The Efficiency of a Swoxid Prototype and an Antimicrobial Nanofiber Membrane as Point-Of-Use Water Filters

By

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DECLARATION

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Abstract (995 words)

Clean water is a scarce resource that numerous individuals lack access to. Resultantly, these individuals resort to using surface waters which leaves them exposed to waterborne diseases if they do not clean the water source adequately. Furthermore, these individuals are also exposed to chemical pollutants from agricultural, domestic and industrial waste which drains into rivers as well as downstream from waste water treatment plants (WWTPs). Furthermore, WWTPs which do not remove chemical pollutants efficiently. There are several methods to clean water, one of the most popular being filtration. In this study, the efficiency of two potential point-of-use (POU) filters was determined.

The first filter, namely the Swoxid prototype, is comprised of a ceramic membrane functionalised with titanium dioxide (TiO_2) and requires ultra violet (UV) light for activation. The mechanism of TiO_2 is that when it is activated by UV light, hydroxyl ($OH^{\cdot}$) radicals are generated, which inactivate bacteria and denature chemical pollutants.

To determine the efficiency of the Swoxid prototype to inactivate bacteria, water from a highly polluted source, namely the Plankenburg River, was filtered through the filter in both the absence and the presence of sun exposure. The bacteria tested were the typical indicator organisms namely the coliforms, faecal coliforms, heterotrophic bacteria, *Enterococcus* spp., *Salmonella* spp. and *Shigella* spp. For the first Swoxid prototype, there was complete removal of bacteria under exposure to the sun except for the heterotrophic bacteria and the coliforms. In the case of the coliforms, no bacteria were observed for the first few days; however on the fourth or the sixth day, growth was observed. This suggests that for the first few days, the bacteria are in a stationary phase, likely due to the time required for DNA repair. For most of the bacteria tested, the results of the trials where the first Swoxid prototype was exposed to the sun were comparable to the controlled conditions. This suggests that the removal of bacteria by the Swoxid prototype is mainly due to filtration and not the inactivation of bacteria by the radicals that are generated by the TiO_2-UV reaction. The second Swoxid prototype was effective at removing most of the bacteria; however, there was regrowth of the heterotrophic bacteria and coliforms. The results of the Swoxid prototype were comparable to the UV control, suggesting that inactivation was mainly due to UV exposure and not radical degradation.

Scanning electron microscopy was used to view the effect of radicals on the membrane of *Staphylococcus aureus* Xen 36. The results showed that the radicals generated by the TiO_2-UV reaction may oxidize the lipid bilayer of bacterial membranes leading to the formation of cracks on the surface of the membrane of bacteria.

The Swoxid prototype was also effective in removing several micropollutants, i.e. benzotriazole, codeine, diclofenac, efavirenz and sulfamethoxazole from spiked and river water. However, because the results were comparable to a commercial UV-filter for many of the micropollutants that were tested, it could be that the removal was mainly due to UV bombardment and not removal of the micropollutants by the radicals.

Future improvements and amendments to the Swoxid prototype should include an increase in the quantity of TiO_2 used to functionalise the membrane.

The second filter membrane prototype comprised of a poly (D,L-lactic acid) (PLA) nanofiber membrane functionalised with an antimicrobial solution (biocide) containing copper (Cu(II)) and zinc (Zn(II)) ions. The proposed mechanisms of Cu(II) and Zn(II) entail disrupting the membrane of microorganisms, decreasing the membrane potential and binding to sulfur-containing proteins and DNA. Ten varieties were fabricated that included low, medium and high biocide loading and low and high density membranes.

As part of the initial experiments, leaching experiments were performed using inductively coupled mass spectrometry (ICPMS) to determine whether or not the quantity of Cu(II) and Zn(II) that leach is below the limit set United States Environmental Protection Agency (USEPA) and South African standards for drinking water. The results showed that the concentration of Cu(II) and Zn(II), which the maximum that leached were 33.6 µg/L and 100.5 µg/L, respectively were indeed below the limits set in the drinking standards of both the EPA and South Africa. The results also showed that membranes with a higher density leached more Cu(II) and Zn(II) than their lower density counterparts. Moreover, the results showed that more Zn(II) leached from the membranes than the Cu(II) which suggests that the Cu(II) is more tightly retained by the PLA fibres.

The membranes were then exposed to *Escherichia coli* Xen 14 and *S. aureus* Xen 36 and the decrease in bioluminescence was observed over time using the XENOGEN VIVO VISION In Vivo Imaging Lumina System (IVIS). The experiment showed that the membranes with the highest biocide and higher density were most effective at inactivating the metabolism of bacteria.

Therefore subsequent experiments entailed filtering reverse osmosis (RO) water spiked with 10^7 *Escherichia coli* Xen 14 and *S. aureus* Xen 36 through the antimicrobial nanofiber membranes to determine the log reductions of the bacteria. However, the membrane with the highest biocide, density and fibre diameter only led to a 1.5 log reduction. The design of the nanofiber membrane was modified to consist of one layer of PLA nanofibers and a second layer containing PLA and the biocide. Three varieties were fabricated consisting of

three different densities and controls containing no biocide. Bacteria were only removed when the high planar density membranes were stacked on top of one another with the highest log reduction being 5 when three membranes were used.

The nanofiber membranes were also characterized before and after filtration using scanning electron microscopy (SEM). It was determined that filtration does not have a significant effect on the structure of the membrane as the nanofibers were still intact. The pores were, however, larger than bacteria in size and that explains the poor filtration results when just one membrane is used. Moreover, it was discovered through electron dispersive spectroscopy (EDS) that the copper binds more tightly to the membrane than the Zn(II) as the difference in the content of copper before and after filtration was very little. In addition to the structure of the antimicrobial nanofiber membrane, the structure of the bacteria after filtration was also viewed. There were several bacteria with damaged outer surfaces, particularly those that were located near what appeared to be the biocide.

The design of the antimicrobial nanofiber membrane can be improved by increasing the density of the membrane and ensuring smaller pore sizes. Additionally, the membranes should be designed in such a way that the Cu(II) leaches more readily at concentrations that are still below the limits set in the EPA and South African Drinking Water Standards.

Abstrak (958 woorde)

Skoon water is ‘n skaars bron en is ontoeganklik vir vele mense. Gevolglik is hierdie mense oorgelaat om oppervlakwater te gebruik wat hul blootstel aan waterverwante siektes, indien die water nie doeltreffend gesuiwer word nie. Daarbenewens is hierdie individue ook blootgestel aan chemiese besoelstