the treatment of aqueous solutions containing humic acid as model foulant as well as dyeing wastewater effluent. It was reported that the modified membrane considerably reduced fouling.

Hu et al. (2016) used polyvinyl alcohol to improve membrane anti-fouling properties and membrane resistance to chlorine exposure, on a TFC RO membrane. The membrane was treated with aqueous glutaraldehyde (GA) followed by polyvinyl alcohol (PVA) aqueous solution. Modification of the membrane was observed through the change of membrane physio-chemical properties. Fouling tests were conducted using BSA aqueous solution. PVA content of modifying solution was altered to improved salt rejection and water flux of the membrane. Results revealed that that PVA modified membranes presented resilient resistance to negative effects imposed by the chlorine disinfectants. Furthermore, the modified membrane was tested using an industrial effluent from a textile factory with a conductivity of 0.319 S/m; and COD of 135.4 mg/l. It is indicated that the modified membrane could improve rejection and resistance to fouling in tertiary treatment of industrial effluent.

2.8 UF, NF, RO process parameters

2.8.1 Salt rejection

Reverse osmosis systems are used to remove dissolved salts; measuring salt rejection is, therefore, a direct way to monitor the performance of such systems. Salt rejection describes the percentage of the feedwater TDS that has been removed in the permeate water. The way to monitor salt rejection is to measure permeate water conductivity. The permeate water conductivity should be measured for each pressure vessel daily. This helps to determine if a high salt passage problem is universal (indicating membrane damage); if it is isolated to a certain stage (possible fouling) or if it is isolated to an individual pressure vessel. Probing of individual pressure vessels can also be carried out to isolate a salt rejection problem to an individual membrane element (Kucera 2011).

The flux observed salt rejection is calculated according to the following formula (Zhang et al. 2019):

$$R = \frac{C_f - C_p}{C_f} \times 100 \qquad \text{(Equation 3)}$$

C_f (µS/cm), C_p (µS/cm) and R (%) are the feed conductivity permeate conductivity and salt rejection respectively (Brusilovsky et al. 1992; Farrokh Shad et al. 2019; Hacıfazlıoğlu et al. 2019b).

2.8.2 Flux

Water flux is typically expressed as volume per area per unit of time. Flux is used to express the rate at which water passes through the surface of a reverse osmosis membrane. The flux of an RO membrane is directly proportional to temperature and pressure. The solute flux is the amount of total dissolved solids that has permeated a given area of the membrane per unit of time. Solute flux depends on the concentration gradient and not driving pressure. Therefore, with increasing driving pressure, the concentration of solute in the permeate decreases due to constant salt leakage and increased water flux (Kucera 2011; De Souza et al. 2018 Rana et al., 2015; de Souza et al., 2018; Aziz and Kasongo, 2019).

The flux can be calculated according to the following formula (Hu et al. 2016, Bruslovsky et al., 1992; Kasongo et al., 2019; Hacıfazlıoğlua et al., 2019 and Shad et al., 2019):

$$J = \frac{V}{A \times \Delta t} \qquad \text{(Equation 4)}$$

V , A , Δt and J are the volume of permeate (L), the effective area of the membrane (m^2), the interval time (hr) and the permeate water flux respectively.

2.8.3 Pressure

Geankoplis, (2003) says that operating pressure in reverse osmosis is mostly in the range of 1035 KPa to 10 350 KPa. Kucera, (2015) states that operating pressure has a direct effect on the water flux, while its effect on the salt rejection is indirect. When feed pressure increases, the permeation of water molecules through the membrane increases while solute molecule passage stays more or less unchanged, hence the permeate contains a lower concentration of solutes (Geankoplis 2003). An increase in pressure drop generally results from a disruption in the flow pattern through the membrane, usually caused by the accumulation of material on the surface of the membrane (Kucera 2011).

2.8.4 Recovery

The percentage recovery is the fraction of the feed water which becomes permeate water. When the recovery rate is increased, there is less concentrate recycled but rather more collected as permeate. A high recovery rate can also cause soluble salts to precipitate. However, if the percentage recovery is too high for the RO design then it can lead to larger problems due to scaling and fouling (Baker 2004; Kucera 2015).

2.8.5 Temperature

Assume that the temperature of the feed being treated is an essential parameter to observe in the RO process. It is known that the effect of temperature on the salt rejection and water flux is complex. Passage of both solute and solvent (water) increases exponentially with increasing temperature. It has been reported that the water flux doubles as the temperature is increased by $30^{O}C$; meanwhile, the salt rejection declines slightly as the temperature increases (Porter, 1989; Baker, 2004).

2.8.6 pH

The stability of polyamide thin film composite membrane is susceptible to pH of the feed solution. However, the PA TFC membranes can operate within a pH ranging from 2 to 11. It should be noted that at a higher temperature it is required to operate within narrower ranges of pH. The pH also affects the rejection efficiency of the membrane. Highest rejections of most species are found

to be in the pH range of 7 to 7.5. The reason for the low salt rejection in the scenario of higher or lower pH operating condition is unfortunately not well explained in the literature; however, it can be attributed to stems from the ionic state of the rejected ions, as well as some changes on the molecular level with the membrane. On the other hand, the pH barely affects the water flux as it stays somewhat constant over the range of pH (Baker 2004; Kucera 2011)

2.9 Membrane surface characterization

Zhao et al. (2013) state that characterization of the membrane after modification is critical to confirm whether the changes of composition (FTIR, NMR and XPS), morphological structure (AFM, SEM) and performance are desirable. Different analyses that most researchers use to study the structure of the modified membrane include the following:

2.9.1 Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR)

Fourier transform infrared spectroscopy is a quantitative and qualitative analysis method which allows the direct study of the chemical composition of material surfaces (Schmitt and Flemming 1998).

2.9.2 Atomic force microscopy

According to Chennamsetty et al. (2006), the atomic force microscopy (AFM) is used to investigate the surface morphology of the membrane. The analysis offers a simple and fast method of detecting the surface structure of a wide range of materials. It allows obtaining of 3D topographic data with a high vertical resolution. Moreover, AFM analysis also allows the calculation of average roughnesse's of the surface, the maximum peak-to-valley distances and the surface areas of the membranes (Cahill and Freger 2017).

2.9.3 Nuclei magnetic resonance

Nuclei magnetic resonance (NMR) analysis can be conducted on a solution state basis or solid-state basis. Both methods are good in analysing the chemical composition and they require signals from different chemical sites being analysed to be resolved from each other in some ways (Duer 2008). Cahill and Freger (2017) state that solid-state NMR spectroscopy allows studying the molecular motion and molecular-level free-volume characteristics of the thin-film polymers.

2.9.4 X-ray photoelectron spectroscopy (XPS)

Cahill and Freger (2017) conducted XPS analysis and studied C, N, and O core levels of aromatic rings, amide groups, and carboxylic acid groups. The results revealed an increase in the amide linkages relative to the pendant carboxylic acid groups in the thin film composite RO membrane formed through interfacial polymerization with the addition of dimethyl sulfoxide (DMSO).

2.9.5 Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM)

Micro-techniques often used in the characterization of morphology and structure of modified membranes include SEM and AFM. Straight observation of the features of surfaces and cross-section of modified membranes with the assistance of micro-technique can directly reveal the difference between morphologies and structures before and after modification (Zhao et al. 2013).

2.10 Physicochemical properties of MPs

2.10.1 Molecular Weight and Size

The molecular weight (MW) is the molecular mass of a compound. MW can be used for the rejection prediction of non-charged and non-polar compounds in low pressure applications. It is the most used parameter showing molecular size, although it is not a direct measurement of size. The diameter projects the molecule onto a membrane surface. More size descriptors are molar volume (MV), molecular length, molecular width, molecular depth and equivalent molecular width. Geometrical descriptors of molecular length and widths with the Stokes radius of the same molecule is included. (Kiso et al. 1992; Ozaki and Li 2002; Van der Bruggen et al. 2002).

2.10.2 Solubility

The solubility of a compound in water reflects its affinity for water. The more soluble it is in water, the more efficient the compound remains in the aqueous solutions, and less adsorption on the membrane surface takes place. The water solubility is the first indication of the effect of a compound on its passage through a membrane (Yangali-Quintanilla et al. 2008).

2.10.3 Acid dissociation constant

The acid dissociation constant (K_a) is an equilibrium constant that measures the ability of an acid to donate a proton to a base. The greater the value of K_a is, the stronger the acid. In dilute aqueous solutions, water is the reference base. The pK_a is defined as -log K_a. The product of the

aqueous acid and base dissociation constants of conjugated pairs are equal to K_w, the ionization constant for water. The percentage of dissociated species of a solute can be determined and classified as ionic (negatively or positively charged fraction) or non-ionic (neutral fraction) (Nghiem et al. 2005).

2.10.4 Dipole moment

The dipole moment is defined as a vectoral property of individual bonds or entire molecules that characterises their polarity (Van der Bruggen et al. 2002; Kimura et al. 2004)

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2.10.5 Octanol-water partition coefficients

The octanol-water coefficient (K_{ow}) is used to describe hydrophobicity and hydrophilicity. The octanol-water partitioning coefficient is a measure of the equilibrium concentration of a compound between octanol and water. In the case of dissociable species, such as acids and bases, the partitioning can be affected by the equilibrium distribution between the neutral and ionic forms, which can vary significantly with the pH of the aqueous phase. For an acidic drug in low pH solutions, the acid molecules are in neutral form (fully not-dissociated) and the total partition coefficient (D) is the same as the partition of neutral species, $K_{OW,\,N.}$ In the limit of high pH values, the acids are deprotonated (fully dissociated) and assumed to form ionic species (either free ions or ion pairs) (Ozaki and Li 2002; Chen and Lin 2016).

2.11 Characteristics of UF, NF and RO

2.11.1 Molecular Weight Cut-off

The molecular weight cut-off (MWCO) of the membrane is a parameter that indicates the relative size of membrane pores. MWCO corresponds to the MW of a solute with retention of percentage. The MWCO is taken as a measure for the retention properties of the membrane. The MWCO, although being a useful parameter for evaluating the rejection of MPs, may not be relied on for a precise prediction of their rejection by UF/NF/RO membranes. Some authors observed the rejections of some MPs to be less than 90% although the molecular weights of those compounds were larger than the MWCO of the membrane inspected. Thus, they suggested considering MWCO only for semi-quantitative prediction of MP rejection by RO membranes (Van der Bruggen et al. 2002; Kimura et al. 2004; Bellona et al. 2008; Yangali-Quintanilla 2010).

2.11.2 Surface Charge

Membranes in contact with an aqueous solution acquire an electric charge by various mechanisms:

- dissociation of surface functional groups;
- adsorption of ions from the solutions;
- adsorption of polyelectrolytes;
- ionic surfactants and macromolecules;

These mechanisms can take place on the interior pore surface as well as the exterior surface of the membrane. The Donnan exclusion mechanism is based on the electrostatic interactions between ions and membrane surface charge. The co-ions (which has the same charge as the membrane) are repulsed by the membrane surface and to satisfy the electroneutrality condition, an equivalent number of counter-ions are retained which results in salt retention.

Membrane surface charge is usually quantified by zeta potential measurements. Studies have shown that the pH influenced the charge of a membrane due to the disassociation of functional groups. The surface charge of the RO and NF membranes are negative, providing selective removal of charged MPs. Many MP in water is also negatively charged, the negative surface charge enhances the removal of ionic MPs (Elimelech et al. 1994; Childress and Elimelech 2000; Bellona et al. 2008; Bartels 2009).

2.11.3 Hydrophobicity and hydrophilicity

hydrophilic is when a surface has a high affinity for water, a surface with low affinity, for water is hydrophobic. The contact angle provides a measure of the hydrophilicity of the membrane surface. A drop of liquid is placed upon a flat and smooth surface and the contact angle is measured. The adsorption of MPs may be related to a change in hydrophobicity/hydrophilicity of the membrane surface. A change in the contact angle may be a method to measure adsorption (Yangali-Quintanilla 2010).

2.12 Membrane rejection mechanisms

2.12.1 Steric hindrance

The rejection of uncharged MPs by RO and NF membranes is influenced by steric hindrance/size exclusion. Authors investigated various MPs and concluded that compound rejection was correlated with molecular width in addition to hydrophobicity. Another study confirmed that rejection of uncharged MPs increased linearly with the molecular weight and width. Another study revealed that steric hindrance may not be the only factor to quantify the rejection of MPs. They observed good rejection of polar/charged MPs where electrostatically hindered transport enhanced solute rejection (Nghiem and Schäfer 2004; Snyder et al. 2007; Bellona et al. 2008; Lee et al. 2008; Silva et al. 2012a; Nguyen et al. 2013; RM Harrison 2017; Krzeminski et al. 2019)

2.12.2 Electrostatic repulsion

Electrostatic repulsion is explained by the repulsive force between negatively charged compounds and negatively charged membrane surfaces. Various studies have shown electrostatic repulsion effects between charged compounds and membranes (Kimura et al. 2004; Nghiem and Schäfer 2004; Kim et al. 2007b)

2.12.3 Adsorptive interaction

The rejection of MPs during membrane applications is an important factor during the adsorption of hydrophobic compounds onto membranes. Authors investigated NF/RO membranes and found that the adsorption of hydrophobic MPs was significant for neutral compounds and ionisable compounds with an electrostatically imposed neutral presence. Another study reported that estrone can adsorb onto the membrane to some extent and concluded that both size exclusion and adsorption are essential to maintain high initial retention by NF membranes. Steroid hormones were investigated and results indicated that at the early stages of filtration, adsorption of hormones to the membrane polymer was the dominant removal mechanism (Nghiem et al. 2002, 2004a; Xu et al. 2006; Schäfer et al. 2011). Different studies have demonstrated that RO and NF membranes can remove low concentrations of MPs present in water samples. These studies have also shown that physicochemical properties of solutes and membrane characteristics may explain transport, adsorption and removal of neutral organic compounds by different solute-membrane mechanisms such as size/steric exclusion, hydrophobic adsorption and partitioning (Kiso et al. 1992; Elimelech and Childress 1996; Childress and Elimelech 2000; Ozaki and Li 2002; Van der Bruggen et al. 2002; Kimura et al. 2004; Nghiem et al. 2005; Xu et al. 2006; Kim et al. 2007b; Bellona et al. 2008; Bartels 2009; Yangali-Quintanilla 2010; Silva et al. 2012a; Nguyen et al. 2013; Krzeminski et al. 2019).

2.13 Endocrine-disrupting compounds (EDC)

An endocrine-disrupting compound is defined by the United States Environmental Protection Agency (USEPA) as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process.” (Diamanti-Kandarakis et al. 2009)

Investigators are discovering trace levels of pharmaceuticals and human hormones in water associated with wastewater treatment plant (WWTP) effluents. Like the steroid hormones, pharmaceuticals as environmental contaminants have not received a great deal of attention until the link was established between a synthetic birth-control pharmaceutical (ethinylestradiol) and impacts on fish. It is now well established that pharmaceuticals and human hormones are ubiquitous contaminants of wastewater effluents. Most often, these compounds occur at sub-mg/L concentrations. While pharmaceuticals and personal care products (PPCPs) are a mostly well-defined group of compounds, endocrine-disrupting chemicals (EDCs) are an extremely diverse group of compounds that interfere with the functioning of natural hormones in animals. It is difficult to determine which chemicals should or should not be classified as endocrine disruptors (Kim et al., 2007a).

Diamanti-Kandarakis et al. (2009) observed that endocrine-disrupting chemicals (EDCs) were originally thought to exert actions primarily through nuclear hormone receptors, including estrogen receptors (ERs), androgen receptors (ARs), progesterone receptors, thyroid receptors (TRs), and retinoid receptors (RRs).

From a physiological perspective, Diamanti-Kandarakis et al. (2009) notes that an endocrine-disrupting substance is a compound, either natural or synthetic, which, through environmental or inappropriate developmental exposures, alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment. Synthetic chemicals used as industrial solvents/lubricants and their by-products [polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxins], plastics [bisphenol A (BPA)], plasticizers (phthalates), pesticides [methoxychlor, chlorpyrifos, dichlorodiphenyltrichloroethane (DDT)], fungicides (vinclozolin), and pharmaceutical agents [diethylstilboestrol (DES)], that is highly heterogeneous natural chemicals found in human and animal food (e.g., phytoestrogens, including genistein and coumetarol) can also act as endocrine disruptors. Leusch et al. (2006) argue that the endocrine system is a biochemical communication system that regulates body function and responses via

chemical messengers, the hormones. Exposure to even low levels of hormonally active agents (termed endocrine-disrupting compounds or EDCs) can result in the widely significant dysfunction of the endocrine system in a wide range of species from different animal phyla.

2.13.1 Endocrine system

According to Leusch et al. (2005), the endocrine system is composed of diverse glands which control hormone metabolism. Hormones regulate a diversity of biological roles such as growth, metabolism, tissue function, sexual development and behaviour, and development of the immune system. Most hormones bind to specific membrane receptors on target cells, triggering a cascade of biochemical reactions that eventually lead to the intended effect (e.g. synthesis of a specific protein, or development of a certain tissue type). Some lipophilic hormones (such as steroid and thyroid hormones) however bind directly to intracellular receptors. This receptor-hormone complex interacts with transcription-control sequences of the DNA, thus modulating RNA and protein synthesis of specific genes.

According to de Jager et al. (2011), the endocrine glands secrete hormones, which circulate around the body via the bloodstream and modulate cellular or organ function by binding with receptors in the target cells. The receptors in the target cell, once activated by binding of the hormone, regulate the functions and processes in the tissue through interactions with the cell's DNA or other complex intracellular signalling processes.

Genthe et al. (2010), Jager et al. (2013) and Van Zijl et al. (2017) further state biological actions of hormones, including estrogens, androgens, progesterone, thyroxin and the neuro-steroids pregnenolone and dehydroepiandrosterone (DHEA), are mediated via high-affinity protein receptors inside the target cells. Steroids circulate in the bloodstream bound to carrier proteins or to serum albumin. They are fat-soluble and readily cross the cell membrane, interacting with diametric receptor proteins, which in the case of estrogens are estrogen receptor (ER)-ct and ER-p*. The steroid-receptor complex binds to target regions of DNA termed "response elements", which activate the cascade of reactions, and the response to the steroids. Naturally, EDCs have been seen to exert their effects exclusively by genomic mechanisms, acting as steroid agonists and binding to the receptor. Recent evidence, however, indicates that some estrogenic compounds do not only have estrogen-receptor mediated effects, but non-genomic pathways may be significant in the target cell response.

2.13.2 Endocrine Disruption (ED)

According to Harrison (2008, 2010), the effects in humans for which links with exposure to endocrine disrupters have been suggested including the following:

- Temporary reduction in sperm counts and quality;
- Increased incidence of testicular and prostate cancer;
- Increased Incidence of Cryptorchidism and Hyphopodium;
- Altered sex ratios;
- Increased incidence of female breast cancer;
- Neurological effects;

Harrison (2008) also stated that endocrine disruptors have been postulated as the cause of many adverse effects on the health of various species of animals in the wild with mammals, birds, reptiles and fish and marine molluscs as examples.

At least four major categories of adverse biological effects may be linked to exposure to endocrine disruptors (Hanselman et al. 2003; Aneck-Hahn et al. 2009; Thomas Zoeller et al. 2014).

- Cancer;
- reproductive and developmental alterations;
- neurological effects; and
- immunological effects;

Endocrine systems that may be involved include the thyroid, adrenal, pituitary, and gonadal system. This includes cognitive effects, which have been observed in animals and humans. The hypothesis that EDCs can cause cancer in humans is based largely on the clear association between exposure of females in utero to diethylstilboestrol (DES), a potent synthetic estrogen taken by pregnant women to avoid miscarriage, and subsequent onset of reproductive organ cancers (Genthe et al., 2010; Van Zijl et al., 2017)

All aspects of reproductive function are controlled by various endocrine communicating systems that employ many protein/peptide and steroid hormones, growth factors and other signalling molecules that affect target gene cell expression and / or protein synthesis.

2.14 Natural and synthetic steroid hormones

According to Larsson et al. (1999) and Joakim Larsson et al. (2002), environmental estrogens are natural or synthetic substances present in the environment, which imitate the effects of endogenous oestrogen. Estrogenic substances were identified in effluent water from a Swedish sewage treatment works receiving mainly domestic wastewater. Substances found include the synthetic oestrogen used in contraceptives, the natural estrogens, and the weaker non-steroidal oestrogens.

According to Leusch et al. (2005a), the type of MSWWTP influent will differ from country to country, states to states, city to city. This will have a direct effect on the MBR-RO system and results thereof. Factors that need to be investigated that will determine the EDC levels in the influents are social factors that can have an impact on the presence of hormones in the environment, with much higher use of the pill as a form of contraceptive by women of reproductive age in Western Europe (48.2%) than in North America (15.5%) (Leusch et al. 2006).

2.14.1 Female (Estrogen) Hormone

The four most important endogenous estrogens are 17α-estradiol (17α-E_2), 17β-estradiol (17β-E_2), estrone (E_1) and estriol (E_3), while the most commonly used synthetic estrogen is ethinylestradiol EE (Van Wyk et al. 2003). Estrogenic activity is shared by many steroidal and non-steroidal compounds. A ranking of endogenous estrogenic potency is as follows: 17β-E_2>E_1>E_3. Each molecule is a 3 to 18-carbon steroid and contains a phenolic ring and a β-hydroxyl group/ketone in position 17 of the D-ring (Van Wyk et al. 2003). The phenolic ring acts as a selective, high-affinity binding point to the estrogen receptors: α and β.

According to Leusch et al. (2006), the quantification of specific organic chemicals, such as EDCs and steroidal estrogens, may be present at low concentrations (ng/L to pg/L) in both treated and untreated sewage effluent and may present specific technical difficulties. Difficulties can often result in a wide variation in any measured concentration values and, at worst, can be significantly biased in the value of ‘true’ concentrations. The measurement of uncertainties is greatest with domestic sewage and tends to diminish with increasing quality of sewage treatment.

Racz and Goel (2010), concur with previous authors stating that the estrogenicity is determined by its ability to bind to the estrogen receptor, thereby mimicking or blocking the activity of natural estrogens. The human estrogen receptor contains a gap in the ligand-binding domain which is near twice the size required for E_2, thus providing space for a variety of other molecules to bind

with the estrogen receptor. Although the human estrogen receptor and the estrogen receptors of aquatic species do not have identical functional activities, all vertebrate species are probably similar in their nonspecific ligand-binding domains. The authors also mentioned that the estrogenicity is dependent on the size and degree of the branching alkyl group and its position on the phenol ring. This feature of estrogen receptor proteins might be responsible for some small loss of estrogenic activity in wastewater as non-estrogenic compounds may bind to estrogen receptors and chemical matrix effects can limit estrogen uptake.

According to Tisdale et al. (2019), 17β-estradiol also has effects on several tissues and organs, including adipose tissue, bone, muscles, blood vessels, the gastrointestinal tract, the brain, the lungs, and the pancreas. Although 17β-estradiol levels naturally fluctuate throughout the menstrual cycle, unusual changes in serum levels of estradiol are linked to conditions such as coronary artery disease, stroke, and forms of cancer.

Wyk et al. (2003); Nghiem and Schäfer (2004); Leusch et al. (2006) and Swart et al. (2011) report that the analysis of sewage is particularly complex, and studies often vary regarding sampling strategies and analytical techniques. However, these studies have indicated reasonably consistent ranges of natural steroidal hormones. Reported variations in concentrations vary from E_3 being detected at the highest concentration (50–260 ng/L), followed by E_1 (<0.5–50 ng/L), and 17β -E_2 (<0.5 – 50 ng/L). These quantities are consistent with the quantities of E_3 and E_1 present in urine produced during pregnancy, which is respectively about 2 and 1 orders of magnitude larger than that of 17β-E_2. Sewage treatment plants receive both natural and synthetic EDCs from urban and industrial dischargers. They also undergo a variety of treatment processes of varying efficiency, such that many of the compounds are not removed efficiently and are ultimately discharged into surface waters.

2.14.2 Male (Androgen) Hormone

According to Costa et al. (2014) and Diamanti-Kandarakis et al. (2009), male hormones are called “androgens” but it is surprising to add here that the female human body also produces a certain amount of this hormone. In addition, the hormones do not go unaffected as they have more than two hundred functions to perform. Androgens come in two chief forms, namely testosterone and androstenedione. No doubt, the level for androgen is high in the male body and it must perform an important part in bringing out male traits and reproductive hormones for males. In women, these hormones undergo conversion into estrogen, the female hormone.

Adrenal glands, fat cells and ovaries produce androgens in women. Generally, the female body can produce androgens in greater and of course smaller amounts. Hormones in women can result in reproduction disorder. Androgens in women are the hormones that initiate the puberty thus stimulation of pubic and underarm hair. Androgens also contribute to the wellbeing of many organs in the female body. Moreover, they are critical to the synthesis of estrogen and sexual desire and sexual satisfaction. This hormone also regulates body functions during menopause and afterwards.

According to Tisdale et al. (2019), testosterone is the principal hormone in the male reproductive system. It is responsible for the regulation and growth of male sexual characteristics and the production of sperm, but it also affects tissues such as bone and muscle and plays an important role in the immune system.

In women, increased production of this hormone may result in thinning of hair, excess hair growth and acne. Some women with increased production of androgen hormone may develop polycystic ovary syndrome (PCOS). This disorder is distinguished by deficient or irregular menstrual period, a disorder of sugar in the blood, and infertility as well as the above-mentioned disorders. If they go, untreated then woman suffering can develop more disorders such as diabetes, high levels of cholesterol and hypertension accompanied by cardiac disorders(Costa et al. 2014).

In addition, to PCOS woman can also develop adrenal gland related disorders. An increased level can also result in adrenal and ovarian tumours. Low libido diminishes feelings of health, and fatigue are due to lowered androgen production and can occur at any age however, it is observed at the time of menopause transition or before menopause. To treat abnormal production of androgen, a combination of testosterone and estrogen is prescribed as medication that can be taken orally or injected. These medications are observed to increase well-being and energy in women who experience androgen abnormality. These medications come with severe side effects like increased risk of endometrial and breast cancer, toxicity of the liver and blood.

Testosterone patches are used to help women with low libido as mentioned in current studies. They say that these patches can help women who have their libido removed surgically. Studies also have shown that using a high dosage for testosterone can cause sexual satisfaction in women. These patches have also been shown to normalize feelings of distress in women.

Testosterone can be used for the treatment of AIDS. The findings still are in process and researchers are working to achieve the desired goal. Testosterone is also considered practically beneficial for premenstrual syndrome (PMS) and diseases related to immunity. Scientists have found that women going through PMS may have lowered levels of androgens (i.e. testosterone) in their bodies and can find relief with the use of testosterone production-boosting supplements (Costa et al. 2014; Diamanti-Kandarakis et al. 2009; Cell et al.).

2.14.3 Evaluation of natural steroid hormones in sewage

According to Van den Berg and Slabbert (2012), sewage treatment plants receiving domestic inputs are suspected of being an important source of natural and synthetic estrogens contaminating the aquatic environment. Cycling women can secrete between 10 and 100 µg estradiol (a natural hormone), estrone, estriol (hormone metabolites) and 17α-ethinylestradiol (synthetic birth control contraceptive) a day. The concentration of estrogen (mainly estriol) secreted by pregnant women can be as high as 30 mg a day. Even a few ng/ℓ of some of the estrogenic chemicals can cause reproductive disturbances in aquatic life. Since sewage effluents are discharged into most environmental waters in South Africa, it is essential to assess their estrogenic potential to ensure useful results for risk assessment and water quality management.

2.15 Measurement techniques EDCs in water

According to Barbosa et al. (2016) and Genthe et al. (2010), in order to assess whether endocrine disruptors are present in water one can do one of two things:

- one either carries out individual tests for each of the chemicals thought to have endocrine disruption capabilities as well as the potential to occur in an area under investigation, or
- test the water sample for endocrine disrupting activity using one or more of the available bioassays.

The former option is not practical, as the general population is thought to be exposed to hundreds of endocrine disruptors. The latter option becomes more of a feasible option where one obtains biological measures of exposure or biomarkers. Bioassays are valuable tools to measure total estrogenic and androgenic activity resulting from all the endocrine-disrupting chemicals present in a water body, including unknowns. Both biological (in vivo and in vitro) and biochemical (in vitro) methods are used to determine endocrine-disrupting chemicals activity and effects Occurrence of individual chemicals is determined by chemical analysis.

The screening of endocrine-disrupting chemical activity is mostly made by in vitro methods. In vitro methods determine the interaction of a chemical with the endocrine system at a cellular level using, for example, cell cultures or enzymes based on the binding of the endocrine-disrupting chemicals to a specific receptor. In vivo experiments, on the other hand, measure endocrine-disrupting chemical effects in the whole animal by measuring a variety of endpoints such as the increase in uterus weight. The major advantage of this type of methodology is that it considers absorption, metabolism and excretion. However, in vivo test methods are expensive and time-consuming and often require the sacrifice of test animals (Genthe et al. 2010; Van Zijl et al. 2017).

2.15.1 In Vitro Bioassays

Czernych et al. (2017); Kase et al. (2018) and Abbas et al. (2019) stated that various *in vitro* methodologies are used worldwide. These include the yeast estrogen screen (YES) assay, the two-hybrid recombinant yeast cell bioassay (TRCBA), the estrogen receptor (ER) binding assay, the enzyme-linked immunoassay (ELISA), the E-screen cell proliferation assay, the ER-CALUX assay, the DR-CALUX assay, the Carp-HEP assay, and the T47D-KBIuc cell line, to mention a few. They can be divided into three categories of assays, depending on which endpoint of biological response they measure(Genthe et al. 2010):

- receptor binding assays
- reporter-gene assays
- cell proliferation assays

Only a few of the above-mentioned in vitro methods are currently practised in South Africa (Pool et al. 2002; Swart and Pool 2007; Truter et al. 2015; Makene and Pool 2019)

Hu and Aizawa (2003), developed a quantitative structure-activity relationship (QSAR) model describing the binding affinity of phenolic compounds, including estrogens, to the estrogen receptor. This model incorporates expressions for bulk effect, polarity effects, and hydrogen-bonding effects. Bioassays such as the yeast estrogen screen (YES) and the ER-CALUX, use reporter genes to quantify relative overall estrogen activity of a sample (Racz and Goel 2010).

The recombinant yeast (YES) screen assay (Routledge and Sumpter 1996b) is a rapid, cost-effective and widely used assay based on modified yeast cells which possess the human estrogen receptor. The activated receptor binds to the estrogen response element located on a reporter plasmid, in tandem with a sequence coding for (3-galactosidase).

In the presence of estrogenic agents, the cells begin to express p-galactosidase. The enzyme is excreted into the culture medium where it reacts with its substrate CPRT to liberate chlorophenol red. The resulting colour change from yellow to red is readily measured spectrophotometrically, compared to a standard curve and the estrogenic potency of the sample expressed as Estrogen Equivalencies (EEQ). (Beck et al. 2003; Balsiger et al. 2010)

Balsiger et al. (2010) mentioned that a four-hour yeast bioassay (YES) for the direct measure of estrogenic activity in wastewater without sample extraction, concentration, or sterilization, is possible.

The MVLN assay (Demirpence et al. 1993; Hoogenboom et al. 2001) is a reporter gene assay that measures growth of cells in response to agents that interact with the estrogen receptor and is also sensitive to agents that do not interact with the protein. The assay utilises MCF-7 breast cancer cells that have been stably transfected with the Vit-Luc reporter gene. The MVLN cell line expresses the endogenous estrogen receptor of MCF-7 and at the same time contains an exogenous estrogen-responsive reporter (luciferase). Therefore, the estrogen specific transcription activity of a test chemical is directly related to the activity of luciferase measured in the lysate of treated MVLN cells.

E-screen (MCF-7 cells) assay (Cell et al.; Hoogenboom et al. 2001; Körner et al. 2004; Diamanti-Kandarakis et al. 2009): the E-screen is a cell proliferation assay. These assays are generally based on, either breast cancer- or genetically engineered- cell lines that require estrogen for growth. The assay compares the number of cells present after a specific duration of exposure (e.g. five days) to an estradiol standard curve. The MCF-7 cell line is normally used, although the T47-D cell line is equally sensitive. A constant source of estrogen-free water to use for controls remains a problem. It is also more expensive and time-consuming than other in vitro methods which therefore limits its use for large-scale screening programs. This method has been successfully implemented by the

Liver slice HEP-Vtg (Shilling and Williams, 2000): instead of using isolated cells, this reporter gene assay can also be performed using liver slices (trout or Xenopus laevis), which is more representative of the organ in vivo. This method is like the fish HEP-Vtg, except that it uses liver slices instead of hepatocytes. Vitellogenin is quantified by ELISA at the end of the exposure period. (Genthe et al, 2008)

The recombinant yeast (YES) screen assay has been proposed as an applicable assay for the screening of estrogenic activity because it is easiest to perform, robust and inexpensive. For these reasons, the YES assay has been used successfully to assess estrogenic activity in environmental samples, However, the yeast cell response can be affected by the sample matrix, especially in wastewaters of industrial origin (Coldham et al. 1997; Rutishauser et al. 2004; Nelson et al. 2007).

2.15.2 In Vivo Bioassays

In vivo assays presently suitable for monitoring in South Africa are the zebrafish assay and Xenopus lavis assay for determining estrogenic effects (Leusch and MacLatchy 2003; Bovee et al. 2007; Swart et al. 2011). These methods are, however, very expensive and time-consuming according to Genthe, 2008. Catfish Vitellogenin assay: vitellogenin (Vtg) is the most widely used biomarker of exposure to estrogenic chemicals in fish. The most common way of measuring Vtg induction is the ELISA (enzyme-linked immunosorbent assay). The methodology of using catfish Vtg coupled with ELISA detection technique has been successfully developed. In South Africa, there is increasing evidence that our aquatic systems are being polluted with EDCs. As a result, there is a serious need to develop a battery of screening tests for not only anti- and estrogenic but also anti- and androgenic effects relevant for local conditions(Swart et al. 2011; Manickum and John 2014a; Truter et al. 2016; Archer et al. 2017a; Manickum 2019).

Chapter 3

METHODOLOGY

3 METHODOLOGY

This chapter describes the research methodology, experimental pilot-pilot plant set-up, analytical techniques and experimental protocols used in this current research work. Membrane modification with grafting polyvinyl alcohol (PVA) through cross-link glutaraldehyde on a TFC PA RO membrane, to enhance the resistance to flux decline, during the treatment of MBR secondary effluent, will be described in this section. Thereafter, the selected natural steroid micropollutants, 17β-estradiol (T) and testosterone (T) with physicochemical properties are discussed, followed by the pilot-plant PFD and experimental plant conditions. Lastly, the UF, NF and RO membranes characteristics to be used in the RO plant, are described.

3.1 Full-scale MBR

WWTW with a full-scale MBR plant is in the City of Cape Town (CoCT), South Africa receives its wastewater from the largest informal settlement in the city. The current population estimate (PE) for the WWTWs were estimated from population growth projections since the last national census campaign in 2011, which resulted in a PE of 390 000. Raw sewage from the WWTW served as the feed, after filtration, for the MBR. The MBR included a bioreactor that was divided into three zones (anaerobic, anoxic and aerobic) to enhance the removal of organic compounds and nutrients. Bioreactor output was filtered in a membrane tank by a commercially available, plate and frame type, with hollow fibre and submerged UF membranes. The MBR system incorporates ZeeWeed® 500 ultrafiltration membranes (GE Zenon, but now trading as Suez Water Technologies and Solution, Trevose, PA, USA), producing 18 megalitres of effluent per day.

Photograph 3. 1: Full-scale MBR plant with six trains linked in parallel feeding the RO pilot-plant attached (left), biological reactor and the MBR submerged plant (right).

The MBR was operated continuously with secondary effluent fed directly into the RO pilot plant, passing through two pre-filters to remove colloidal solids before passing through the membranes, in once-through mode.

3.2 Reverse osmosis (XLE) membrane surface grafting modification

During the pilot-plant study on-site, membrane fouling became a great concern. A commercial anti-scalant was used during all experimental runs, but the variation in MBR effluent quality was noted. During the second month of test-work, the prefilters were blocked daily with colloidal material (dissolved solids). On investigation, it was discovered that two of the train units had leakages due to UF hollow fibre membrane damage in the MBR that needed urgent replacement.

A reverse osmosis membrane surface grafting modification study on the XLE membrane with MBR effluent was investigated. This part of the project was done off-site in the laboratory. An RO virgin membrane was cut to a specific size for membrane surface modification prior to fouling experiments. PVA solutions were used as modification agents. Membranes were tested before and after membrane surface modification in a bench-scale RO system. A static adhesion test was performed to study biofouling.

3.2.1 Experimental procedure for modification (PVA)

1. PVA was grafted onto the surface of the virgin membrane through covalent bonding initiated by GA. Modification procedure was like the one described by Hu et al., (2016).
2. An RO spiral wound TFC membrane was cut into flat sheets with dimensions of 18 cm length and 12 cm width, giving a surface area of 0.216m^2
3. A membrane sample was immersed in DI water over a period of 12 hours; the water was manually replaced every hour to remove preservatives from the membrane sample. DI water was used to thoroughly rinse the membrane which was allowed to dry at room temperature thereafter.
4. The membrane was then wrapped around a rectangular frame, in such a way that the layer faced the inner part of the frame to allow thorough contact with the modifying agent solution.
5. GA solution was prepared by diluting 25 wt.% GA solution to 0.05 wt.% with pH adjusted to 3.05 with dilute H_2SO_4. 100 mL of the prepared solution was added into the rectangular frame where the membrane had been fixed for 5 min and the excess solution removed.
6. The membrane was then manually rinsed with DI water and allowed to dry completely.
7. PVA solutions of different concentrations were then prepared (0.5 g/L, 0.1 g/L, 0.15 g/L and 0.2 g/L).

8. A fixed amount of PVA was added to 1L of DI water and placed on a heating element with a magnetic stirrer used to mix the solution for 30 min at 75 ˚C until the PVA was dissolved completely.
9. The PVA solution could cool and the pH was adjusted to about 3.05 using diluted sulphuric acid.
10. The prepared PVA solution was then poured on the coated membrane around the rectangular frame for 3 min and the solution was removed and placed in an oven at 50 ˚C for 6 min.
11. DI water was used to rinse the modified membrane, and the membrane was further placed in DI water at 45 ºC for at least 10 hours; the water was manually replaced every hour to remove unreacted molecules from the surface.
12. The membrane was stored in DI water before experimental runs.

3.2.2 Modification steps in brief

1. The membrane was rinsed with de-ionised water.
2. It was left to dry under room temperature conditions.
3. Once dried, it was wrap to the frame.
4. A 100 ml GA solution were added to the membrane and allowed to stand for 5 min.
5. After 5min, the GA solution were removed from the membrane and rinsed with DI water.
6. The membrane dried
7. 50ml of PVA solution were added to the frame and allowed 3 min contact time with the membrane. Thereafter the membrane was rinsed and placed in the oven at 50 ^{O}C for 6 min.

Table 3.1: Membrane surface modification

	PVA
Experiment	**Concentration (g/L)**
1	0.05
2	0.01
3	0.15
4	0.2

3.2.3 Biofouling experiment

Bio-fouling experiments were conducted to evaluate membrane surface resistance to biofouling using the gram-negative E. coli bacteria in solution.
Membranes with better permeations were selected and further studied in terms of resistance to bacterial growth and biofilm formation. E. coli bacterial solution was used as a model foulant. Bacterial growth measurement was similar to that described by Wang et al., (2015) with minor modifications.

1. The membrane was cut into small sizes (2 cm x 2 cm) and placed under UV for a half-hour.
2. The membrane was placed into a nutrient broth (NB) of ≈ 0.47 OD for a period of 3 hours.
3. The membrane was removed and rinsed with fresh broth and the solution collected was diluted in series (2-10 folds).
4. 100 µL of diluents were plated on LB Agar medium and incubated overnight.
5. The plate count method was used to determine the number of E. coli bacteria in contact with the membrane.
6. The same NB of E. coli suspension, without being in contact with the membrane, was diluted in series (2-10 folds) and platted on LB agar medium and incubated at 37 ˚C for 20 hours.

The number of E. coli bacteria not in contact with the membrane was also determined as mentioned above, and this allowed calculating the mortality ratio (R) according to the formula:

$$R = \left(\frac{B - A}{A} \right) \times 100 \qquad \textbf{(Equation 3)}$$

Where B is the number of viable bacteria not in contact with the membrane surface and A is the number of viable bacteria in contact with the membrane surface for given contact time.
Biofouling effect was further investigated by measuring pure water flux of membranes before and after incubation of 2.1×10^8 CFU.mL^{-1} E. coli bacteria suspension for 30 hours.

3.2.4 Water flux Test

Two membranes each from the two modifications procedures investigated in this research were selected based on their perm selectivity. These membranes together with the unmodified membranes were used to test water flux after exposure to E. coli cell suspension for 30h under 5 Bar and room temperature, using DI water.

The following procedure was followed regarding water flux data collection:

- After exposure of the membrane to bacteria cells, membranes were rinsed and placed into the RO cell.
- DI water was added to the system
- The operating conditions of the RO system were set to 5bar feed pressure, 19 cm/s cross velocity
- The flux of the contaminated membrane was recorded every 30min for a period of 6 hours.

Table 3.2: Experimental summary

Experimental run	Membrane
1	Unmodified membrane
2	Unmodified membrane (duplication)
3	0.15 g/L PVA membrane
4	0.15 g/L PVA membrane (duplication)

3.2.5 RO system

The experiments were carried out on lab scale using a bench-scale RO cell. The feed used was acquired from an existing MBR WWTP and make-up synthetic feed, composed of specifically identified foulants. A low-pressure high flow rate hydra-cell pump was used to pump the water through the cell. Permeate was discharged into a holding flask and the brine was recycled to the feed tank. LabVIEW software was used as the controller where the velocity of the system is controlled and the pressure around the cell is monitored using a hydraulic pump. The feed pressure was regulated in order to achieve a constant flux and readings were recorded.

Table 3.3: RO system operating conditions

Initial Conditions	Feed solution
Flux: 60 L/m^2.hr Pressure: 5 bar Cross velocity: 19 Cm/s Temperature: Ambient	Synthetic Real effluent (membrane with best perm selectivity)

3.2.6 Membrane characterization analysis

A qualitative analysis (SEM) and semi-quantitative analysis (ATR-FTIR) were used to characterize the membrane before and after modifications. Both analyses were done externally.

3.3 Reverse osmosis pilot-plant

3.3.1 Quantitative Design

An explanatory field experimental approach will be was used to conduct investigations for the research. A reverse osmosis pilot-plant was used for the study. Quantitative statistical methods such as the t-test were applied to test the validity of the results. The research was structured around the MBR secondary effluent coming from domestic sewage waste. The concentration levels of the feed and permeate in terms of EC, turbidity, 17β-estradiol, and testosterone in the sewage water were the independent or random variables, which will affect or determine the efficiency of the reverse osmosis process. The dependent variables were the same concentrations in the products and the by-products, and the process efficiency.

Ordinal and primary data in the form concentrations, to the parts per million levels, were used for the quantification of the research results. Correlation were established among variables such as the presence of the human and pharmaceutical endocrines in the product of the reverse osmosis process, pH, temperature, flow rate, pressure and other important parameters or constituents of importance.

Due to membrane characteristic properties, the null hypothesis states that the membranes will perform differently in terms of the human natural hormonal activity mentioned above in their respective effluent to produce a quality permeate clean enough to be augmented for potable application. This means that the 17β-estradiol concentration would be equal to 0.7ng/l as stipulated by the regulation. The alternative hypothesis, therefore, states that the concentration of 17β-estradiol in the permeate is higher than 0.7ng/L and is not below the recommended standard for potable application and in turn for augmentation.

3.3.2 RO Pilot-Plant Design

The research methodology entailed a pilot plant that was designed, built and commissioned on-site. The contaminant removal efficiency was evaluated at a WWTW designated for possible agricultural, recreational, and potable reuse of wastewater effluents. This treatment plant consisted of a full-scale Membrane Bioreactor (MBR) plant receiving wastewater from a densely populated residential area followed by UF/NF/RO pilot plant. The pilot plant consisted of three different TFC polyamide membrane modules, in parallel, which were subjected to various experimental running conditions. Secondary MBR effluent was used to feed into the pilot plant (Figure 3.1). Batch, 8 hours, once through mode experimental runs were conducted on the pilot-plant with individual membranes at any given time.

Photograph 3. 2: RO Pilot-plant installation next to the MBR plant with RO plant piping connection.

Permeate (C_P) and feed (C_F) conductivities were used to evaluate the salt rejection of membrane as shown in Eq. (1) (Rana et al., 2015; de Souza et al., 2018; Aziz and Kasongo, 2019). Different operating conditions of flux and percentage recovery were used during the experimental runs. Table 1 shows a summary of the experimental conditions. The permeate flux (J) was calculated using the volume of permeate (V) collected through the active surface area of membrane (A) for a given period of time (Δt), as shown in Eq. (2) (Bruslovsky et al., 1992; Kasongo et al., 2019; Hacıfazlıoğlua et al., 2019 and Shad et al., 2019), as shown in Chapter 2.

$$R = \left(1 - \frac{C_p}{C_F}\right) \times 100 \qquad \text{(Equation 5)}$$

$$J = \frac{V}{A.\Delta T} \qquad \text{(Equation 6)}$$

The pilot plant consists of the following units:

- Feed pump which draws a feed stream from the feed tank which contains secondary effluent from the MBR.
- Two bag filters: each with five and one micropore sizes to remove colloidal solids.
- High-pressure pump that elevates the flow rate and pressure to the set levels prior to entering the membrane modules.
- Three membrane modules fitted in parallel to each other. Each module consists of a different type of membrane, which allows simultaneous testing of all three membranes with the same feed.
- Biological agent dozing to prevent microbiological growth.
- Sulphuric acid dozing to keep pH constant
- Three sampling points, one at each membrane outlet stream.
- A permeate recycle line going back into the feed stream increase feed rate to improve the efficiency of the process.
- The brine is recycled to the MBR process.
- Permeate to the product tank, that can be used clean the plant

3.3.3 Experimental Procedure

The RO feed was be obtained from the UF/MBR effluent stream at a Municipal Sewage Waste Water Treatment Plant in the City of Cape Town. This MSWWTP receives more than 95% of its wastewater from domestic sources. The secondary effluent was be fed directly into the reverse osmosis pilot plant as shown in Figure 3.1. This feed stream was first filtered by a five-micron bag filter followed by one micron. The filtered stream was pumped to the membranes. A high-pressure pump will increase the pressure and the feed rate to the required levels. The feed was split into three streams, each feeding a membrane module. The three membrane modules were parallel to each other.

Once the feed entered the membrane part of it diffused towards the centre while the other part continued to flow along the length of the membrane. The part that flowed towards the centre was collected as permeate. The liquid that had larger molecules flowed towards the end of the pressure vessel and was discharged as brine. Both permeate and brine were sampled. The permeate stream was split into a recycled and product stream. The recycled re-entered the process at the RO feed, and the product stream was fed into the permeate tank. The brine was returned to the MBR waste stream.

Two types of experimental runs were performed. Continuous experiments were conducted in an indefinite time period, whereas the batch process took place over a daily eight-hour period. The daily eight-hour periods were performed firstly, to identify and solve the normal process challenges. During these runs an eight hourly composite sample was taken continuously by instituting a needle valve after each membrane, to collect permeate samples (Figure 3.1). Two permeate and two brine samples were collected for each membrane. A total of twelve samples were collected. One feed sample was also be taken during the experiment. Each composite sample was reduced and kept in a 500 ml glass flask. It was stored on ice for transportation to CPUT Laboratory for SPE extraction (see Appendix 1 for the experiment parameters and conditions). Extracted samples were sent to the Ecophysiology Laboratory at the University of Stellenbosch for different screening test (see Appendix 1, biochemical and biological tests).

The screening tests were divided into two parts, namely in vitro ELISA and YES bioassay screening test. The yeast test (YES) was analysed for estrogen (17β-estradiol) hormonal activity. The last in vitro test was more specific and tested for specific EDC's in terms of concentration using an ELISA Kit. It also tested for concentrations of β-estradiol and testosterone. It is important to measure β-Estradiol below the recommended 0.7ng/L requirement for potable use. All three tests are crucial in terms of identifying hormonal activity, and to specify the concentration.

In addition to the above results the following readings were taken daily for the different membranes: Feed electronic conductivity (EC), permeate EC, feed temperature, permeate temperature, feed flow, permeate flux, feed pressure, feed pH, and permeate pH. (see Photograph 3.3, showing the pilot plant activities).

Photograph 3.3: (from top to bottom) Pilot plant on wheels linked directly to the MBR effluent.

The eight-hour tests were repeated to generate about 50 analyses. The results were then processed statistically. The average concentration of the endocrines was compared to the minimum value of 0.7ng/l. The alternative hypothesis states that the endocrines are more than this concentration. Should the alternative hypothesis be rejected for not having enough evidence to accept it at about 95% confidence level, it will be concluded that the reverse osmosis can be used to reduce the endocrines in the domestic sewage water system to the levels that the sewage water can be cleaned to augment the potable water reservoirs.

3.3.4 UF/NF/RO membranes

High-pressure membranes examined in this project included the NF270 (Dow Chemical Co., Filmtec NF270-4040, Midland, MI) polyamide TFC loose nanofiltration (NF) membranes, UA60 (TriSep, 4040-UA60-TSA, Goleta, CA) piperazine-based TFC loose ultrafiltration (UF) membranes, and the XLE (Dow Chemical Co., Filmtec XLE-4040, Midland, MI) polyamide TFC low pressure reverse osmosis (RO) membrane. RO and NF membranes are considered thin-film composite comprising three layers: 0.2 μm polyamide, 40 mm polysulfone and 120 mm polyester support web. The characteristics of these membranes are presented in Table 8.

Table 3.4: Pilot-plant operating conditions

Parameters	**Operating Conditions**		
Membrane module	XLE	NF270	UA60
Recovery (%)	50; 75	75	75
Flux (L/m^2hr^1)	25; 30	30	30
pH	uncontrolled; 6.5	uncontrolled	uncontrolled

3.3.5 Water analysis

Inorganic reagents (NH_3, P, PO_4 reagents), as well as the COD reagent obtained from Hanna instruments, were used for the analysis of feed, brine and permeate water. Lovibond RD 125 COD Reactor and a multiparameter photometer were respectively used for COD and inorganics analysis. pH/conductivity meter Model 3540 and the EC meter were used to measure the pH and the electro-conductivity (EC) as well as the turbidity. Deionized (DI) water obtained from a Millipore water purification system was used for water analysis.

3.3.6 Estrogenic and androgenic steroid hormones

Target compounds selected for this study included natural steroidal hormones; an endogenous estrogen, **17β-estradiol (E_2),** [1,3,5(10)-Estratrien-3,17β-diol] and an endogenous androgen, **testosterone (T),** [4-Androsten-17β-ol-3-one]. The physicochemical properties are presented in Table 9, (Nguyen et al. 2013), where the chemical structure shows that both E_2 & T have two oxygen-containing functional groups, which take the form of primary or secondary alcohol or a ketone (Nghiem et al. 2004b). 17β-estradiol and testosterone have very low solubility in water. Their K_{ow} values suggest their hydrophobic nature and moderate to high binding to organic colloids and macromolecules in water.

3.3.7 Sample Collection and Solid Phase Extraction (SPE)

Sampling was carried out during the months of May, June and July as well as October, November and December. The sampling points were: (1) municipal wastewater raw-sewer (influent); (2) MBR influent; (3) MBR effluent; and (4) permeate of UF/NF/RO element. Grab samples were taken once weekly of both influent and effluent. To avoid frequent fluctuations in concentrations, each sample taken from the pilot plant was an 8-hour composite sample taken for the duration of each experimental run. All water samples were collected in amber glass bottles (2.5 L), covered with tin foil and placed on ice and kept in a cool box, and transported to the laboratory for testing. There was no contamination or contact with the plastic lid of the bottles. Once they reached the laboratory, the effluent samples were filtered through 1.0 µm pore size glass fibre filter paper (Whatman GF/B), then the filtrates were stored in a refrigerator at 4 ^{O}C, and the solid phase extraction (SPE) was performed within 48 h.

3.3.8 Enzyme-linked immunosorbent assays (ELISAs)

17β-Estradiol (E_2) & Testosterone (T) concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits. Inter- and intra-assay variation for steroid hormone ELISAs are negligible, as shown by Swart and Pool (2007) who determined inter-assay variation at 5.6% (n = 3) and intra-assay variation between 0.6% and 2.5% (n = 3). Thus, the accuracy of the ELISAs reduces the need for expensive and time-consuming replication and provides for a rapid screening of several samples. All reagents required for the assays are supplied with the kits. 17β-estradiol (E_2) & testosterone (T) levels were determined in the C18 SPE extracts of water collected using commercially available ELISA kits (E_2 and T, DRG International Inc., USA;) according to the manufacturers' instructions. Assay ranges of the kits are estradiol 9.7 – 2000 ng/L and testosterone 83 – 16000 ng/L. The extracted samples in ethanol (1000× concentrated) were diluted (E_2, 1/10; T, 2/10) in a 0.1 % w/v human serum albumin, 0.9 % NaCl solution and

assayed (Swart and Pool 2007). The diluted samples were then assayed using the kit and the data obtained were plotted on the same graph as the standard curve to determine if the curves were parallel. The kits were assayed for intra-assay reproducibility by assaying replicates of the same sample on a single assay plate. The OD was determined at 450 nm using a plate reader. A standard curve was drawn using the reading obtained for the standards; the concentrations of the samples were read off this curve. After factoring in the concentrated samples that were applied to the ELISAs (Faul et al. 2014), the effective lower level of quantification (LOQ) for each was reduced to 0.97 ng/L (E_2) and 4.15 ng/L (T), respectively. Truter et al. (2015), had the following detection limits for: E_2 at 0.37ng/L, after a solvent blank correction.

3.3.9 In Vitro Recombinant Yeast Estrogen Screen (YES)

The recombinant yeast-based screen followed the protocol described by Sohoni and Sumpter (1998). *Saccharomyces cerevisiae* transfected with the human estrogen receptor (hER) gene and a plasmid containing an estrogen response element-linked *lac*-Z gene was used. Successful binding of ligands in the water samples (steroids) to the receptors in the yeast cells initiated the expression of the *lac*-Z reporter gene which encoded for the enzyme β–galactosidase in the assay. The β–galactosidase then metabolised chlorophenol red galactopyranoside (CPRG), which resulted in a colour change of the assay medium, indicating a dose-dependent activity of the ligands to bind to the estrogen receptor. The assay medium was prepared as described by Sohoni and Sumpter (1998). The yeast was incubated in assay medium containing no CPRG for 48 hours under 26ºC on an orbital shaker. The concentrated wastewater extracts (500x) were serially diluted and 10 µL was spiked into 96-well sterile flat-bottomed plates with low evaporation lids (Costar, 3370, Sigma). The previously incubated yeast culture was then included in the new assay medium containing CPRG at a concentration of approximately 8x105 cells/mL. The seeded assay medium was then added at 200 µL/well into the assay plate to provide a final concentration of the water extracts ranging from a 50x to a 1.56x. A concentration of 1x was depicted as an un-concentrated water sample. For the raw wastewater samples, serial dilutions of the samples were made with MeOH to obtain a concentration range of each sample ranging from 12.5x to 0.39x in the assay due to cytotoxicity observed in the 50x and 25x concentrated sample. For the effluent (permeate) water samples, serial dilutions of the samples were made with MeOH to obtain a concentration range of each sample ranging from 50x to 6.25x due to the lower observed estrogenicity in these samples compared to raw wastewater samples. All samples were analysed in triplicate in the same assay plate, and each assay was repeated twice.

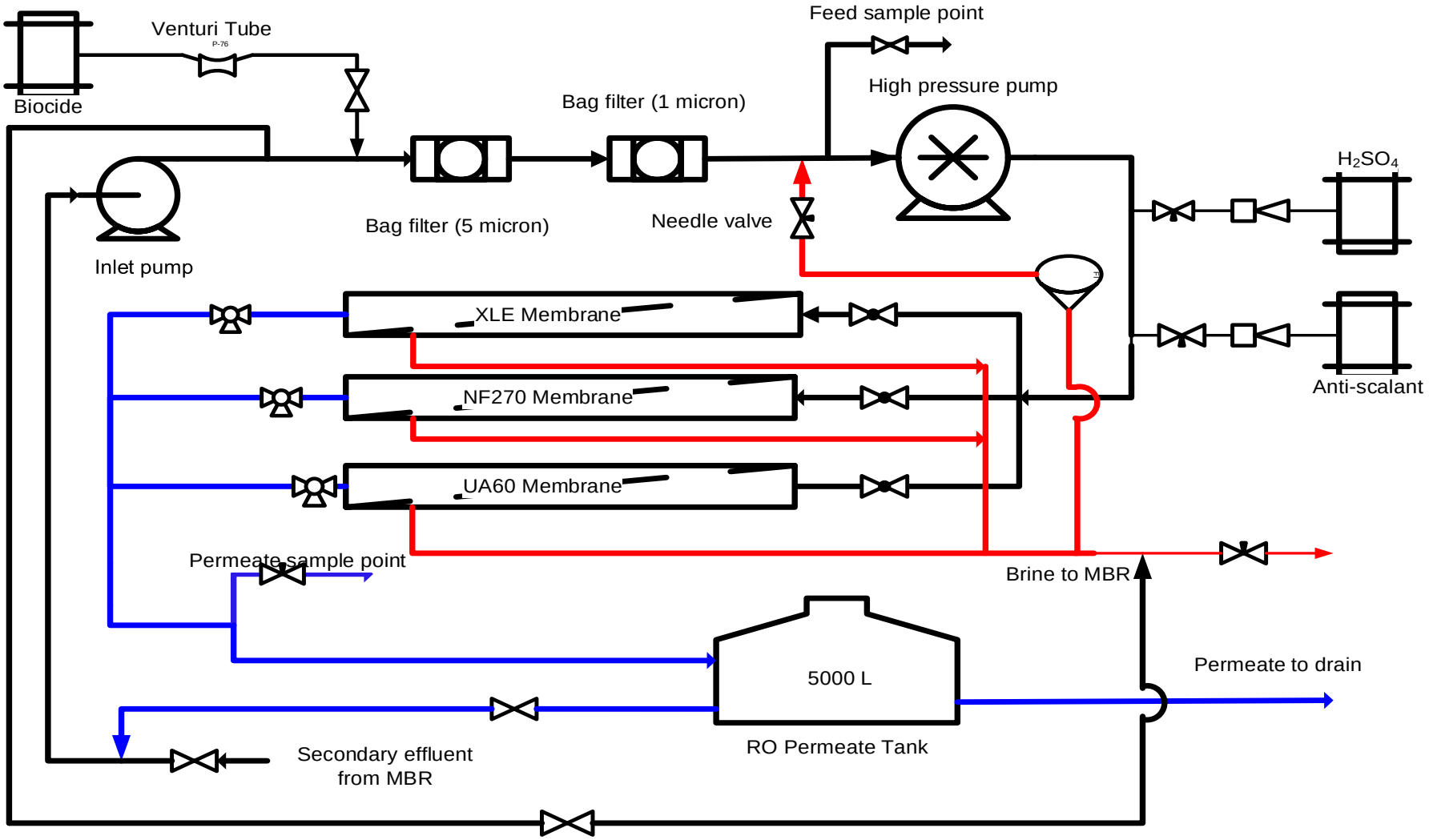


Figure 3.1: Process flow diagram of the UF/NF/RO pilot-plant

A standard curve for the steroid hormone 17β-estradiol (E_2; CAS 50-28-2; Sigma) was included for each assay plate in 12 serial dilutions, ranging from 1.0 to 2700.0 ng/L. Blank wells were also included in each assay plate containing only an assay medium without any hormone spike or water sample extracts. The assay plates were then allowed to incubate on a shaker for 72 hours at 30ºC under dark conditions (Archer 2018).

3.3.10 Exposure

The C18 surface water extracts and C18 (Milli-Q) negative controls were assayed at a 5×concentrated state (1×concentrated denotes the state in nature) in sterile 96-well low evaporation lid flat-bottom plates (Costar, Corning, USA). The C18 extracts were diluted to the appropriate concentration in ethanol and 10 µl of these diluents was dispensed in duplicate per assay plate. As standards, each plate furthermore contained a 12-point twofold serial dilution of E_2 (0.1 nM – 4.88 pM: YES), dispensed into duplicate wells. Once the ethanol was evaporated from assay plates in a laminar flow cabinet, 200 µl assay medium with yeast was dispensed per well (approximately 8×106 cells). The lids of assay plates were subsequently sealed with autoclave tape and incubated at 32 °C in the dark for approximately 72h. The environmental samples were screened in two independent experiments. Estradiol equivalents values (expressing the relative potency of water samples) were calculated per assay plate using the plate-specific regression functions (Truter et al. 2015).

3.3.11 Quantification of estrogenicity

Upon 72 hours of incubation, the YES assay plates were measured for colour change using a spectrophotometer. The absorbance was measured at 570 nm for colour change of CPRG caused by steroid hormone-mediated β–galactosidase production, and 620 nm for turbidity change and cytotoxicity. The thresholds for cytotoxicity in the samples were determined using equation 3:

$$\text{Cytotoxicity} = \text{Median Blank}_{620nm} - (3*\text{stdev Blank}_{620nm}) \quad \text{(Equation 4)}$$

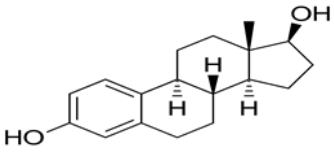
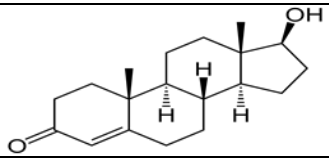
In order to correct for turbidity in the wells, a corrected absorbance (CA) was calculated for each sample in the assay using equation 4:

$$\text{Corrected absorbance (CA)} = (OD_{570nm} - [OD_{620nm} - \text{blank}_{620nm}]) \quad \text{(Equation 5)}$$

Table 3.5: Properties of three membrane modules

Membrane Component	Texture	Type	Rejection %	Effective Area (m^2)	MWCO (Da)	Maximum Pressure (bar)	Maximum Temp. (^0C)	Max. Permeate Flowrate (m^3/hr)
RO	TFC Polyamide	Filmtec XLE-4040	99% NaCl	8.1	< 200	6.9	45	9.8
NF	TFC Polyamide	Filmtec NF270-4040	>97% MgSO4	7.6	400	4.8	45	9.5
UF	TFC Piperazine	TriSep 4040-UA60-TSA	80% MgSO4	8.2	1000	7.6	45	11.4

Table 3 6: Physiochemical properties of a selected estrogen and androgen compound

Analytes	MW (g/mol)	Formula	CAS Number	Solubility (mg/L)	Dissociation Constant pK_a	Classification	Partition. Coefficient (log K_{ow})	Chemical Structure
17β-estradiol (E_2)	272.38	$C_{18}H_{24}O_2$	50-28-2	13	10.4	natural hormone (estrogen)	4.01	
Testosterone (T)	288.42	$C_{19}H_{28}O_2$	58-22-0	23.4	17.4	natural hormone (androgen)	3.32	

Physiochemical information was obtained from Nghiem and Schäfer (2004); Yoon et al. (2007); Nguyen et al. (2013); Krzeminski et al. (2019)

where OD570nm and OD620nm refer to the optical density of the sample measured at 570nm and 620nm respectively, and Blank620nm refers to the median optical density measured for the blank wells in each assay plate at 620nm. Water samples were only considered for further analysis if the corrected absorbance was above a detection threshold using equation 5:

Detection = Median $blank_{CA}$ + (3*stdev $blank_{CA}$) (Equation 6)

For the YES, the CA of water samples above the detection threshold of the assay were then log-transformed and expressed as a percentage of the maximum log-absorbance value calculated in the E_2 standard curve using equation 6:

Log % max E_2 (sample/standard curve) = (log-CA_{sample} / log-CA_{E2} max) * 100 (Equation 7)

A non-linear calibration curve was then constructed for the E_2 standard curve of each individual assay plate by plotting the calculated log % max E_2 of the E_2 dilution series against its known concentration (in ng/L). An E_2-equivalent concentration (EEQ; ng/L) for each water sample was then calculated from the generated trend-line of the calibration curve and corrected for their dilution factors to obtain a final EEQ concentration of each water sample (in ng/L) (Archer et al. 2017b). Coefficients of determination (r^2) of 0.965 or better were observed for the dose-response curves.

3.4 STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad Prism (v. 5.00) and Microsoft Excel 2010. The variation between individual samples was assessed using an unpaired t-test. For the determination of significant variation between sampling and membranes, a one-way analysis of variance (ANOVA) was performed. Significant variance was achieved with $P < 0.05$

Chapter 4

Surface grafting of polyvinyl alcohol (PVA) cross-linked with glutaraldehyde (GA) to improve resistance to fouling of aromatic polyamide thin-film composite reverse osmosis membranes using municipal membrane bioreactor effluent

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4 MEMBRANE SURFACE MODIFICATION

4.1 Introduction

The MBR effluent is a real stream consisting of various MPs, which is problematic due to membrane fouling. There is not enough literature available to explain the fouling phenomenon with a full-scale MBR connected directly to an NF/RO pilot plant. In this study, the results are presented on the resistance to fouling of RO membranes with surface modification using a lab-scale RO unit. Using a real secondary effluent feed is problematic due to all the unknown pollutants that will interfere with the grafting process as well as the increase of membrane fouling that will negate the research success. It is very important to use an MBR model feed in order to establish a base case.

4.2 Membrane surface modification using PVA: membrane characterization

Modification of the polyamide thin-film composite reverse osmosis membrane was conducted by bonding PVA cross-linked with glutaraldehyde, to the membrane top selective layer. ATR-FTIR analysis was used to study the physiochemical structure of unmodified and PVA modified membranes.

4.3 ATR-FTIR analysis

ATR-FTIR was used to verify the grafting of PVA chains onto the TFC membrane surface. The ATR-FTIR was used to analyse membrane surface functional groups and illustrated that grafting succeeded. The peaks at 1240, 1482, 1510 and 1589 cm^{-1} in Figure 4.1 (left and right) are characteristics of the polysulfone support layer of the membrane, as described by Wei et al. (2010) and Cheng et al. (2013). The small but clear peaks at 1610 cm^{-1} of membranes spectra in both figures correspond to the amide I group (C=O stretching), which were ascribed to the polyamide top layer of the membrane (Liu et al. 2015). The presence of these peaks showed that the structures of the membranes were not affected after modification (Lin et al. 2016). The spectra of PVA modified membranes show an adsorption band at about 1725 cm^{-1}attributed to the presence of PVA molecules attached to the polyamide layer (Hu et al. 2016). The bend of the peak at about 1030 cm^{-1} on the modified membranes spectra shows the cross-linkage of the PVA molecules and GA onto the surface of the membrane (Tang et al. 2016).

4.4 Membrane morphology and structure

Membrane morphology and structure were investigated using SEM. Top views of all membranes in Figures 4.2 and 4.3 presented ridge and valley surface structure for all membranes (Freger et al 2002). PVA modified demonstrated denser surface as compared to the unmodified membrane. The level of disproportion in the structure differed with a change in concentration of the modifying agent. PVA modified membranes became more compact as the concentration of PVA increased from 0.05 to 0.2 g/L, causing a surface negative charge decrease (Hu et al. 2016). Modifications conducted by other researchers (An et al. 2011) showed that a less dense surface ensured smoothness; while the use of GA and PVA caused denser surfaces, hence roughness. Cross-section images of PVA modified membranes in Fig.3 demonstrated the impact of the modification on the surface of the membrane. The thickness of the top selective layer of membrane increased with an increase in PVA concentration. Modifications of membranes using PVA resulted in an increase of the thickness of the selective skin layer of the TFC membrane as the modifying solution concentration increased.

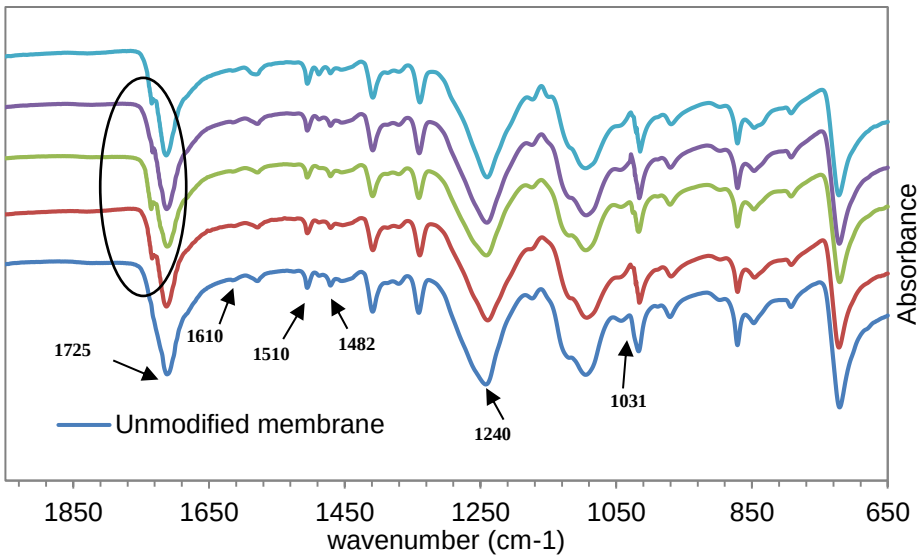

Figure 4.1: ATR-FTIR spectra of PVA modified membranes

4.5 The flux of PVA modified membranes during the fouling test

Figure 4.4 displays the time-dependent flux of the modified membranes compared to the unmodified membrane during the filtration of the synthetic feed solution. The same operating conditions with initial permeate flux of 57.5 $L/m^2.h^1$, 19 cm/s cross-flow velocity, and 5 bar pressure were used for all experimental runs. The flux was normalized at 25℃.

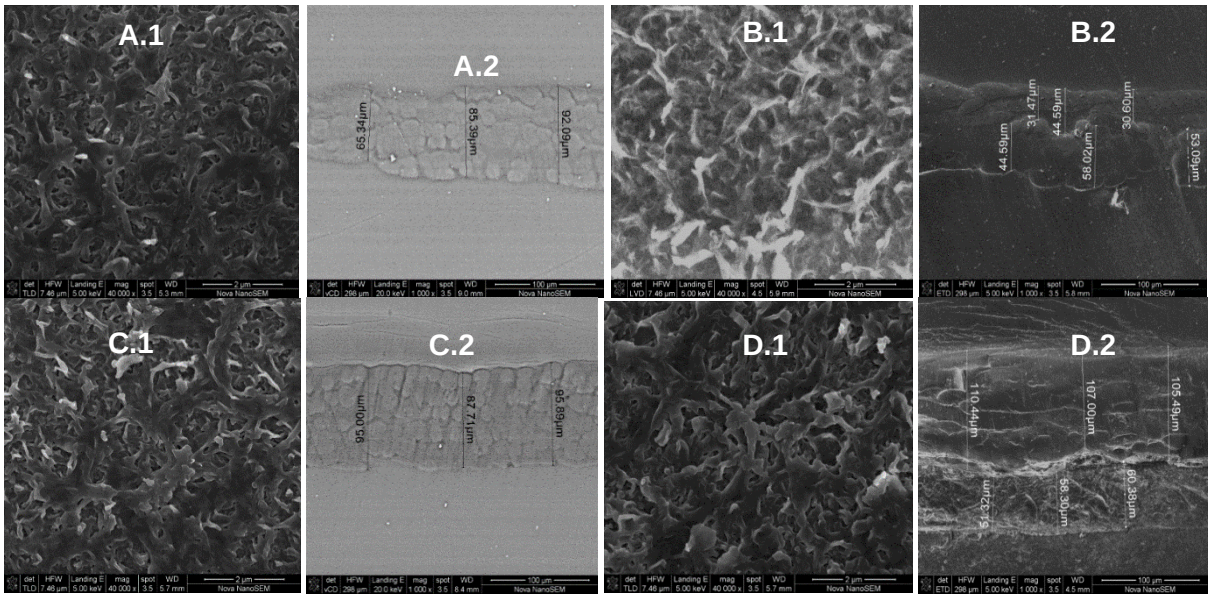

Figure 4.2: SEM images of PVA modified membranes (top view & cross-section): (A) 0.05 g/L PVA, (B) 0.1 g/L PVA, (C) 0.15 g/L PVA and (D) 0.2 g/L PVA

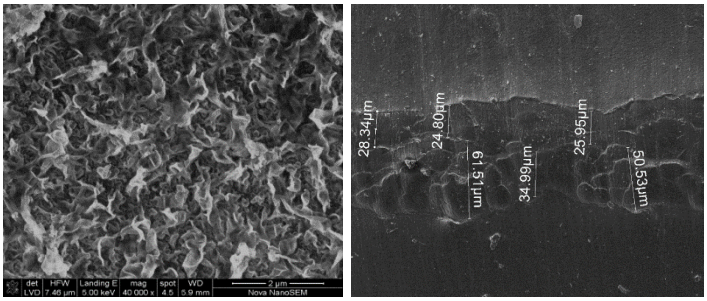

Figure 4.3: SEM images of the virgin membrane: top view (left) and cross-section (right)

The persistent decrease in the normalized flux, for all graphs in Figure 4.3, proved the presence of fouling (Cheng et al. 2013). Over time the flux decline is more noticeable, and the flux difference between the unmodified and the modified membranes, increases. The distance between flux curves on each plot differ. The normalized flux of modified and unmodified membranes seemed to be similar in the first 500 min; thereafter the difference was more noticeable. The unmodified membrane flux declined the most. Among PVA modified membranes, the 0.15 g/L. PVA membrane declines the least. Flux decline rate indicates of fouling severity, thus higher flux decline indicated that sever fouling happened (Yu et al. 2013). Thus, it can be deduced that the fouling was more severe with the unmodified membrane and less with modified membranes, with increasing concentration. It is known that the reduction in flux decline after modification is due to the lower hydraulic resistance to water permeation of the surface of the membrane where the fouling layer is formed (Yu et al. 2013). Thus, it can be said that PVA modified membrane permitted the formation of a lower hydraulic resistance at the surface of the membrane during filtration.

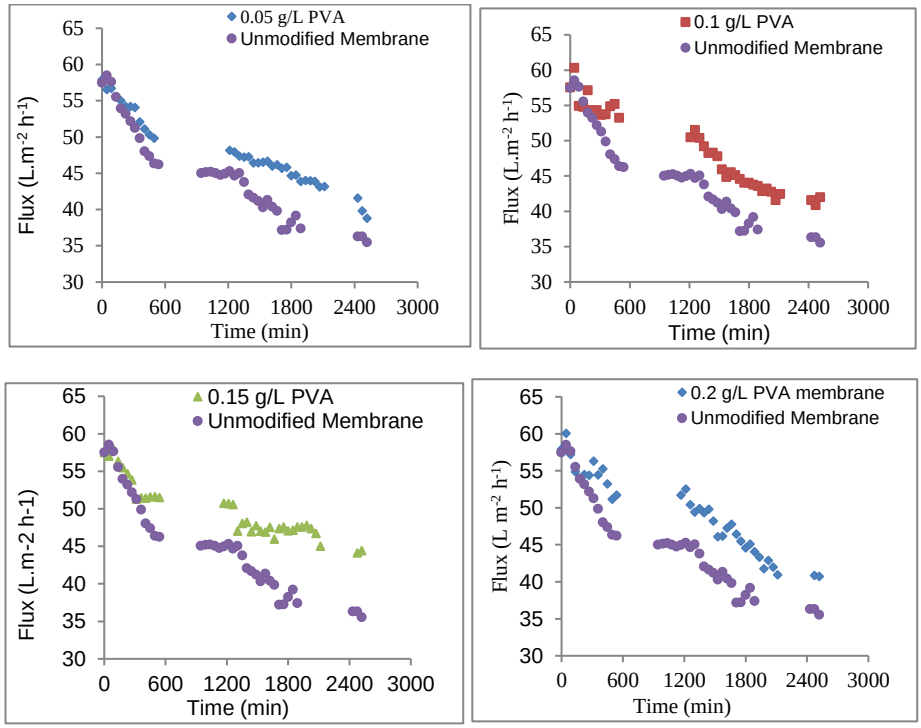

Figure 4.4: Time-dependent flux of unmodified and PVA modified membranes (bottom) during filtration of the synthetic feed

4.6 Membrane anti-fouling properties

Antifouling properties of the membranes were evaluated using the FDR and the FRR as reported by other studies (Zhao et al. 2015; (Shen et al. 2017). The FRR also revealed the cleaning properties of membranes after fouling tests. The highest FDR and lowest FRR were obtained from the unmodified membrane.

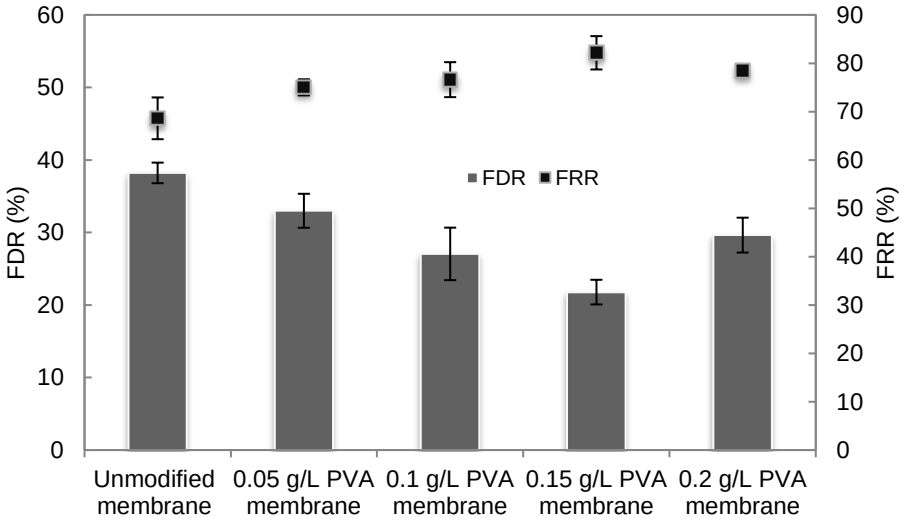

Figure 4. 5: FDR during filtration of the synthetic feed and pure water FRR of membranes

The flux declines ratio (FDR) 38.21 % before modification while the lowest value of the FDR was 21.78% among PVA modified membranes. Lower flux decline rate shows that the membrane possessed better resistance to fouling (Hu et al. 2016). The pure water flux of the unmodified membrane after fouling experiments was recovered only to 68.61%, while the FRR of PVA modified membranes were higher, and increasing with an increase in the concentration of the modifying solutions. Higher flux recovery indicates that fouling occurring, is more reversible (Mahdavi et al. 2017). Thus, it can be said that fouling of modified membranes was more reversible with increasing concentration of the modifying agents up to 0.15 g/L PVA.

4.7 Membrane anti-biofouling properties

The 0.1 g/L PVA modified membrane and the unmodified membrane were selected to perform biofouling tests and evaluate membrane anti-biofouling properties using *Escherichia coli* (E. coli) bacterial solution. The value of the 0.1 g/L PVA modified membrane was 36.75%, whilst the value of 33.80% was obtained for the unmodified membrane. A higher sterilization ratio shows how much the membrane possesses anti-microbial property to obstruct multiplication of microorganisms tested against the microorganism tested (Zhang et al. 2013). The unmodified and 0.15 g/L PVA modified membranes were further tested for water flux in the RO process after exposure to E. coli bacteria cell suspension for 30 hours, and values are reported in Figure 4.6. Pure water flux of the membranes was measured at the same initial conditions and compared. Although R values of both unmodified and 0.1 g/L PVA modified membranes were similar, showing a similar capacity to obstruct reproduction of microorganisms; results presented in table 4, however, showed that the PVA modified membrane had a higher resistance to adhesion of microorganisms to the surface of the membranes.

Table 3: Values of membrane sterilization ratios obtained from the selected membranes

Membrane	A (x 10^6 CFU/mL)	B (CFU/mL)	Sterilization ratio, R (%)
Unmodified membrane	106	16x 10^7	33.75
0.1 g/L PVA modified M	100	16 x 10^7	36.80

The water flux of the unmodified membrane decreased from 62 to 34 $L.m^{-2}hr^{-1}$; while the selected PVA modified membrane decreased from 49 to 36$L.m^{-2}hr^{-1}$. It implies that the flux of the unmodified membrane was recovered to only 54%, while the flux of the PVA modified membrane flux was recovered to 74%, thus showing poor adhesion of micro-organisms on the modified membranes.

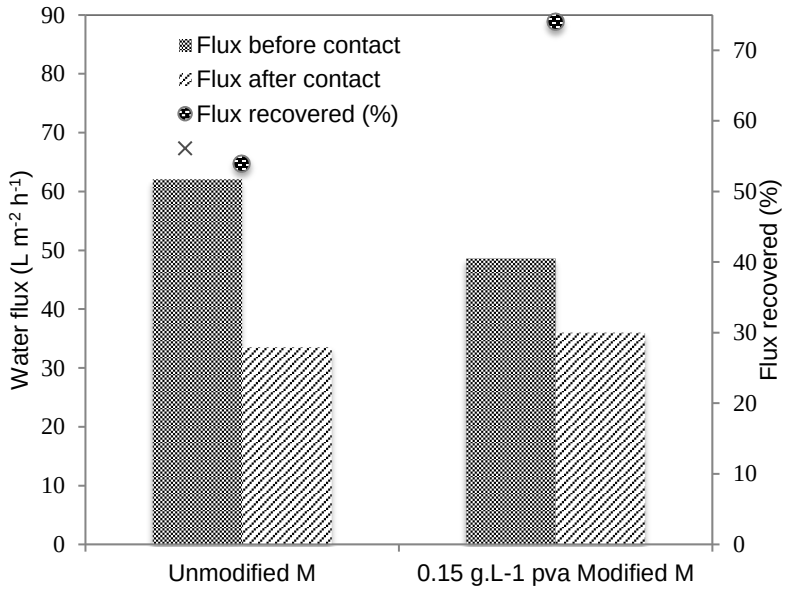

Figure 4.6: Pure water flux of membranes before and after the biofouling of E. coli bacteria

4.8 Summary

In this study, it has been shown that cross-linkage of glutaraldehyde and polyvinyl alcohol was successfully grafted onto the active layer of the TFC RO membrane surface. The chemical structure of unmodified and modified membranes were determined by chemical analysis. ATR-FTIR spectra revealed noticeable new peaks as well as an increase in their intensities highlighting the successful grafting on the membrane surface. Top-view SEM images clearly show a change in density whereas cross-section SEM images show changes in the thickness of the top layers of the modified membranes. This top layer thickness increases with the increase of grafting solution concentration. Pure water flux of PVA modified membranes varied in descending order with an increase of the grafting solution concentration. The flux decline ratio decreased, and flux recovery ratio increased for the modified membranes as grafting solution concentration, increase. During the biofouling test with E. coli bacterial solution, the sterilization ratio increased after modification. In conclusion, these modified membranes showed high resistance to fouling and great potential as an RO purification step for municipal secondary MBR effluent.

Chapter 5

Removal of inorganics from municipal MBR effluent using thin-film composite membranes: Effect of operating conditions

This chapter will be submitted:
Aziz M; Ojumu TV; Kasongo G; The removal of selected inorganics from domestic MBR secondary effluent using aromatic polyamide thin-film composite UF/NF/RO membranes: Effect of operating conditions; 10TH International Conference on Environmental Pollution and Remediation (ICEPR'20), submitted 23 January 2020 [Paper ID: ICEPR'20-111]

5 THE REMOVAL OF TARGETED INORGANICS

5.1 Introduction

This study aimed at evaluating the effects of operating conditions in the removal of inorganics from MSWWTWs MBR secondary effluent using three membranes, namely UF, NF, and RO. Experimental runs were conducted on a pilot plant in a continuous system, varying the pH, as well as the permeate flux and the percentage recovery in the case of RO membrane. Chemical analysis of different inorganics was conducted to calculate the percentage removal.

5.2 Salt rejection and TDS removal

The performance of membranes was assessed by measuring the TDS and salt rejection, with the pilot plant operating at 25 L/m^2 hr^1 flux, 75% recovery and pH uncontrolled in phase one, then 6.5 pH in phase two of experimental runs. Figure 5.1 shows the permeate salt rejection and Figure 5.2 the TDS as a function of time, obtained for all three membranes during experimental runs on the pilot plant. RO system salt rejection was the highest, in the range of 94.4–96.6% with controlled, and 89.2– 91.4% with uncontrolled pH. The UF and NF membrane performed as expected, but a controlled pH of 6.5 resulted in a better salt rejection for both type of membranes. The UF and the NF did not reduce the TDS concentration as much as the RO membrane. The results indicated that the performance of the membranes was stable throughout the duration of the experimental study. The stable conditions could be also due to the online anti-scalant dosing throughout all experimental 8-hour runs. No sign of flux decline throughout this period was experienced. A study using a thin film composite polyamide RO membrane within a similar pilot-plant set-up confirmed that the dosing of antiscalant minimize scaling and influence the delay in flux decline (Aziz and Kasongo 2019). It is normal that the salt rejection % of RO membrane was found higher because RO has a denser skin than NF and UF membranes. Since salt rejection is due to the rejection of ionic species present in water, the mechanisms that can be attributed to salt rejections are size exclusion, charge and ionic electrostatic interactions of the ions in the membranes' surface. It is reported that monovalent ions in the feed water can generally pass through the membrane more easily than divalent ions (Bartels et al. 2008) because divalent ions are larger than those of monovalent ions. According to the literature, the surface of NF and UF membranes are negatively charged at pH values higher than 4 (Al-Amoudi and Lovitt 2007; Al-Amoudi et al. 2008). The pH of the wastewater used in this work was between 6.3 - 7. It is understandable that at pH of 6.5, NF and UF membranes present negative charge density on the membrane surface. Because divalent ions are generally larger than monovalent ions, the main mechanism of ion rejection by membranes is a sieving mechanism (Sert et al. 2017).

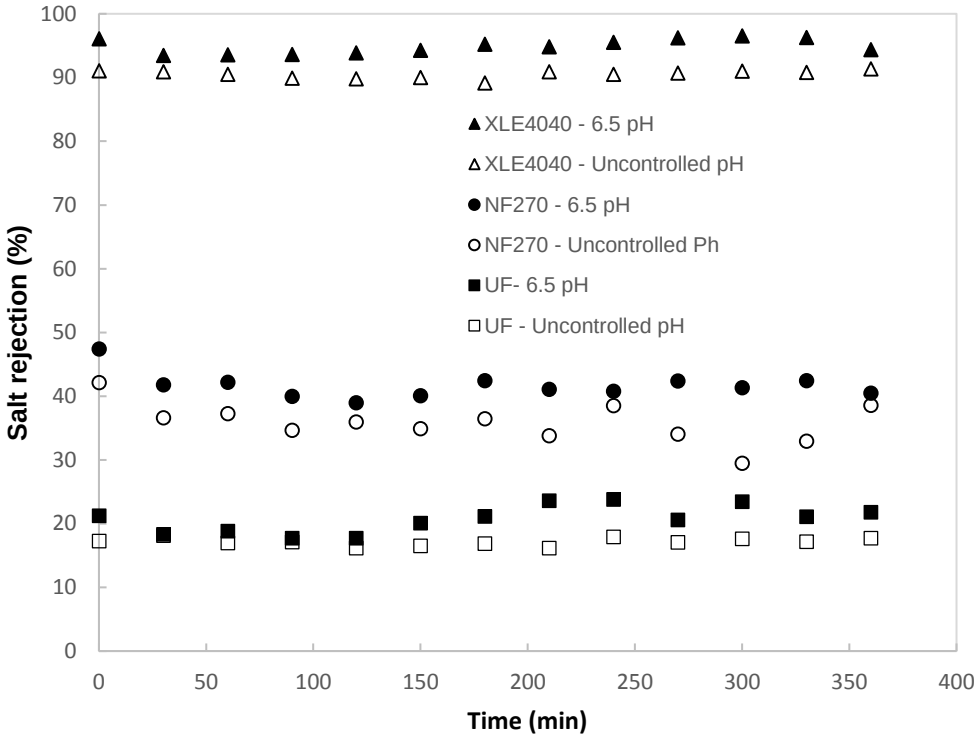

Figure 5.1: Effluent salt rejection obtained with the three membranes for experimental conditions with uncontrolled pH and 6.5 pH

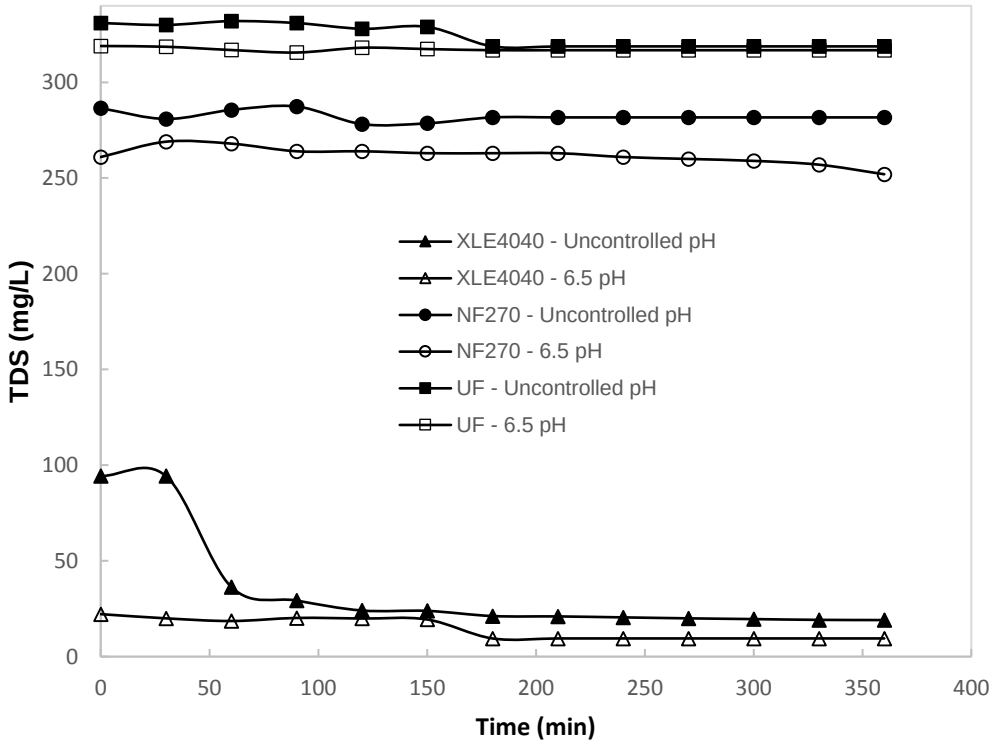

Figure 5.2: Effluent TDS obtained with the three membranes for experimental conditions with uncontrolled pH and 6.5 pH

5.3 COD removal

The removal of COD for all tested membranes is presented in Figure 5.3. The COD percentage removal with the RO membrane was significantly higher than the NF and UF membranes ($p = 0.009$ for RO, $p = 0.013$ for NF and $p = 0.018$ for UF) for, uncontrolled pH and pH of 6.5 at 92 and 99 %, respectively. The effect of pH on the COD removal with the NF and UF membranes appeared to have the opposite effect as compared to the RO membrane, as with a controlled pH higher percentage was achieved with the latter membrane. The pH range with uncontrolled pH experimental runs was between 6.7 and 7.1 as indicated in Table 3, while experimental runs with controlled pH maintained the latter at 6.5. The COD removal has been reported to increase with increasing the pH, which was in part attributed to the rise in the hydroxide ions concentration, resulting in an increase of the production of hydroxyl free radicals (Al-Bastaki 2004). This may, therefore, suggest that the increase in pH was a predominant factor in the removal COD when using the UF and NF membrane. Changes in properties such as pH have been reported by other researchers to affect contaminant removal which was found to be substantially lower when operated without pH control (Saichek and Reddy 2003).

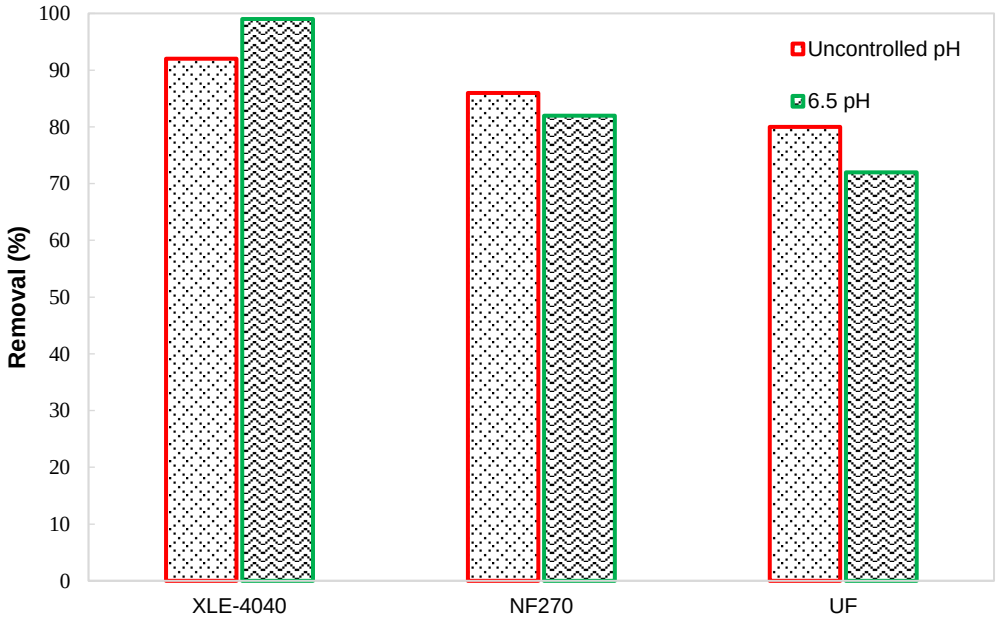

Figure 5.3: COD removal

The better removal of COD achieved with a controlled pH when using the RO membrane may be explained by the fact that a controlled pH results in a higher and more sustained osmotic flow which caused greater COD removal (Saichek and Reddy 2003); as well as the surface of the membrane, which became less negative with the decrease in pH as compared to experimental runs with no adjustment of pH (Chan et al. 2007).

5.4 Removal of inorganics

The permeate quality of the UF, NF and RO membranes in terms of inorganics of interest is summarized in Figures 5.4, 5.5 and 5.6. The common inorganics found in wastewater emanate from domestic and industrial products which include pesticides, preservatives, surfactants and perfluorochemicals in the form of human waste, pharmaceutical residues, as well as steroidal hormones excreted by humans (Shad *et al.*, 2019). Therefore, the salts were selected in order to evaluate the correlation of anionic, neutral and cationic solutes with membrane-type and pH. The percentage removal of phosphate (Figure 5.4) was: 40, 89 and 94% and phosphorus (Figure 5.5): 58, 90.5, and 96% with the UF, NF and RO membranes, respectively. There was, therefore, a significant difference in the removal of selected inorganics and as well as the COD observed with the three membranes (p = 0.001 for uncontrolled pH, p = 0.043 for 6.5 pH at α = 0.05). Phosphorus removal is visibly higher than phosphate due to the size exclusion and chemical charge. Phosphorous is a neutral molecule, which differs from phosphate a multivalent anion, that may increase electrostatic repulsion with the surface of the membrane (Chu et al. 2017).

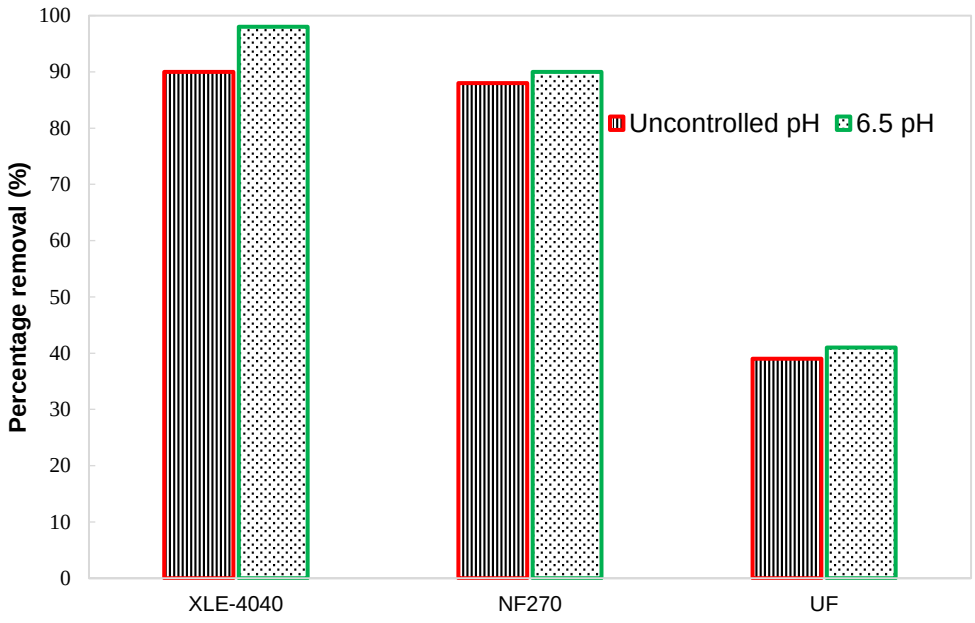

Figure 5.4: Phosphate removal with UF, NF and RO membranes

The slight reduction of both phosphate and phosphorous with pH change indicates that pH adjustment affects only slightly the removal of these physicochemical properties regarding the three membranes (p > 0.05 for both phosphorous and phosphate). Unlike ammonia, the adjustment of pH had a significant effect, especially for the RO and NF membranes (p = 0.018 at α = 0.05), where the removal percentage increased from 62 to 99% and 52 to 87% respectively, when changing from 6.5 pH to uncontrolled pH. This could be explained by the

fact that the pH adjustment (pH 6.5) shifted the equilibrium of ammonia, resulting in higher removal of ammonia (Figure 5.6) (Chan et al. 2007).

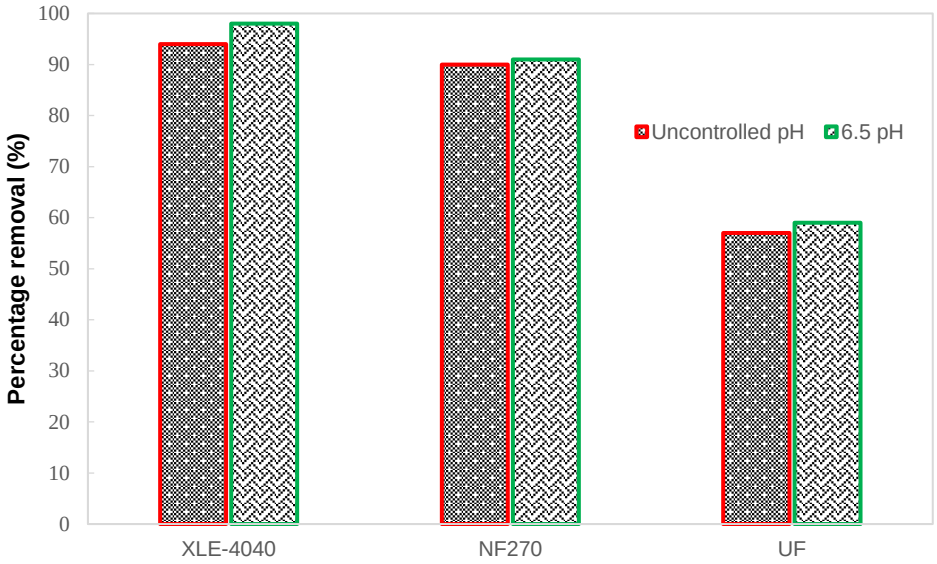

Figure 5.5: Phosphorous removal with UF, NF and RO membranes

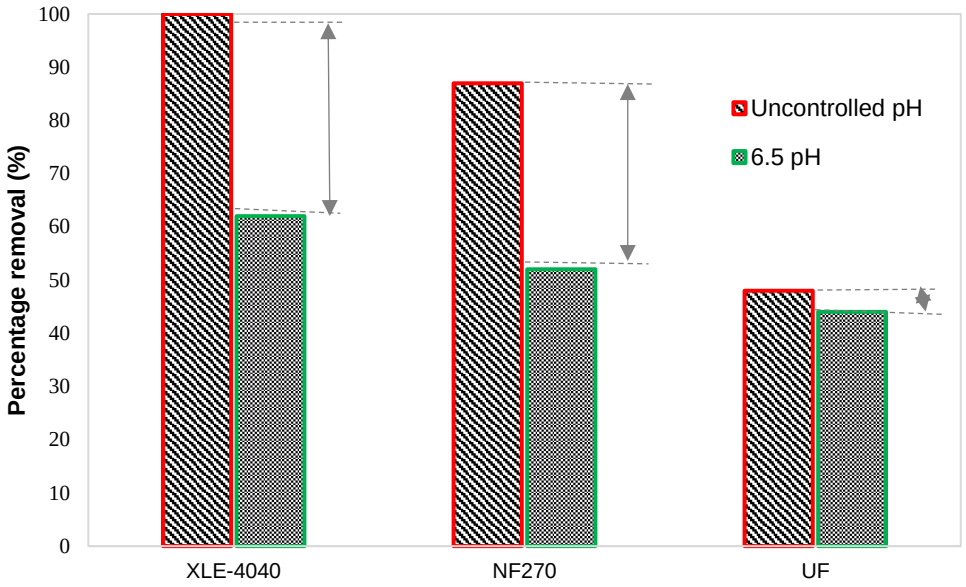

Figure 5. 6: Ammonia removal with UF, NF and RO membranes

5.5 Treated effluent application

The permeate obtained with the three membranes were compared to water quality requirements for reuse in cooling systems as well as irrigation. Basic parameters of the permeate obtained in this study are presented along with the criteria for specific reuse in Table 5.1. According to the results, important changes were observed in the inorganic concentrations and COD with the three membranes. The RO permeate met all conditions required for reuse

in both applications, irrigation and cooling system. Interestingly, the RO permeate was found to be unrestricted for any type of irrigation according to requirements presented by other workers (Emongor et al. 2005; Üstün et al. 2011; Hansen et al. 2016). The physiochemical properties of the NF effluent suggest that the effluent is suitable for use in industrial cooling but may be restricted for a specific application in irrigation as some of the parameters such the TDS (255 mg/L) fall out of the required range for unrestricted irrigation water quality. The UF, in contrast, appeared to be suitable only for reuse in cooling systems.

Table 5.1: Characteristics of water quality obtained with the different membranes and reuse criteria for wastewater in different applications

Parameter	Irrigation (Emongor et al. 2005; Üstün et al. 2011)	Cooling system (Asano et al. 1988; Hansen et al. 2016)	UA60	NF270	XLE
COD (mg/L)	< 50	< 30	16	10	2
NH_3 (mg/L)	< 6.08	< 1	0.62	0.28	0.17
P (mg/L)	< 1.5	-	1.8	0.79	0.21
PO_4 (mg/L)	< 2	< 7	2.07	0.91	0.45
TDS (mg/L)	< 200	-	300	255	19
pH	6.5 – 8.4	6.8 – 7.2		6.5 – 7.05	
EC (µS/cm)	< 250	< 1445	471	355	37
Turbidity (NTU)	< 2	< 36	-	-	0.08

5.6 The effect of flux and recovery on RO (XLE) membrane

The effects of permeate flux and recovery were evaluated using the XLE - RO membrane. The system's performance was evaluated in terms of the percentage reduction of contaminants. Table 5.1 shows the percentage reduction at different flux and recovery conditions. For the experimental runs conducted at constant pH, the highest percentage reductions were achieved at 25 L/m^2.hr and 75% recovery. The highest percentage reductions obtained for Ammonia, Nitrate, Nitrite, Phosphate and Phosphorous were 98%, 100%, 83%, 97% and 98 %, respectively. Although, the findings unmatched expectations (suggesting a slight decrease in permeate inorganics concentration when increasing flux, on the basis that ion leakage across the membrane remains fairly constant (Shad et al., 2019)) the phenomenon may be explained by the increase in percentage recovery (75%) which allows for the mass of ions at the surface of the membrane to be blended with more permeate, resulting in a lower concentration of inorganics in the permeate.

The percentage reduction of the different inorganics tested increased from nitrite, nitrate, phosphate and ammonia removals, respectively, to phosphorous removal. Higher rejection of multivalent ions can be explained by the size of multivalent ions, which are larger than monovalent ones. Therefore, an increase in ion charge causes an increase in electrostatic interactions with membranes which determine the contaminant removal mechanism (Chu et al. 2017).

Table 5.2: inorganics percentage removal using XLE-4040 membrane

Operating conditions →		30 L/m^2.hr flux		25 L/m^2.hr flux	
↓		% Recovery			
Water pH	Inorganic	**75**	**50**	**75**	**50**
6.5	NH_3	92	97	98	97
	NO_3	100	87	80	100
	NO_2	63	60	83	82
	PO_4	90	97	90	97
	P	92	97	98	97
Uncontrolled	NH_3	80	87	94	92
	NO_3	68	76	63	63
	NO_2	61	55	86	71
	PO_4	98	86	98	88
	P	80	87	94	92

Figure 5.7 describes the results obtained for COD removal under different experimental conditions, the water recovery percentage and fluxes were varied, along with the pH. The highest % removal was obtained (99%) when the system ran at 75 % recovery and 25 L/m^2.hr^1 of flux, with a controlled pH. Overall, the change in operating conditions contributed to a slight difference in the removal percentage of the COD as observed with the inorganics observed in Table 5.2. This suggests that the accumulation of organic matter in the treatment of the effluent with XLE membrane can be effectively reduced with a controlled pH and by adjusting the flux and recovery.

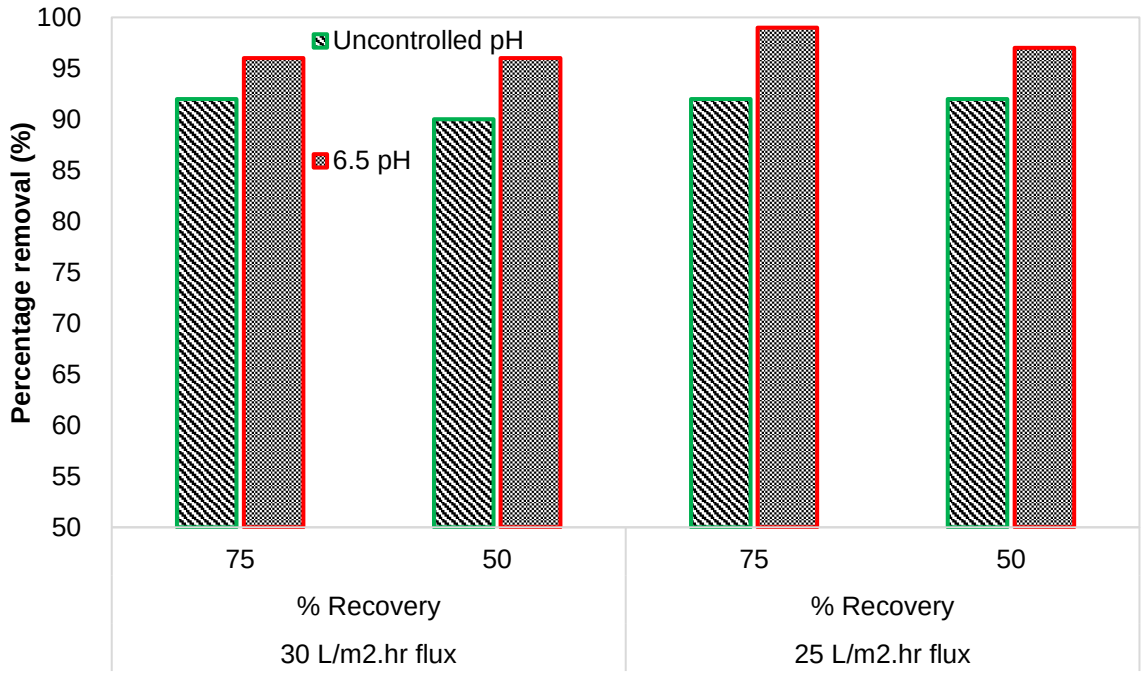

Figure 5.7: COD removal using XLE RO membrane

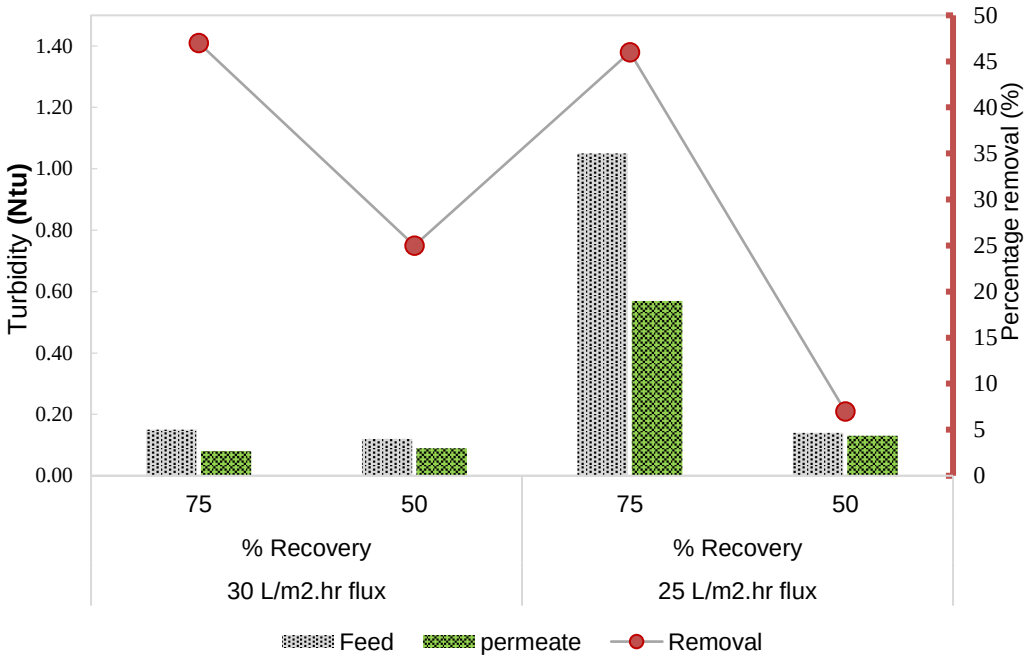

Figure 5.8: Turbidity removal using RO membrane with controlled pH at 6.5

Figure 5.8 shows levels of turbidity obtained after and before treatment of secondary effluent under different conditions, where the lowest turbidity is obtained under conditions of 75 % recovery and 25 L/m^2 hr^1 permeate flux; where the highest turbidity in the permeate is obtained at 50 % recovery and 25 L/m^2.hr^1. The high turbidity observed in the feed samples indicates the presence of finely divided organic and inorganic matter, soluble coloured organic compounds and microscopic organisms which were likely to be high. Photograph 5.1 shows the different stages of organic and inorganic removal and how the samples differ for each treatment process.

Photograph 5. 1: Samples showing the turbidity at different steps of the MBR/RO process

5.7 Conclusion

In this study, the effects that pH, permeate flux and recovery have on the removal of inorganics in the treatment of MSWWTWs MBR effluent with UF/NF/RO process have been shown. The effects of percentage recovery, as well as permeate flux, were tested with the best performing membrane, RO (XLE). The pH adjustment was found to be one significant factor governing the reduction of most inorganics tested as well as the COD for all three membranes, RO (XLE), NF (NF270) and UF (UA60). The percentage reduction was found to exhibit different behaviour regarding the control of pH for the different inorganics. The RO was able to reduce the COD by 99 % with a controlled pH and adjusted flux of 25 L/m^2 hr^1 and water recovery of 75 %, respectively. This demonstrated that the control of pH and adjustment of flux and recovery, especially for the RO membrane, could be an option to consider for the improvement of inorganics removal in the advanced treatment of domestic secondary effluent. It was shown that the quality of water obtained with the RO could meet quality requirements for many potential potable and non-potable reuse applications.

Chapter 6

Combining MBR and UF/NF/RO membrane filtration for the exclusion of estrogenic and androgenic steroid hormones for potential indirect water reuse application

This chapter has been published as:
Aziz M & Ojumu TV; 2020; Exclusion of estrogenic and androgenic steroid hormones from municipal membrane bioreactor wastewater using UF/NF/RO membranes for water reuse application, *Membranes,* 10 (37), 1-18 [ISSN 2077-0375/DOI:10.3390/membranes10030037]

6 Natural Steroid Hormones

6.1 Introduction

In this study, MWWTWs wastewater treated by a full-scale membrane bioreactor (MBR) was used directly as the influent to reverse osmosis in an RO pilot-plant for the removal of 17β-estradiol (E_2) and testosterone (T) as a potential indirect water recycling application. Three commercially available UF/NF/RO membranes, namely UA60, NF270 and XLE, were selected for this investigation. Estrogenicity and androgenicity were assessed using the enzyme-linked immunosorbent assays (ELISA) and the recombinant yeast estrogen receptor binding assays (YES).

6.2 ELISA analysis of 17β-estradiol (E_2) and testosterone (T)

Estradiol was detected in all influent samples analysed (Figure 6.1). The highest E_2 concentration was detected in the raw influent sample (80.22 ng/L), followed by the average MBR influent (7.61 ng/L) and effluent (4.84 ng/L). The MBR effluent (RO influent) for May, June and July was 5.35, 3.39 and 6.71 ng/L, respectively.

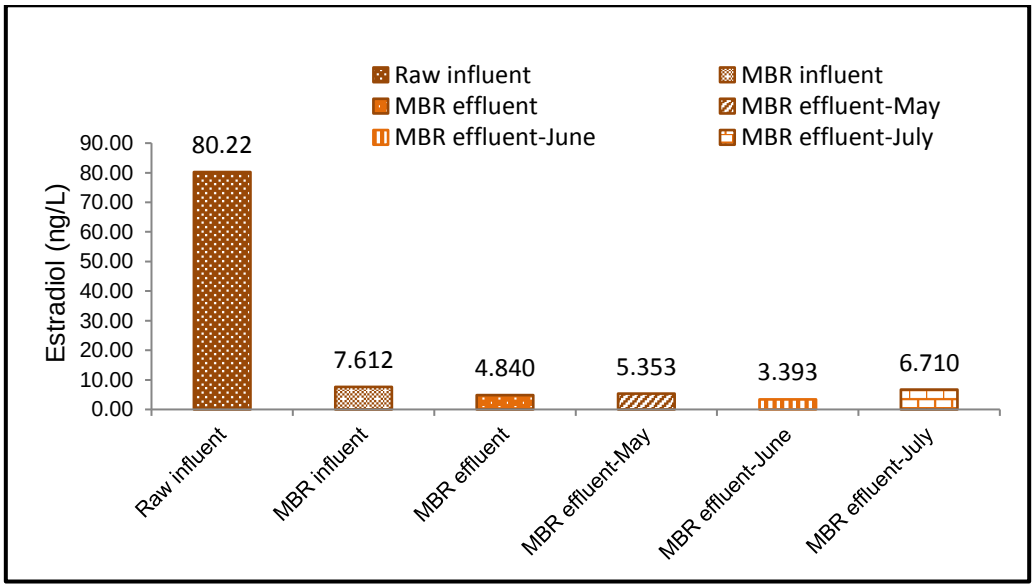

Figure 6.1: The mean of 17β-estradiol (E_2) levels (ng/L) measured in water collected from influents at six stages within the MSWWW including MBR & RO, during winter: May, June and July.

The highest concentrations of estradiol were found in the raw influent (Figure 6.1), which was confirmed by Faul et al. (2013), who measured E_2 at the sewage inlet plant in Windhoek, Namibia at 78ng/L. Raw and MBR influent and effluent revealed a significant removal of

estradiol during the purification process (Figure 6.2). The concentrations of estradiol in the RO effluent (the permeate water) were below the level of detection (LOD) for the XLE and NF270, but not for the UA60 membranes.

High testosterone levels, ranging from 281 - 135 ng/L, were detected in the raw feed inlet (Figure 6.6). Testosterone removal is highly effective during the sewage water treatment process (anaerobic and the MBR aerobic tank). Testosterone concentrations were, on average, much higher than those of estradiol. This agrees with the excretion concentrations of the natural steroid hormones by humans and animals (Shore and Shemesh 2003) and is similar to trends shown by Leusch et al. (2006) for MSWWTPs in Australia and New Zealand. Estradiol and testosterone are lipophilic and poorly soluble in water and 17β-estradiol exerts its physiological effects at a lower concentration than other natural steroids (Shore and Shemesh 2003).

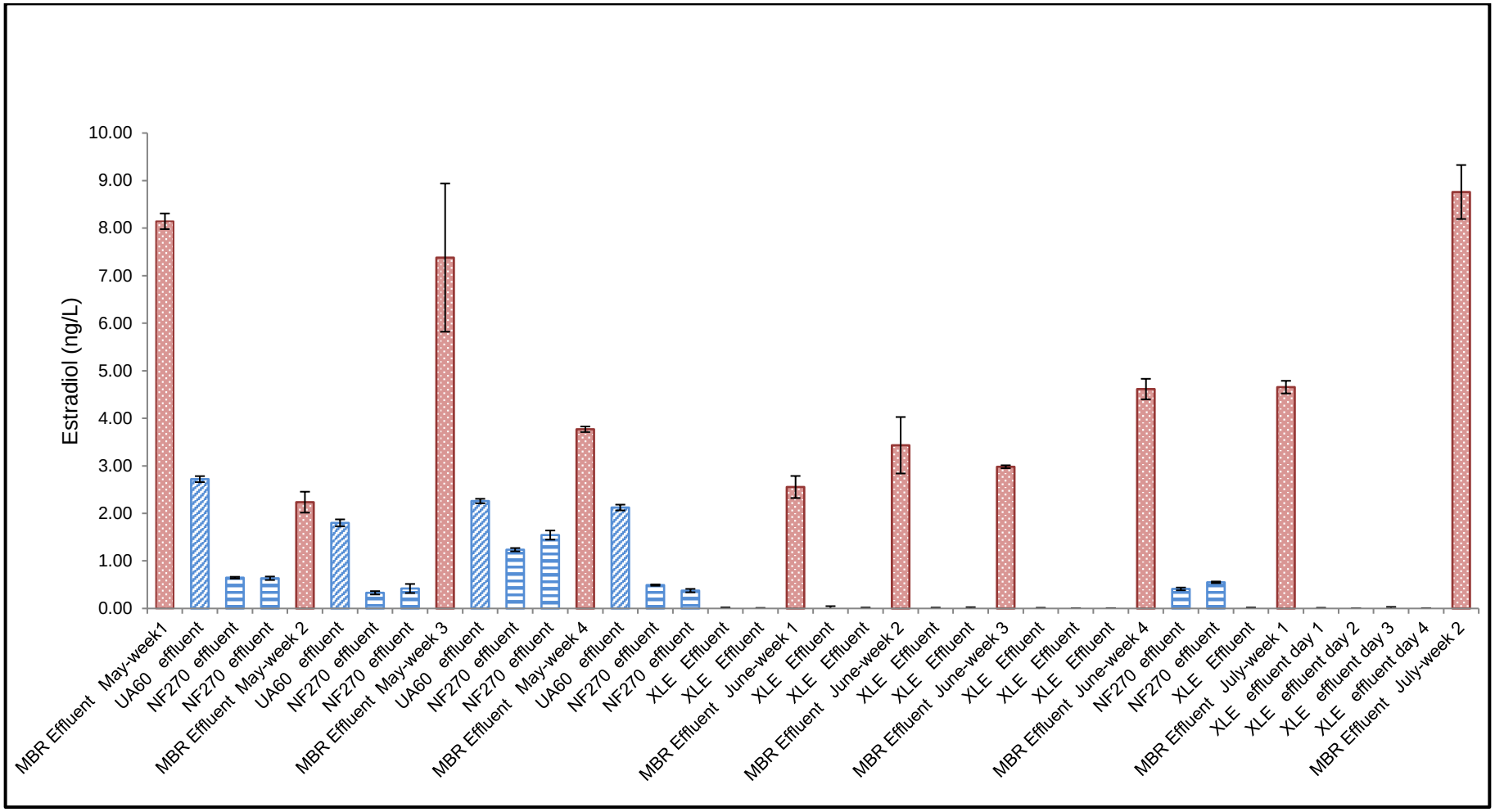

Figure 6.2: The ELISA mean of 17β-estradiol (E_2) levels (ng/L) measured in water collected from influent and effluents at various stages within the MSWWW including MBR and RO, during winter: May, June and July. Error bars denote SD; *n* = 2, error bars show maximum levels detected

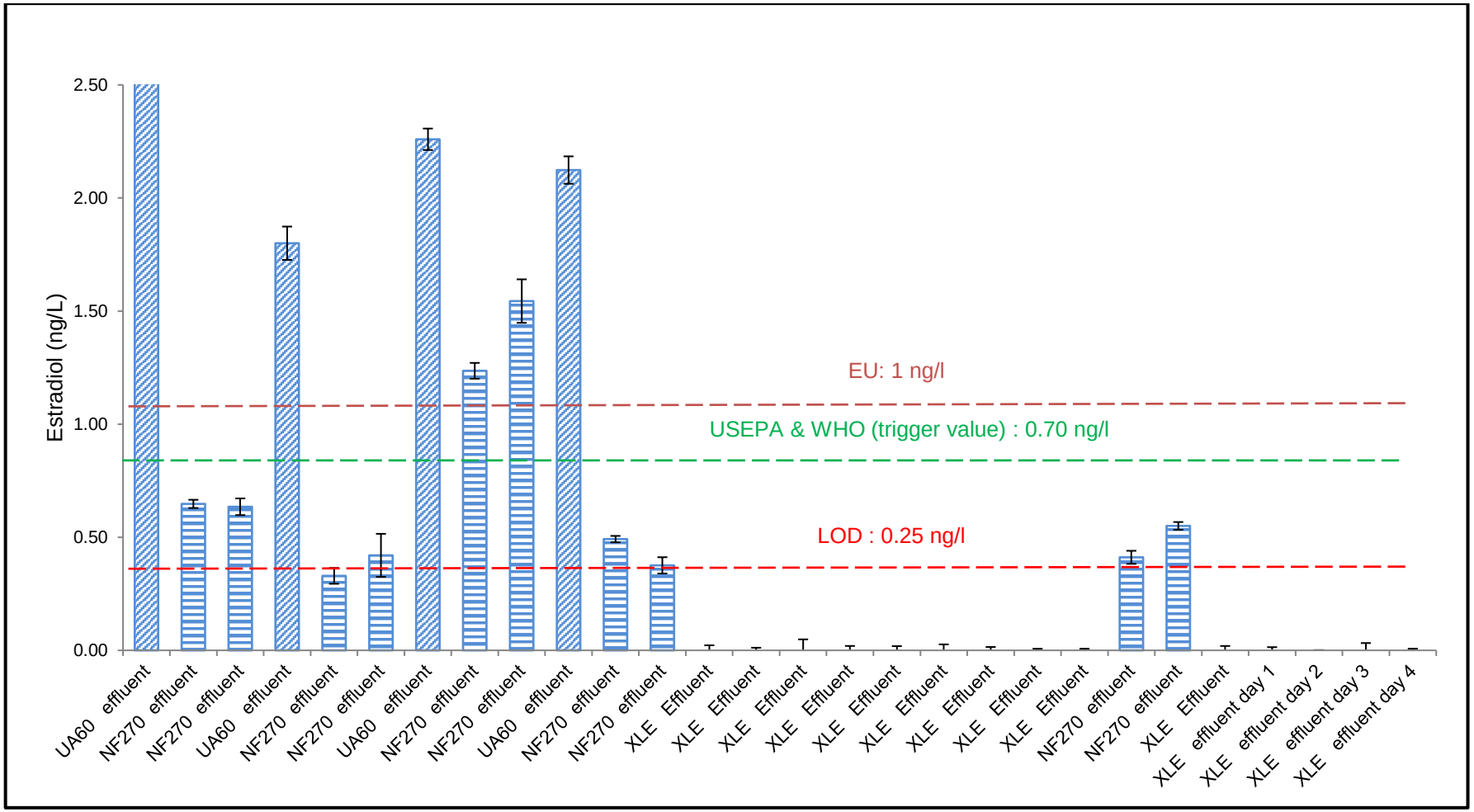

Figure 6.3: The ELISA mean of 17β-estradiol (E_2) levels (ng/L) measured of the effluents of the UF, NF and RO membranes processes under various pilot plant conditions. Error bars denote SD; *n* = 2, error bars show maximum levels detected. [EU, 1 ng/L modulate fish production (Shappell et al. 2007); USEPA and WHO, 0.70, trigger value for drinking water (Genthe et al. 2010), LOD: 0.25 ng/L

A 91% removal of estradiol was recorded in the anaerobic (anoxic) tank, where the raw influent was reduced from almost 80.22 to 7.61 ng/L. The lowest % removal (36) was measured by the MBR aerobic (oxic) tank, where the MBR influent was reduced from 7.61 - 4.85 ng/L, only. UF, NF and RO had an expected % removal of 54, 84 and 97. The change in MBR influent and effluent can be seen in Figure 6.4, where the error is notable. This is an indicator confirming the fluctuation of the inlet streams.

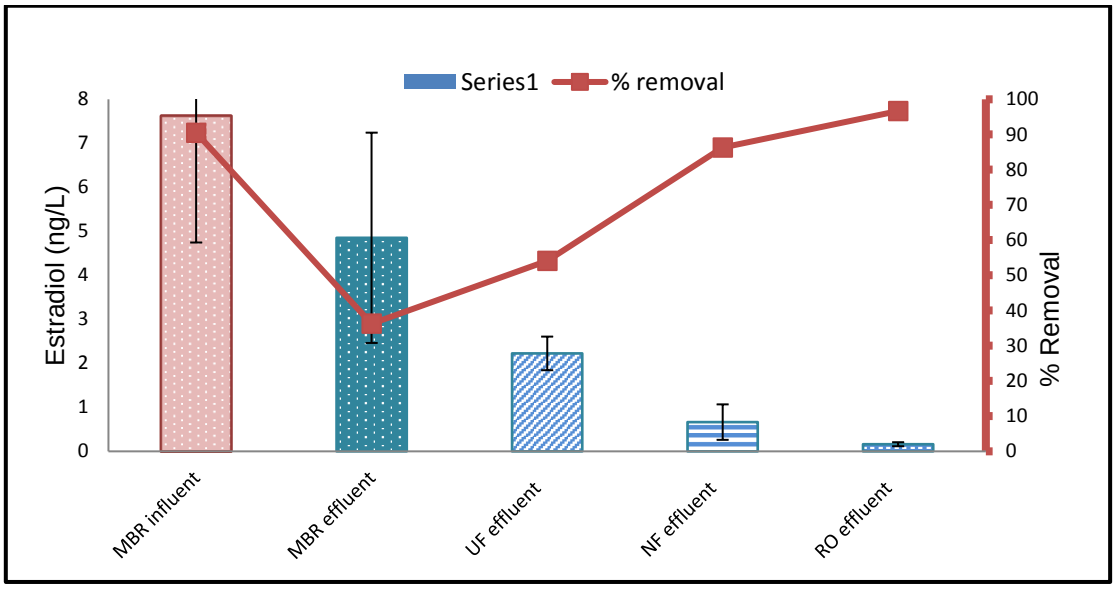

Figure 6.4: The mean of 17β-estradiol (E2) levels (ng/L) measured in water collected from influents at five stages within the MSWWW including (MBR and RO feed and permeate) during winter: May, June and July (Figure 6.2 without the raw feed). Error bars denote SD; *n* = 2, Error bars show maximum levels detected

E_2 was completely removed to below level of detection (LOD) for all XLE treatment processes with its removal efficiency of >93%. This agrees with previous results reported by Lee *et al*, (2008), for secondary processes. The UF, NF and RO effluents in sequence with MBR process were conserved to give very good efficiencies for the removal of 17β-estradiol and testosterone. Figure 6.4 showed that the E_2 concentration for the effluents of MBR, UF, NF and RO in sequence with MBR process had very low E_2 concentrations of 4.85, 2.22, 0.67 and 0.16ng/L. NF and RO effluents had significantly reduced E_2 concentration compared with the influent, 7.61 ng/L. (p = 0.007 at for UF, p = 0.00027 for NF, p = 0.00016 for RO α = 0.05). This is consistent with a similar study of MBR/NF and MBR/RO membrane effluent rejection (Comerton et al. 2008). MBR is considered a relatively better treatment process for the removals of steroids compared to conventional activated sludge processes alone (González et al. 2007; Méndez et al. 2017). Likewise, micropollutants (MP), such as E_2 and T, can be removed by size exclusion and adsorption mechanisms using ultrafiltration (UF), nanofiltration

(NF) and reverse osmosis (RO). It was showed that steroid hormones such as E_2 can be removed by MBR/RO processes by 99%. (Auriol et al. 2006; Snyder et al. 2007; Lee et al. 2008),

Testosterone (T) was detected in all feed samples analysed (Figure 6.5). The highest T concentration was detected in the raw influent sample (281.3 ng/L), followed by the average MBR influent (135.0 ng/L) and effluent (118.7 ng/L). The MBR effluent (RO influent) for May, June and July was 117.2, 116.9 and 116.4 ng/L, respectively.

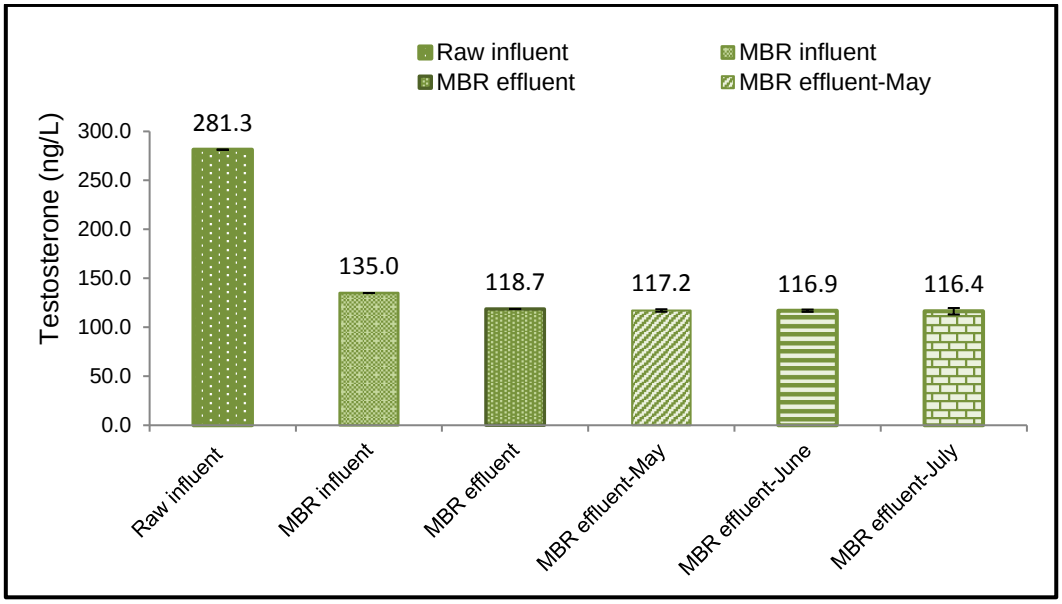

Figure 6.5: The mean of testosterone (T) levels (ng/L) measured in water collected from influents at six stages within the MSWWW including MBR and RO, during winter: May, June and July.

Testosterone concentrations showed greater variation between the different samples with the highest as mentioned before with a raw influent concentration of 281.3 ng/L (Figure 6.6) and lowest concentration after the MBR/RO process with an RO effluent (Figure 6.7) of 11.4 ng/L. Testosterone levels measured corresponded well with those measured by Stalter et al. (2011) in Switzerland and Germany (21 - 400 ng/L) and Manickum et.al. (2014) in South Africa (11 to 343 ng/L), while Fernandez et al. (2007) in Canada and Chang et al.(2011) in China, observed much lower concentrations (21 to 76.7 ng/L). However, the disparity in concentrations measured by Leusch et al. (2006) is much more extreme (113 – 4300 ng/L). The mean MBR effluent before UF/NF/RO treatment was quite high with an average value of 118ng/L (Figure 6.6 and 6.7). Testosterone was almost completely removed in all the effluent samples after treatment with UF, NF and RO membranes, with approximately 12 ng/L remaining (Figure 6.8). This represents a removal efficiency of more than 90% (Figure 6.9), which is the same as recorded by Chang et al. (2011) in China.

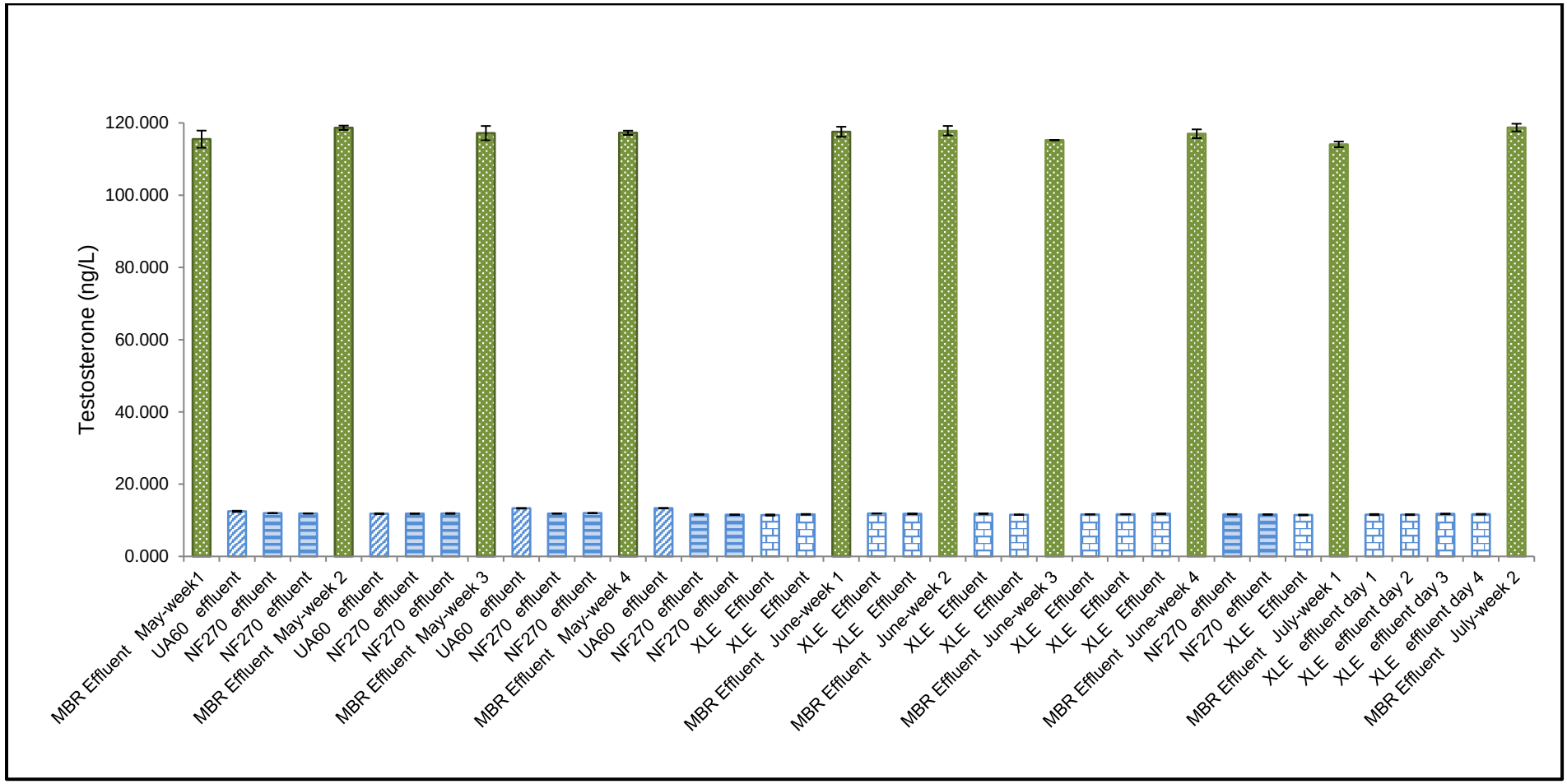

Figure 6.6: The ELISA mean of testosterone (T) levels (ng/L) measured in water collected from influent and effluents at various stages within the MSWWW including MBR and RO, during winter: May, June and July. Error bars denote SD; *n* = 2, error bars show maximum levels detected

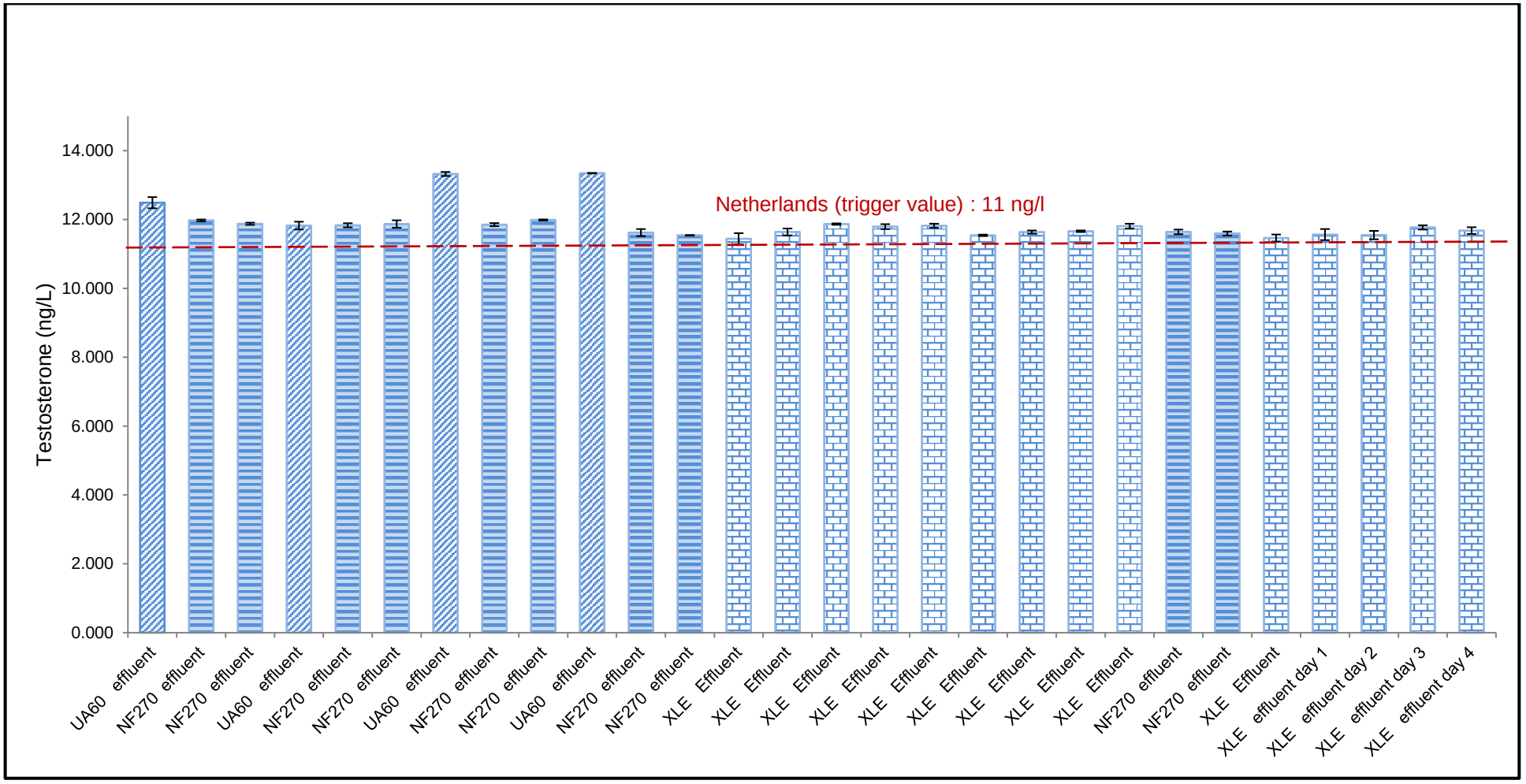

Figure 6.7: The ELISA mean of testosterone (T) levels (ng/L) measured of the effluents of the UF, NF and RO membranes treatment at various pilot plant conditions. Error bars denote SD; *n* = 2, error bars show maximum levels detected (Netherlands, 11ng/L, trigger value for drinking water Brand et al. 2013)

A 91% removal of Testosterone (T) was recorded in the anaerobic (anoxic) tank, where the raw influent was reduced from almost 281.3 - 134.9 ng/L. The lowest % removal (13) was measured by the MBR aerobic (oxic) tank, where the MBR influent was reduced from 134.9 to 116.9 ng/L, only. UF, NF and RO had an expected % removal of 89, 90 and 90.

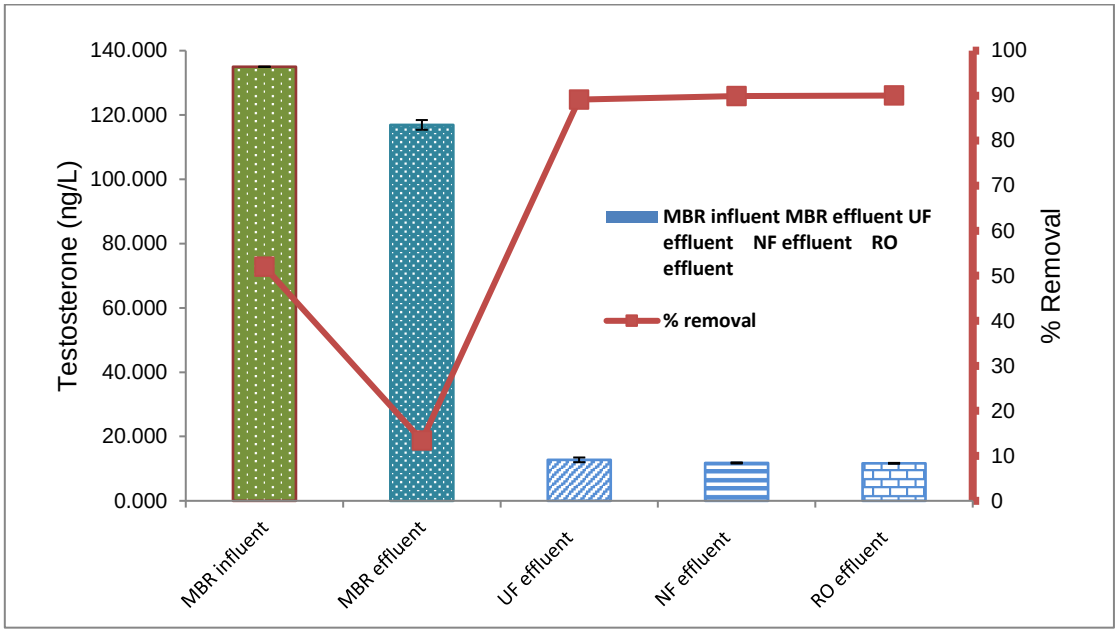

Figure 6.8: The mean of testosterone (T) levels (ng/L) measured in water collected from influents at five stages within the MSWWW including (MBR & RO feed and permeate) during winter: May, June and July. Error bars denote SD; *n* = 2, error bars show maximum levels detected

The mean E_2 concentration (Figure 6.4) for the UF, NF and RO effluent are 2.22, 0.66 ng/L and 0.16, respectively. Figure 6.5 showed that the T concentration for the effluents of MBR, UF, NF and RO in sequence with MBR process had lower T concentrations of 116.9, 12.75, 11.78 and 11.66ng/L, respectively. UF, NF and RO effluents had the significantly reduced T concentration compared with the influent, 134.9 ng/L. ($p = 3.13 \times 10^{-20}$ for UF, $p = 4.67 \times 10^{-18}$ for NF, $p = 4.51 \times 10^{-18}$ for RO $\alpha = 0.05$). The results were consistent with the previous study indicating the downstream levels of the dams in Namibia with E_2 and T concentrations of 7.2 and 19 ng/L, respectively (Faul et al. 2014).

The three processes; MBR/UF, MBR/NF and MBR/RO exhibited relatively similar T removal % (Figures 6.7). In the UF/NF/RO stages following the MBR treatment, the removal % of all the T effluents were crowded into a very high but narrow range (e.g., 89% for UA60, 90% for both NF270 and XLE). According to Yangali-Quintanilla et al. (2008) and Sahar et al. (2011), the residual natural organic matter (NOM) increases the membrane removal potential by increasing the negative surface charge of the membrane which therefore increases the electrostatic repulsion. It is also possible that these new conditions lead to contaminants rejection as a result of increased hydrophobic interactions with the membranes.

6.3 The effect of flux on testosterone

The MBR/UF and MBR/NF systems were run at the same flux, but the MBR/RO system was run at two different fluxes (Table 5.2). An analysis of T removal rates by all systems imply that these filtration techniques can remove T to a very high extent (Figure 6.7), although the results in all the applied fluxes were above the limit of quantification (LOQ) and on par with the Dutch drinking water trigger level of 11 ng/L (Brand et al. 2013). Regardless of their high removal rates, however, T concentrations also exceeded the limit of detection (LOD). These results show that several molecules of T managed to penetrate the UF, NF and RO membrane, and therefore, it was concluded that UF/NF/RO cannot serve as an absolute barrier to testosterone. In addition, flux had no effect on testosterone removal. Sahar et al. (2011) correspond with these findings in their investigation of the effect of three fluxes when removing MPs with CAS-UF/RO and MBR/RO systems.

6.4 YES, analysis of 17β-estradiol (E_2)

During the recombinant yeast estrogenicity bioassay screening (YES) test, no estrogenic activity was measured in the extraction control samples, thus contamination of the cartridges during the extraction process can be excluded. Yeast growth was checked at an absorbance of 620nm. Compared with the reference, none of the samples showed a decrease in cell density, therefore no cytotoxicity was present. The sample was considered positive for estrogenic activity when three or more consecutive observations were above the level of detection (LOD) of the assay. The estrogenic activity (EEQs) of the samples is based on the EC_{50} value of the dose-response curves obtained for 17β-estradiol (E_2) and the test sample.

The level of detection (LOD) was calculated for each bioassay and experiment using the mean activity of the negative control and adding threefold its a standard deviation. As the LODs varied between bioassays and membrane experimental runs, they were not all shown for the sake of clarity. However, in general, only results above the LODs were considered. In a few cases, such as estrogenic activity, lower activities were shown because of their ecotoxicological relevance (low effect threshold) and for comparing membrane effectivities.

Estrogenicity (binding to the human estrogen receptor [ER]) was detected in all feed samples analysed (Figure 6.9). The raw influent had the highest proportion of estrogenic activity with 34.94 ng/L E_2 equivalents (EEQs), followed by the average MBR influent (1.18 ng/L EEQs) and effluent (0.63 ng/L EEQs). The average MBR effluent (RO influent) for October, November and December was 0.38, 0.74 and 0.44 ng/L EEQs, respectively.

Although EEQs (Figure 6.10) followed a similar trend as E_2 concentrations (Figures 6.2 and 5.3), the YES EEQs was a bit lower than E_2 concentrations measured using ELISA. This is

consistent with the previous study indicating higher E_2 ELISA concentrations (Manickum and John 2014b). The YES and ELISA assay screening methods are the same, generating similar results for E_2 as shown before, so no YAS (yeast androgen screening) for T was done because the results are expected to be similar.

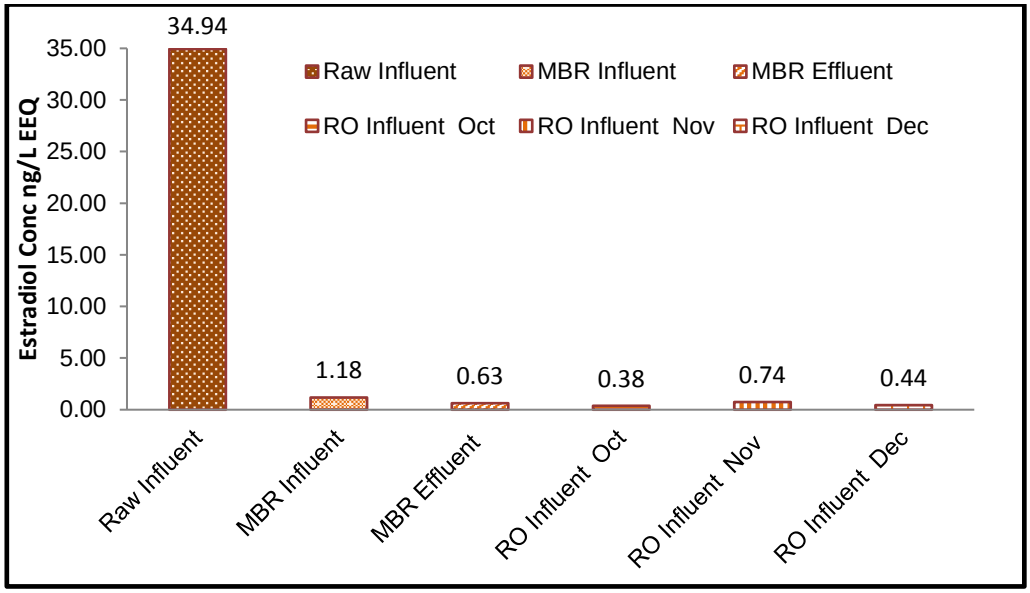

Figure 6.9: The 17β-estradiol (E_2) levels (ng/L EEQs) measured in water collected from influents at six stages within the MSWWW including MBR and RO, during summer: October, November and December.

Variations in the pilot-plant operating conditions in Table 5.2 (flux and pH) did not have any visible effect on the removal of E_2 and T for the MBR/UF, MBR/NF and MBR/RO processes. Rasak et al. (2007) commented that the pH and pressure had a noticeable influence on the rejection of organic compounds while using a lab-scale cell with synthetic feed, where this study used a pilot-plant with a real-time MBR feed-in once-through mode.

The physico-chemical properties of E_2 and T are considered to influence its rejection by UF/NF/RO membranes. This can be observed in Figure 6.2 and 6.3, where the MBR/RO treatment removes all the test samples of E_2 below LOD < 0.25 ng/L, regardless of the specific operating plant conditions. Most of the MBR/NF treatment test samples were below LOD < 0.25ng/L. The rest were visibly between 0.33 – 0.65 ng/L. The MBR/UF treatment shows clearly poor removal of E_2 with all four test samples between 2.12 – 2.72 ng/L.

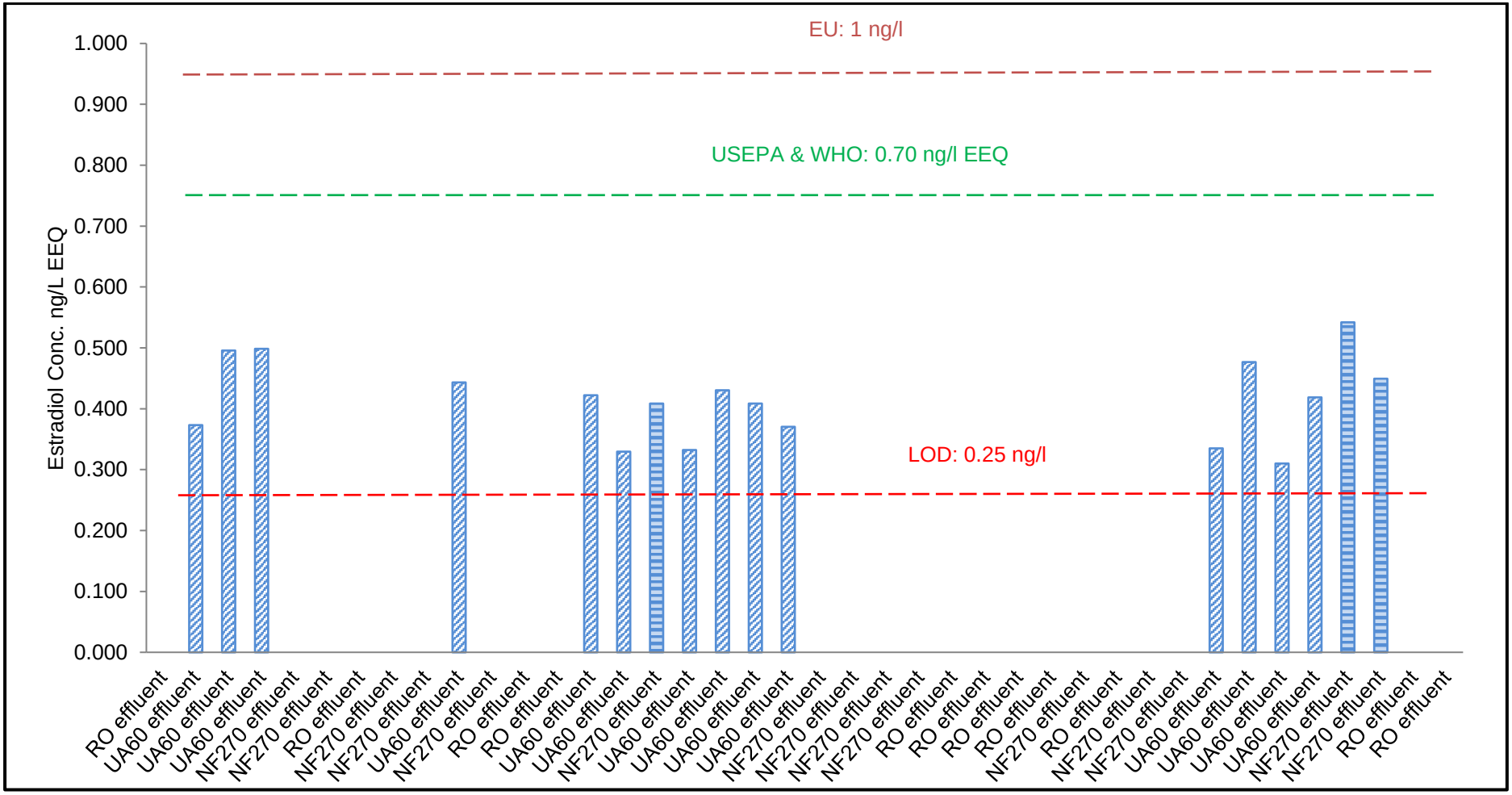

Figure 6.10: The YES of 17β-estradiol (E2) levels (ng/L EEQs) measured in water collected from influent and effluents at various stages within the MSWWW including MBR & RO, during: October, November and December, [EU, 1 ng/L modulate fish production (Shappell et al. 2007); USEPA and WHO, 0.70, trigger value for drinking water (Genthe et al. 2010), LOD: 0.25 ng/L]

The retention of MPs in membrane separation processes depends on the characteristics of both the membrane and the pollutants. The hydrophobicity represented by the partitioning coefficient (K_{ow}) of E_2 and T as well as the adsorption, size exclusion and charge repulsion, would have major influences on the rejection. The molecular size would be the overriding factor in the rejection by the UF/NF/RO membranes (Rasak et al. 2007; Bellona et al. 2008; Ozaki et al. 2008)

Testosterone was poorly removed by all treatment processes with all effluent test samples measuring an average of 12ng/L (Figure 6.6 and 6.7). This could be due to the high dipole moment of the T compound. It is reported that a high dipole moment of a compound would lead to a decrease in the rejection by membranes (Van der Bruggen et al. 2002; Kimura et al. 2004).

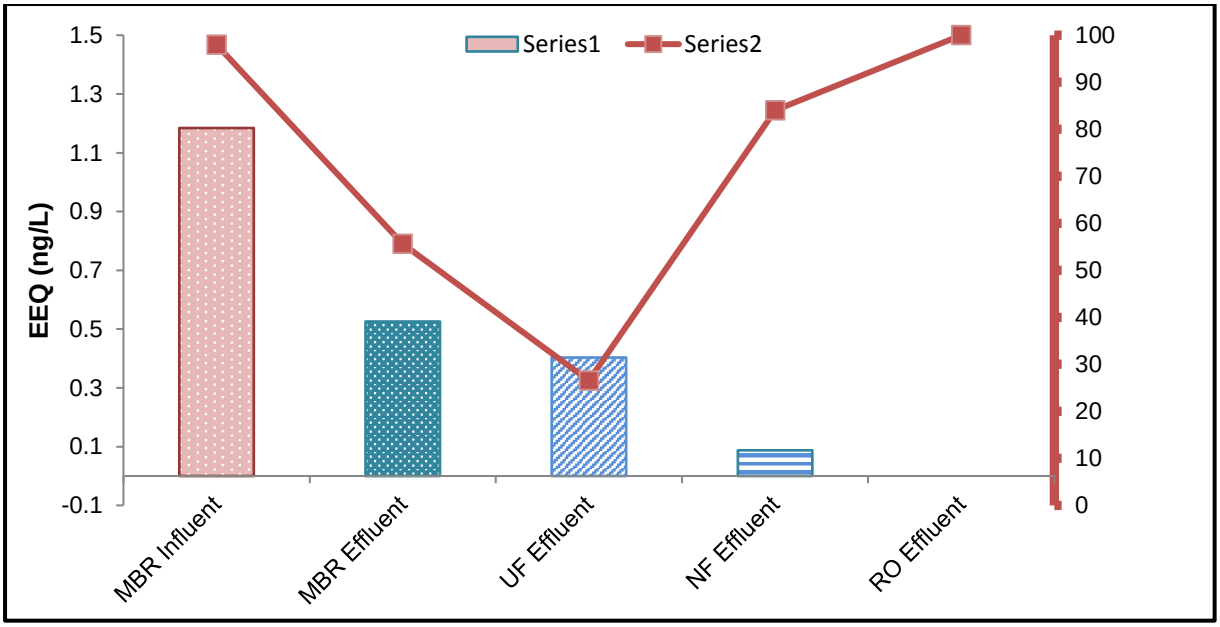

Figure 6.11: The mean of 17β-estradiol (E_2) levels (ng/L EEQs) measured in water collected from influents at five stages within the MSWWW including (MBR and RO influent and effluent) during summer: October, November and December

A 98% removal of estradiol was recorded in the anaerobic (anoxic) tank, where the raw influent was reduced from almost 34.94 - 1.18 ng-EEQ/L. The lowest % removal (55) was measured by the MBR aerobic (oxic) tank, where the MBR influent was reduced from 1.18 - 0.53 ng-EEQ/L, only. UF, NF and RO had an expected % removal of 27, 84 and 100.

Figure 6.12 showed that the EEQ values for the effluents of MBR, UF, NF and RO in sequence with MBR process had very lowest estrogenic activities of 0.53, 0.43, 0.088 ng-EEQ/L and LOD, respectively. NF and RO effluents had significantly reduced estrogenic activity compared with the influent, 1.18 ng-EEQ/L. ($p = 0.187$ at for UF, $p = 0.005$ for NF, $p = 0.007$ for RO $\alpha = 0.05$). This is consistent with studies (Körner et al. 2004; Ihara et al. 2014), which showed highly reduced EEQ values in the effluent from advanced wastewater treatment processes with YES screening assays (Lee et al. 2008).

6.5 Effect of membrane properties on the rejection

The removal of E_2 and T is due to the direct filtration by the UF/NF/RO membranes (Figures 6.11 and 6.12). This is due to steric hindrance and their adsorption onto the membrane polymeric matrix. Adsorption can only contribute to the short-term removal; as the feed is continuously filtered through the membrane, so membrane sites will be saturated with hydrophobic MPs. The charged and hydrophilic MPs do not adsorb to the membrane polymeric matrix and can be effectively removed by UF/NF/RO membranes via steric hindrance and electrostatic interaction mechanisms. Steric hindrance occurs because of the MW (E_2: 272.38 g/mol; T: 288.42 g/mol), which is larger than the membrane pore size (MWCO) of the RO (<200 Da) and NF (400Da). So, rejection increases as the MW of the MPs increases, thus, explaining the poor performance of the NF membrane. The UA60, NF270 and XLE membranes used for this study were negatively charged. Thus, electrostatic interactions occurred between the charged MPs (E_2 and T) and the negatively charged membrane surfaces, resulting in higher rejection compared to neutral solutes of a similar size. This result aligns with those of past studies (Nghiem et al. 2004a; Xu et al. 2006; Snyder et al. 2007; Bellona et al. 2008; Schäfer et al. 2010; Silva et al. 2012a; Nguyen et al. 2013; Krzeminski et al. 2019).

The thin-film composite RO and NF membranes have about the same thickness, but the active layer of the NF is weaker. The rate of E_2 and T transport across the membrane is governed by the diffusion through the active skin layer. Freger et al. (2002) explain that water is lightly soluble in the polymer where the diffusion process between E_2 and T takes place in a polymeric matrix saturated with small amounts of water. The authors also mentioned that the convective flow has only a small contribution to the transport of E_2 and T across the membrane, but the presence of water is thought to play an important role in facilitating the diffusion process (Nghiem and Schäfer 2004). It can be observed in Figures 6.10 and 6.11 where the NF/RO membrane treatment complements MBR treatment very well, with the E_2 and T being removed to below the level of detection (0.5ng/L) with an 84 and 100% removal.

The combination of MBR with UF/NF/RO leads to enhanced removal of MPs. The MBR/RO achieved higher removal efficiencies of E_2 than MBR/NF and MBR/UF. Our observation is in good agreement with previous reports (Alturki et al. 2010; Nguyen et al. 2013) and can be explained by the fact that NF270 and UA60 are loose membranes with a larger pore size and a higher permeability. This is also supported by the low conductivity rejection by the UA60 (10%) and NF270 membrane (41-49%) compared to the XLE membrane (93-95%) (Nguyen et al. 2013). The molecular weights of E_2 and T were considerably smaller than the pore size of the UF (UA60) membrane; therefore, most of the MPs could not be physically retained by size exclusion as shown in Figure 6.10 where the E_2 ng/L EEQ is on par with the LOD =0.25 ng/L EEQ, thus only a 26.5% removal (Figure 6.11). Estradiol retention by the UF was due to adsorption to the membrane surface. This is clearly explained by Nghiem et al. (2004a, 2005) and Neale et al. (2009) that consider the negative charge of the UF membrane and the dissociation constant of the MPs.

McCallum et al. (2008) and Silva et al. (2012b) simultaneously investigated the adsorption and desorption processes occurring during the NF membrane filtration of E_2. They explained that the adsorption of E_2 onto the membrane and its desorption are dynamic processes which meant that when the concentration of hormone in the feed solution was higher than in the membrane, adsorption would occur and the permeate concentration would increase until achieving an equilibrium; if the concentration in the feed solution was lower than the equilibrium concentration in the membrane, desorption would occur, until a new equilibrium was reached.

Neale et al. (2009), have demonstrated estradiol could interact with the bulk organic matter including natural organic matter (NOM) surrogates such as humic acid. It adsorbs to the membranes through hydrophobic interaction, thus increasing the rejection. NF membranes retained Estradiol due to both hydrophobic adsorption and size exclusion, while the UF membrane retained estradiol due to hydrophobic adsorption. This is well demonstrated in Figure 6.11 where the NF (NF270) and UF (UA60) membranes achieved an 84 and 26.5% removal respectively. This result aligns with those by authors (Yoon et al. 2004, 2006, 2007; Silva et al. 2012b), where it can be concluded that estradiol retention by NF was significantly greater than that by UF.

The removal rate of E_2 and T with UF/NF/RO is a function of the partitioning coefficient (log *Kow)*, given as E_2: 4.01 and T are 3.32. This is confirmed by Yoon et al. (2007), who removed 25 MPs with UF and NF membranes, concluding that the retention increases with the increasing of the partitioning coefficient (log Kow). In the same research, a different retention trend was observed by Yoon et al. (2007), for when MPs having a Log *K*ow of >2.8 exhibited a percentage removal of less than 40%, except when MPs with a Log *K*ow < 2.8 showed a percentage removal of more than 75%. This could be the possible reason why T concentration was not removed below the measure 12ng/L (89 removals %) throughout all treatment samples as shown in Figure 6.10 and 6.11.

6.6 Risk assessment of 17β-estradiol (E_2) and testosterone (T)

When analysing samples using bioassays, trigger values are useful to evaluate if more research is needed. Beyond the trigger, value does not imply that a health outcome is expected. According to Brand et al. (2013), trigger value with bioassays for agonistic hormonal activities in drinking water define the level above which human health risk cannot be waived. The trigger values are based on: (i) adequate daily intake (ADI) values of specific compounds, (ii) pharmacokinetic factors defining their bioavailability, (iii) approximations of the bioavailability of unknown compounds with equal hormonal activity, (iv) relative endocrine potencies, and (v) physiological, and drinking water allocation (Van Zijl et al. 2017).

Brand et al. (2013), derived trigger values of 3.8 ng/L EEQ and 11 ng/L for the estrogenic and androgenic activity of drinking water sample bioassays, respectively. The authors concluded that no human health risks were expected from hormonal activity in Dutch water. Although both, Genthe et al. (2010) and Brand et al. (2013) used similar methods to determine the trigger value, Brand et al. (2013) with 3.8 is higher than the 0.7 proposed by Genthe et al. (2010), due to dissimilar safety reasons applied. The more protective trigger value of 0.7 was used in this project.

An approach for managing endocrine disruptors in water is needed in South Africa. Framework for guideline development for endocrine disrupting chemicals making use of a preventative approach was developed for South Africa based on the World Health Organisation (WHO) risk assessment approach. It is normal practice in health risk assessments to assume that toxic substances have some safe level at which no adverse health effects will occur over a lifetime of exposure to the substance (USEPA, 2002; WHO, 2004). This safe threshold is also referred to as the reference dose which is derived from an adequate daily intake (ADI). The trigger value was based on a WHO value of estrogenic

equivalency factor or quotients (EEQ). (Genthe et al. 2010). Jarošová et al. (2014b) discussed about a trigger value of estrogenicity 0.4 ng/L for long term fish exposure. The European Water Framework Directive, annual-average environmental quality standard (AA-EQS) for estrogenicity is also 0.4 ng/L (Van Zijl 2016; Escher et al. 2018).

Despite the moderate- to efficient removal of estrogenicity by the MBR/UF, MBR/UF and MBR/RO treatment, the measured EEQ values still pose a potential adverse health risk. As conventional risk assessment approaches are focussed on acute- or chronic toxicity endpoints, the use of predicted no-effect concentrations (PNEC) and no-observed effect concentrations (NOEC) are mostly incorporated to assess potential lethal toxicity in aquatic wildlife (Hernando et al. 2006, Archer et al. 2017b). However, such an approach is largely focussed on the toxicity of individual chemicals and therefore does not consider the complex mixture interactions of environmental pollutants within a water system. The YES offers a viable option that indicates the net estrogenic potential of a water sample to modulate hormone receptor binding, with the estimated EEQs providing a semi-quantitative assessment of all compounds which may mimic an estrogenic response in a similar manner as E_2. It is, therefore, possible to compare such EEQ values to other toxicological studies (Archer et al. 2017b).

The measured E_2 and T concentrations were severely reduced in the effluent, although during the UF treatment they were not removed completely. This may still pose an environmental and health risk at very low ng/L concentrations levels. A multitude of investigations has shown that the presence of natural steroid hormones in effluents has antagonistic effects on wildlife, including, among others, reduced fertility, abnormal development of male and female secondary sex characteristics, alteration in sex ratio, the feminisation of males and alteration of behaviour (Rodríguez et al. 2007; McCallum et al. 2008). Human and animal health is threatened when excess sewage effluent enters our water sources and effluent is used for irrigation application. In surface water, the effective lower LOQ for each was reduced to 0.97 ng/L for E_2, and 4.15 ng/L for T. (Faul et al. 2013, 2014).

Results of the ELISA for the male steroid hormone T are presented in Figures 6.6 and 6.7. All the effluent samples for the MBR/UF, MBR/NF and MBR/RO, were higher than the lower PNEC of the test as well as the trigger value of 11ng/L by Brand et al. (2013). Bandelj et al. (2006) stated that androgenic constituents in wastewater effluent can result in biological responses in animals and the exposure of mosquitofish to androgenic constituents in paper and pulp effluent has resulted in its masculinization (Manickum and John 2014b).

The limit of detection (LOD) and limit of quantification (LOQ) were calculated as: 3 x standard deviation of the negative control and 6 x standard deviation (SD) of the negative control, respectively (Cai et al. 2012; Jiang et al. 2012), The estrogenicity LOD was calculated as 0.5 ng/L (EEQ). Results of the ELISA and YES for the female steroid hormone E_2 are presented in Figures 6.2. 6.3 6.10 and6.11, respectively. All the E_2 effluent samples with the MBR/UF, MBR/NF and MBR/RO, were lower than the lower LOQ of the test and are less than the PNEC values for fish (1 ng E_2/L) as proposed by Shappell et al. (2007) and Faul et al. (2014.) The PNEC's are derived from the effect levels of the most sensitive test organism (Choi et al. 2008). During In-Vivo Vitellogenin (VTG), induction studies of the PNEC for E_2 are appropriate for the application in risk assessment of aquatic organisms. The PNEC value for long-term exposures (i.e. >60 days) in water is 2 ng E_2/L. Higher PNECs are recommended for short-term (i.e., a few days or weeks) exposures (Caldwell et al. 2012). The authors summarise PNEC below 1ng/L as no risk and above 10 ng/L as high risk. Jiang et al. (2012) confirmed Shappell et al. (2007) that stated a PNEC of 1 ng/L from the England and Wales Environmental Agency (2002).

Stephan et al. (1985), suggested a PNEC of 0.75 ng E_2/L EEQ for protecting aquatic organisms from chronic and full-lifecycle exposures to E_2. Caldwell et al. (2012), and his colleagues recommended a slightly higher PNEC for E_2 (2 ng E_2/L), which was derived from investigating 21 in vivo NOECs (Caldwell et al. 2012). The European Union recommended a PNEC of (0.4 ng/L) E_2 for protecting aquatic life (Wu et al. 2013). From the perspective of protecting aquatic species rather than fishes only, environmental researchers argue that 0.75 ng E_2/L may be more reasonable than 2 ng E_2/L, and 0.75 ng E_2/L may be more protective for aquatic organisms (Wu et al. 2013). An estimated E_2 trigger value of 0.7 ng/L (Genthe et al. 2010) for drinking water standards and 0.4 ng/L EEQ (Jarošová et al. 2014a) estrogenicity for long term fish exposure, have been proposed, above which further monitoring should be considered to establish the identity and origin of the MPs (Archer et al. 2017a). To estimate the exact source of estrogenicity within environmental samples may prove difficult, which was the reason why chemical analysis of known estrogenic micro-pollutants was not considered during this study and this is confirmed by Archer et al. (2017b).

6.7 Summary

In this chapter, it has been shown that RO and NF membrane processes exhibited exceptional removal rates (>95%) for E_2 and T. The influent pH and flux did not influence the rejection of E_2 and T, which was most likely ruled by adsorption, size exclusion and diffusion simultaneously. Size exclusion was seemingly dominant, especially with NF and RO membranes. T with a smaller partitioning coefficient (log Kow) value were most likely adsorbed on the membranes and then passed through it to give a low rejection with all three membranes. It can be confirmed that the MBR/UF, MBR/NF and MBR/RO comply with the USEPA, WHO and EU trigger value PNEC as stipulated. It was found that RO show higher removal percentages when compared with NF and UF. Consequently, domestic wastewater treated by MBR followed with NF or RO is adequate for the effective removal of natural steroids hormones.

Chapter 7

Conclusion

7 CONCLUSION AND RECOMMENDATION

7.1 Conclusion

The findings in this study obtained from both theoretical and experimental work provide insight into full-scale removal of natural steroid hormones from MBR with membrane technology for potential reuse. A database for full-scale MBR with UF/NF/RO that will provide an understanding for design, prediction and improvement for future work and in the process fill the literature void and contribute to the body of knowledge and development of standards. Reclaimed water can be suitably used for a variety of applications. Among the most common applications are irrigation, residential uses, urban and recreational uses, groundwater recharge; aquaculture, industrial cooling water and drinking water production. Currently, in the Western Cape and CoCT which are experiencing unpredicted weather patterns with little rainfall, surface water is not guaranteed, thus alternative avenues need to be pursued. The reuse of municipal sewage wastewater MBR effluent will be beneficial to all.

It can be confirmed that the membrane performance investigated using model and municipal wastewater MBR secondary effluent with RO membranes, could improve, when using various modifying PVA solution concentrations. The modified membranes showed high resistance to fouling and great potential as an RO treatment step for municipal secondary MBR effluent. Experiments with flat-sheet NF/RO membranes displayed similar values of rejection for inorganic and organic solutes compared to NF/RO elements suggesting that the membrane modification results of the flat-sheet membranes may be compared to membrane elements.

The effects of percentage recovery, pH as well as permeate flux, was tested with the best performing RO membrane. The pH adjustment was found to be a significant factor governing the reduction of most inorganics tested as well as the COD for all three UF, NF and RO membranes. The percentage reduction was found to exhibit different behaviour regarding the control of pH for the various targeted inorganics. The RO (XLE) membrane was able to significantly reduce the COD with a controlled pH and adjusted flux, significantly. This demonstrated that the control of pH, adjustment of flux and process recovery, for the RO membrane, could be an option to consider for the improvement of inorganic removal in the advanced treatment of domestic secondary effluent. It was shown that the quality of water obtained with the RO could meet quality requirements for many potable and non-potable reuse applications.

The overall goal was to comprehensively understand the UF/NF/RO with respect to its capacity to remove MPs from MBR secondary effluent. The results highlight the complex range of factors that may influence the affects of estrogenicity and androgenicity during tertiary wastewater treatment. The measured and calculated EEQ values for E_2 and T revealed by ELISA and YES screening, correlated well with existing literature. The assays highlighted the complexity of the environmental samples where a mixture of organic- and inorganic micropollutants are present.

This study evaluates and discusses an integrated full-scale membrane bioreactor (MBR) with an ultra-filtration, nanofiltration and or reverse osmosis pilot-plant with the main aim of producing potential reusable water, but also generating a concentrated waste stream. The recirculation of the UF/NF/RO brine to the MBR minimizes the unwanted discharge of a concentrated waste stream.

Many research papers have been published on the MBR-NF/RO treatment processes for reuse application. Unfortunately, most of the publications use the MBR model effluent matrix (synthetic) feed samples and spike it with known MPs to perform NF/RO rejection experiments in a laboratory-scale environment. However, this only gives partial insight into the removal capacity of the MBR-NF/RO system. Before this study, only a few research papers on full-scale MBR with UF/NF/RO pilot-plant concept was published.

This study showed alignment with the South African Government National Development Plan (NDP) for 2030 (National Planning Commission 2010) by addressing certain goals, such as “conducting exploration on grave problems affecting long-term growth’ and addressing ‘interventions to guarantee environmental sustainability and pliability to impending surprises’. South Africa is a semi-arid country, where population growth and urbanisation leads to an increased potable water demand usage which still relies on environmental surface waters as a drinking water source.

7.2 Recommendation

The investigation into the removal of all estrogen and androgens with membrane technology with MBR effluent needs to be continued. Numerous compounds are recognized for having manifold modes of action for a diversity of physiological and toxicological endpoints, therefore producing further difficulties in mixture interaction studies that need to be unlocked. The knowledge regarding mixture interactions of environmental MPs is very complex. The reflective information present to date needs to be addressed in future environmental studies.

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APPENDICES

APPENDIX A

Experimental Procedure

Experimental Procedure

Pilot plant study using three types of membrane:

1. UA60-4040
2. NF270-4040
3. XLE-4040

Operating conditions:

- Operating Pressure (500kPa)
- Flux rates (25, 30)
- Feed flow rate
- Recovery (50, 75%)
- Rejection

Typical characteristics of influent and effluent to RO:

pH

Turbidity (NTU)

Conductivity (*u*S/m)

TDS (mg/l)

COD (mg/l)

Phosphate (mg/l)

Ammonia (mg/l)

Estrogenic activity and estrogen (hormone) concentrations determination

1. 17β-Estradiol (Estrogen- Female)
2. Testosterone (Androgen – Male)

ESTROGENIC ACTIVITY – (In Vitro biochemical and biological tests)

- The Recombinant Yeast Estrogen Screen Assays (YES)
- Enzyme-Linked Immunosorbent Assays (ELISAs)

APPENDIX B

Solid Phase Extraction (SPE)

Extraction procedure for water samples

This is the procedure according to (de Jager et al. 2011).

Extraction procedure for water samples for the assessment of estrogenic activity in the recombinant yeast estrogen assay Compiled by (Aneck-Hahn et al. 2009)

This protocol describes the extraction of organics from water samples by solid-phase extraction (SPE). These extracts are suitable for the assessment of estrogenic activity and androgenic activity in the bioassays.

Apparatus

- Millipore Milli-Q synthesis ultrapure water system or equivalent system to produce double distilled water (dd H_2O). The system must be equipped with an EDS filter (Cat. No. EDSPAK001, Microsep) to remove endocrine disrupting compounds (EDCs) from the water
- Vacuum pump
- Vacuum manifold, 12 columns (Chromabond® Manifold Cat. No. 730150 or equivalent)
- Glass filtration funnels (500 mℓ)
- Clamps to connect the filtration funnel and the funnel sieve
- Glass vacuum filtration flasks, 1-2 ℓ
- Rubber tubing to connect the flask to the vacuum pump
- 9 Port Reacti-vap evaporator including a heating stirring module and Reacti-vap needles and plugs (Thermo Cat. No. TS-18825 or equivalent)
- Filter forceps (blunt nose), (Millipore, Cat. No. XX6200006 or equivalent)

Consumables/Materials

- Aluminium/tin foil
- Amber glass bottles 4 mℓ (Chromatography research supplies, Cat. No.154515)
- C18 ec (end-capped), SPE cartridge 6 mℓ/500 mg (Chromabond Macherey-Nagel, Cat. No. 730 014 or Oasis HLB SPE cartridges, 6 cc 500 mg, Waters Corp Cat. No. 186000115)
- Glass bottles, 1 ℓ (for sampling purposes, can also be amber bottles)
- Glass Pasteur pipette with a rubber bulb
- Glass wool filters (Macherey-Nagel, Cat. No. 000904 or equivalent)
- pH 0 -14 indicator strips

- Serological pipettes, sterile, 1 mℓ and 10 mℓ (Corning Costar Cat. No. 4012 and 4101, Scientific Group or equivalent)
- Sterile filters, 0.45 µm, 47 mm diameter (MicroSep, Osmonics Cat. No. E04WG047S1 or equivalent)

Reagents

- Concentrated HCl
- Double distilled water
- Methanol – High-performance liquid chromatography (HPLC) grade
- Ethanol – HPLC grade

METHOD

Note: Throughout the extraction method, dd H_2O refers to dd H_2O that went through the EDS filter to remove EDCs from the water.

Collection and pre-treatment of samples

1. Collect 2 x 1 ℓ of aqueous samples such as sewage, surface water, groundwater or tap water in methanol rinsed sample bottles.
   Line the lid on the inside with tin foil to prevent the sample from encountering the plastic lid of the bottle which can be a possible source of EDC contamination.
2. Measure the original pH of the water using the pH strip and make a note.
3. Drop the pH of the sample to 3 by adding concentrated HCl dropwise with a glass Pasteur pipette. Check using pH strips.
4. Bring the sample back to the laboratory as soon as possible and store in the dark at 4°C and extract as soon as possible.

Note: If the samples are sewage samples the extraction process should be started within 4-6 hours of collection or if necessary, they may be stored overnight at 4°C.

5. This step is necessary if you are dealing with raw sewage samples (or similar samples), otherwise, proceed to step 8. Raw sewage samples require pre-filtration.
6. Raw sewage and very turbid samples require pre-filtration. Assemble the glass filtration unit (see Figure 4.7). Place 1-2 glass wool filters between the loading reservoir and the sieve funnel.
7. Connect the filtration unit to the vacuum inlet and pass the entire sample through the unit under vacuum. Once the entire sample has been pre-filtered, rinse the reservoir thoroughly with methanol and dd H_2O and go to step 8.

8. Assemble glass filtration unit (as in Figure 1) and load with 0.45 µm, sterile filters (47 mm diameter).
9. Connect the filtration unit to the vacuum inlet. Pass the sample through the filtration unit under vacuum, 250 – 300 mℓ at a time. You may need to replace the filter if it gets clogged, but this will depend on the sample. Once the sample (1 ℓ) has been filtered, you can proceed to the solid-phase extraction.

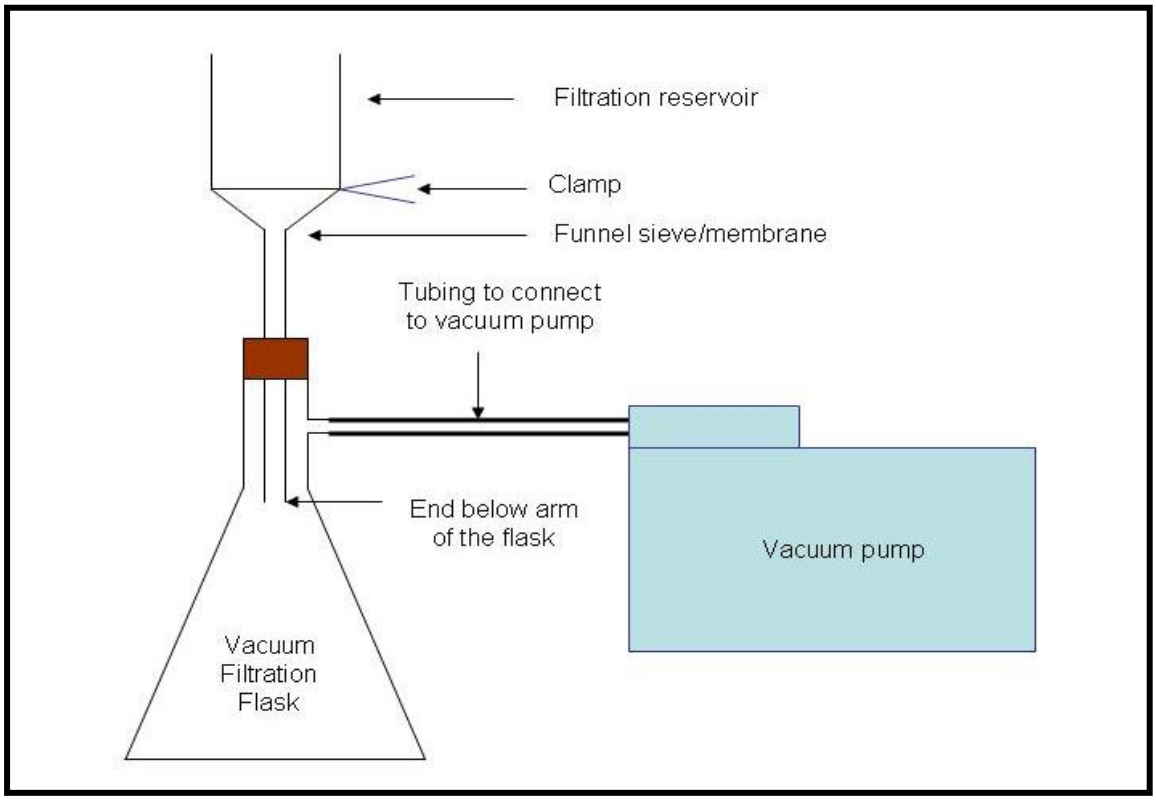

Figure 4. 7: Filtration setup for water extraction procedure (Aneck-Hahn et al. 2009)

Note: Place the membrane for clarifying (removing particles) from the water onto the funnel sieve/membrane and clamp the funnel closed. Place the filtration funnel (with the glass stem of the funnel through a rubber stopper) into the vacuum filtration flask. Make sure the bottom of the stem of the funnel is as deep as possible, preferably below the level of the sidearm of the flask. Connect the arm of the vacuum filtration flask to the inlet of the vacuum pump. Switch on the vacuum pump and add the sample into the funnel. Make sure no liquid gets sucked into the vacuum pump. Once the sample has been filtered, remove the pipe from the arm of the filtration flask and connect to the vacuum inlet of the SPE vacuum manifold (see Figure 28).

Solid-phase extraction – pre-conditioning of the cartridge

Important note: The most commonly used sorbents are porous silica particles bonded with C_{18} or other hydrophobic alkyl groups. Therefore it is important to first condition the cartridge with a water-miscible organic solvent to solvate the alkyl chains. Then equilibrate the cartridge with water or buffer solution. Do not allow the sorbent bed of the SPE cartridge to run dry during the extraction. This can significantly reduce the retention efficiency of the cartridge and result in low analyte recoveries and poor assay to assay reproducibility.

1. Load the SPE cartridges onto the SPE manifold and open the vacuum valves (see Figure 2a and b).
2. Add 5 mℓ dd H_2O to the reservoir of each cartridge and allow to pass through by gravity.
3. Just before the water reaches the top frit (see Figure 3) add 5 mℓ methanol (HPLC grade) and allow to pass through by gravity.
4. Just before the methanol reaches the top frit add 5 mℓ dd H_2O and allow to pass through by gravity and just before the water reaches the top frit, close the vacuum valve at the bottom of the cartridge.

Note: If you have a large sample adaptor you will need to fill the reservoir in step 4 with distilled water.

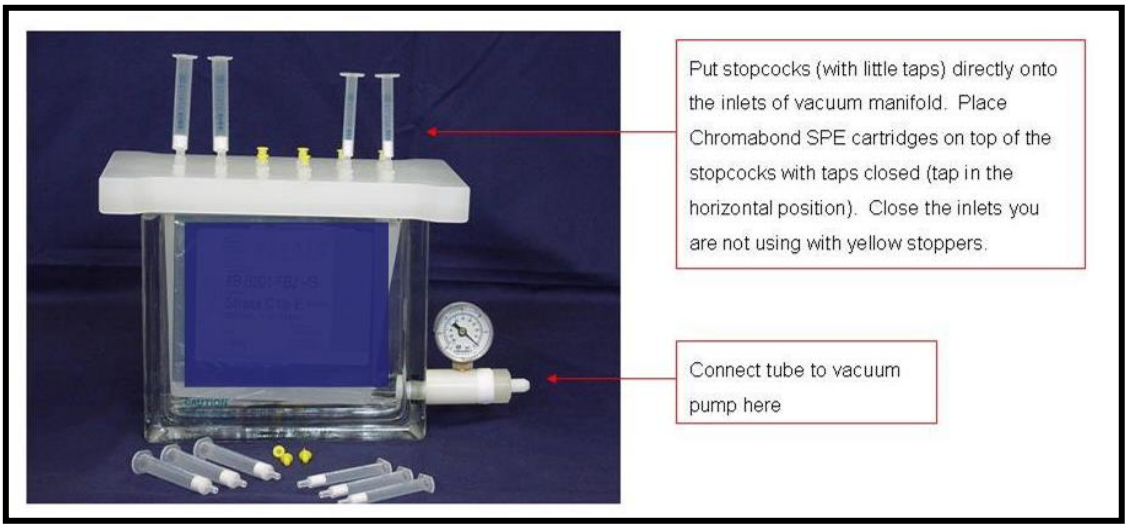

Figure 4. 8: An example of a solid-phase extraction manifold (de Jager et al. 2011)

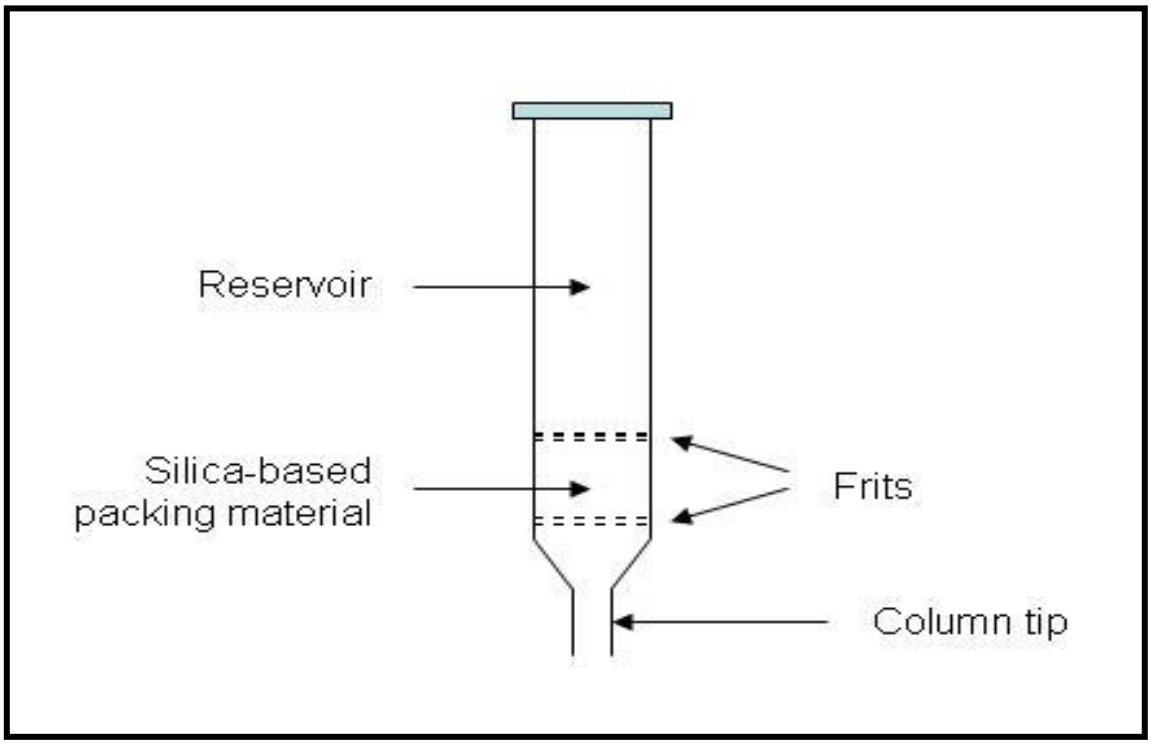

Figure 4. 9:A solid phase extraction column/cartridge (de Jager et al. 2011)

Extraction procedure

1. Connect the SPE manifold to the vacuum trap (see Figure 4.8 and 4.9).
2. If you have a large sampler adaptor, connect it to the top of the SPE cartridge (make sure the seal between the adaptor and cartridge is tight and drop the weight at the other end of the tube into the water sample.
3. If you do not have a sample adaptor, you will need to use a sterile 10 mℓ pipette per sample and gently fill the reservoir of the cartridge with sample (± 5 mℓ).
4. Open the vacuum valve for all samples and gently turn on the vacuum.

Note:

- If you have a sample adaptor, you need to check that each sample is flowing from the sample bottle to the SPE cartridge.
- If this is not the case, you need to check that the seal between the adaptor and cartridge is tight. In order to do this, close the vacuum valve for that sample and tweak the connection until it is sealed tightly.

Caution: NEVER let the sorbent bed run dry while doing this. If necessary, fill up the reservoir with dd H_2O.

5. Adjust the vacuum strength to achieve a flow rate of approximately 10 mℓ/min (equal to ± 3 drops/s). As the process continues you might need to increase the vacuum but do not exceed 70 kPa (20 mmHg).
6. If you don't have a large sample adaptor, gently keep filling the cartridge reservoir with the aqueous sample, preventing the cartridge from running dry.
7. When the vacuum trap is full, close the valves, stop the vacuum, disconnect the trap, empty the contents down the drain and reconnect the trap. Turn the vacuum back on, open the valves and set the flow rate again.
8. After the entire 1 ℓ sample has passed through the column, disconnect the large volume sample if required, leave the cartridge on the manifold (with vacuum) to dry and then close the valves.

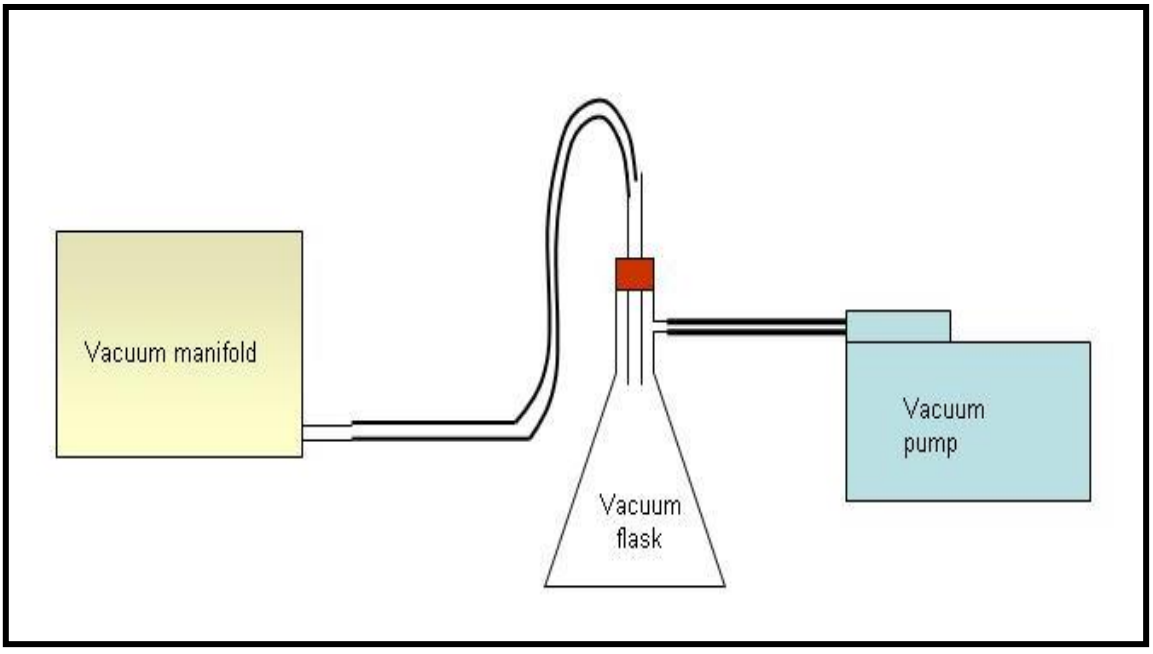

Figure 4. 10:An example of the filtration setup for sample extraction (de Jager et al. 2011)

Note: The vacuum manifold should be connected according to Figure 31 so that the waste can collect in the vacuum flask.

Elution

1. Remove the manifold lid carefully with cartridges still in place and insert a tube rack with a Reacti-vap conical tube or equivalent in the appropriate holes to correspond with the cartridges on the manifold lid.
2. Add 3-5 mℓ methanol to each cartridge reservoir and allow the solvent to percolate through the sorbent bed.
3. Open the valves and allow eluting with gravity alone into the tube.
4. Once most of the methanol has eluted, connect to a vacuum pump and gently turn the vacuum on to remove the remaining solvent from the sorbent bed (about 2-3 minutes). The vacuum should be reduced to 5 mmHg, to prevent the methanol from passing through too quickly.
5. Once all the solvent has been eluted, the samples can be carefully removed from the manifold and placed in the Reacti-vap evaporator in a fume hood to be blown down.
6. The SPE manifold can be dismantled and cleaned thoroughly with methanol and dd H_2O.

   Evaporation and reconstitution
7. In a fume hood, load the Reacti-vap tubes with the eluent into the Reacti-vap evaporator. Lower the needles of the blow-down unit into the tubes and turn on the nitrogen flow to create a gentle flow on the surface of the samples (not too strong to cause splashes).
8. Lower the needles every 30 min to keep a constant, gentle flow on the surface of the samples. It should take approximately 1-2 hours to blow the sample to dryness.
9. Once completely dry, remove the tubes from the unit. Reconstitute each sample by adding 1 mℓ ethanol (sample concentrated 1000x) to each tube. Mix the samples thoroughly by vortexing or sonifying.
10. Place the eluent into sterile glass amber vials (4 mℓ volume) and store at -20°C prior to analysis.

APPENDIX C

Pilot Plant CIP, dosing and storage solution chemicals

Preparation of Pilot Plant CIP, dosing and storage solution chemicals

Preparing the chemical solutions of NaOCl and citric acid for cleaning in place (CIP), sodium metabisulphite for long-term UF/NF/RO membrane storage.

Steps for preparing 100 L of 400 ppm NaOCl from 12.5% NaOCl

1. Using the equation $C_1V_1 = C_2V_2$
2. Concentration of undiluted NaOCl (C1) = 12.5%, therefore C_1 = 125 g/L and V1 = unknown volume
3. 100 L 400 ppm NaOCl is required, therefore C_2 = 0.4 g/L and V_2 = 100 L
4. $C_1V_1 = C_2V_2$
5. (125 g/L) x V_1 = (0.4 g/L) x (100 L)
6. V_1 = 0.32 L per 100 L water
7. In order to prepare 100 L of 400 ppm NaOCl, 0.32 L (320 ml) of 12.5% NaOCl must be added to 99.68 L of water

Steps for preparing 100 L of 1% citric acid

17. 1% citric acid = 1 g/ 100 ml
    = 10 g /L
17. Therefore, 10g/1L = x g/100L
    X = 1000 g
17. In order to prepare 100 L of 1% citric acid, 1000 g (1.0 kg) of citric acid must be added to 100 L of water

Steps for preparing 100 L of 1% sodium metabisulphite

17. 1% sodium metabisulphite = 1 g/ 100 ml
    = 10 g /L
17. Therefore, 10 g / 1 L = x g/100L
    X = 1000g
17. Therefore, in order to prepare 100 L of 1% sodium metabisulphite, 1000 g (1.0 kg) of sodium metabisulphite must be added to 100 L of water

APPENDIX D

Calculations of RO parameters

Sample calculations are based on Unmodified Membrane data obtained

Flux

The permeate flux was calculated using the following formula (Hu et al., 2016):

$$J = \frac{V}{A \times \Delta t}$$

$$\frac{0.033L}{0.00416hr \times 0.013775m^2} = 57.5 L.m^{-2} hr^{-1}$$

The flux was then normalized to account for the change in temperature using the following formula (Water Environment Federation, 2006):

$$Normalized \quad flux = \frac{Actual \quad flux}{1.03^{(T-25)}}$$

$$Normalized \quad flux = \frac{57.5}{1.03^{(24.6-25)}} = 58.2\, L.m^{-2} hr^{-1}$$

Salt Rejection

The observed salt rejection was calculated using the conductivities of the feed ($EC_{(F)}$) and the permeate ($EC_{(P)}$), according to (Kucera 2015):

$$Salt \quad rejection = \left(1 - \frac{EC_{(p)}}{EC_{(f)}}\right) \times 100$$

$$Salt \quad rejection = \left(1 - \frac{28.7}{380.1}\right) \times 100 = 92.45$$

Flux Decline Ratio (FDR)

The flux decline ratio (in %)was recorded to evaluate de severity of fouling using the initial flux of water (J_i) and time dependent flux of water (J_t) all in in $L.m^{-2}\ h^{-1}$ in the following formula (Hu et al. 2016):

$$\left(\frac{J_i - J_t}{J_i}\right) \times 100$$

… (Equation 3)

$$\left(\frac{57.5 - 55.75}{57.5}\right) \times 100 = 3.03\%$$

Flux Recovery Ratio

The flux recovery ratio (FRR) was evaluated after 42 hours RO cell process experiments using the flux of pure water before experiment and the pure water flux after flushing (physical cleaning) of the membrane in the following formula:

$$FRR = \left(\frac{J_{Wi}}{J_{Wf}}\right) \times 100$$

… (Equation4)

$$FRR = \left(\frac{43.91}{62.29}\right) \times 100 = 70.49\%$$

APPENDIX E

Modifying agent solutions preparation – PVA (Sample calculation)

PVA polymer solution and GA solution preparations

Preparation of 0.05 % wt. GA and 0.1 g/L PVA solution

Dilution to a 500 mL solution of 0.05% wt. GA:
GA stock concentration: 25% wt.
Density: 1.06 g/mL

$$V_{required} = \frac{\%required}{Actual\,\%} \times Volume_{solution}$$

$$V_{required} = \frac{0.05}{25} \times 500mL = 1mL$$

Volume of water:

$$V_{water} = 500mL - 1mL = 499mL$$

Therefore, to prepare a 0.05 % wt. of GA 500mL solution, 1 mL from the stock solution has to be added to 499 mL of DI water.

- Adjusting the pH of the solution:

A diluted solution of H_2SO_4 is prepared by adding 2 drops of 98% H_2SO_4 to 100 mL of DI water. Then droplets of the prepared solution are slowly added to the GA solution to obtain a solution of pH 3.

- 0.1 g/L PVA solution:

17.1g of PVA was measured on a weighing balance and added to 1 L of DI water. The solution was then placed on a magnetic stirrer plate and heated to about 70 ˚C until the PVA is completely dissolved. Then add an amount of DI water to compensate for the evaporated water.

APPENDIX F

Salt Rejection and TDS

Table F.1: Total dissolved solids (Pilot Plant data)

Total Dissolved Solids (TDS) (g/L)						
Time	**NF (NF270)**		**RO (XLE)**		**UF (UA60)**	
min	pH = 6.5	Un-control pH	pH = 6.5	Un-control pH	pH = 6.5	Un-control pH
0	261.00	286.50	94.30	22.20	319.00	331.00
30	269.00	280.90	94.30	20.00	318.60	330.00
60	268.00	285.60	36.40	18.60	316.90	332.00
90	264.00	287.40	29.30	20.20	315.60	331.00
120	264.00	278.20	24.10	19.90	318.10	328.00
150	263.00	278.60	23.90	19.40	317.40	329.00
180	263.00	281.70	21.20	9.50	316.80	318.80
210	263.00	281.70	21.00	9.50	316.80	318.80
240	261.00	281.70	20.50	9.50	316.80	318.80
270	260.00	281.70	20.00	9.50	316.80	318.80
300	259.00	281.70	19.60	9.50	316.80	318.80
330	257.00	281.70	19.20	9.50	316.80	318.80
360	252.00	281.70	19.10	9.50	316.80	318.80

Table F.2: Salt rejection (Pilot Plant data)

Salt Rejection (%)						
Time	**NF (NF270)**		**RO (XLE)**		**UF (UA60)**	
min	pH = 6.5	Un-control pH	pH = 6.5	Un-control pH	pH = 6.5	Un-control pH
0	47.41	42.16	91.07	96.08	21.23	17.29
30	41.79	36.63	90.91	93.47	18.35	18.13
60	42.18	37.26	90.51	93.61	18.82	16.97
90	39.97	34.68	89.93	93.65	17.71	17.14
120	38.98	35.96	89.80	93.87	17.72	16.17
150	40.07	34.89	90.02	94.28	20.09	16.51
180	42.45	36.46	89.18	95.23	21.14	16.85
210	41.08	33.79	90.91	94.83	23.59	16.17
240	40.78	38.51	90.51	95.56	23.80	17.94
270	42.37	34.07	90.70	96.23	20.60	17.08
300	41.36	29.51	91.00	96.56	23.44	17.62
330	42.44	32.95	90.80	96.30	21.11	17.17
360	40.49	38.59	91.40	94.40	21.78	17.71

APPENDIX G

ELISA and YES Data

Table G.1: ELISA screening Assay (T and E_2)

ELISA (ng/L)		
	T	**E_2**
MBR Effluent May-week1	115.50890	8.14373
UA60 effluent	12.48927	2.72007
NF270 effluent	11.97439	0.64755
NF270 effluent	11.87660	0.63475
MBR Effluent May-week 2	118.66049	2.23543
UA60 effluent	11.82393	1.79985
NF270 effluent	11.83221	0.32936
NF270 effluent	11.87043	0.42005
MBR Effluent May-week 3	117.17155	7.37939
UA60 effluent	13.32280	2.25943
NF270 effluent	11.85121	1.23597
NF270 effluent	11.98719	1.54416
MBR Effluent May-week 4	117.26990	3.76924
UA60 effluent	13.34600	2.12336
NF270 effluent	11.62060	0.49189
NF270 effluent	11.54158	0.37570
XLE Effluent	11.44109	< 0.25 ng/L
XLE Effluent	11.63933	< 0.25 ng/L
MBR Effluent June-week 1	117.54557	2.55491
XLE Effluent	11.86811	< 0.25 ng/L
XLE Effluent	11.79427	< 0.25 ng/L
MBR Effluent June-week 2	117.86059	3.43297
XLE Effluent	11.81954	< 0.25 ng/L
XLE Effluent	11.54159	< 0.25 ng/L
MBR Effluent June-week 3	115.22976	2.98011
XLE Effluent	11.63502	< 0.25 ng/L
XLE Effluent	11.66001	< 0.25 ng/L
XLE Effluent	11.80691	< 0.25 ng/L
MBR Effluent June-week 4	116.99963	4.61452
NF270 effluent	11.64132	0.41135
NF270 effluent	11.59346	0.55017
XLE Effluent	11.46340	< 0.25 ng/L
MBR Effluent July-week 1	114.05859	4.65546
XLE effluent day 1	11.56276	< 0.25 ng/L
XLE effluent day 2	11.54807	< 0.25 ng/L
XLE effluent day 3	11.77109	< 0.25 ng/L
XLE effluent day 4	11.67896	< 0.25 ng/L
MBR Effluent July-week 2	118.72532	8.75942

Table G.2: YES screening Assay (E_2)

YES (ng/L EEQ)	
	E_2
MBR effluent-Sept	0.62920
RO effluent	< 0.25 ng/L
UA60 effluent	0.37320
UA60 effluent	0.49570
UA60 effluent	0.49850
NF270 effluent	< 0.25 ng/L
NF270 effluent	< 0.25 ng/L
RO effluent	< 0.25 ng/L
NF270 effluent	< 0.25 ng/L
NF270 effluent	< 0.25 ng/L
UA60 effluent	0.44330
NF270 effluent	< 0.25 ng/L
RO effluent	< 0.25 ng/L
RO effluent	< 0.25 ng/L
UA60 effluent	0.42220
UA60 effluent	0.32960
MBR effluent-Oct	0.37860
NF270 effluent	0.40860
UA60 effluent	0.33230
UA60 effluent	0.43040
UA60 effluent	0.40860
UA60 effluent	0.37040
NF270 effluent	< 0.25 ng/L
NF270 effluent	< 0.25 ng/L
NF270 effluent	< 0.25 ng/L
NF270 effluent	< 0.25 ng/L
RO effluent	< 0.25 ng/L
RO effluent	< 0.25 ng/L
RO effluent	< 0.25 ng/L
MBR effluent-Nov	0.74360

RO effluent	< 0.25 ng/L
NF270 effluent	< 0.25 ng/L
RO effluent	< 0.25 ng/L
NF270 effluent	< 0.25 ng/L
NF270 effluent	< 0.25 ng/L
UA60 effluent	0.33500
UA60 effluent	0.47670
UA60 effluent	0.31010
UA60 effluent	0.41870
NF270 effluent	0.54203
NF270 effluent	0.44940
RO effluent	< 0.25 ng/L
MBR effluent-Dec	0.44400
RO effluent	< 0.25 ng/L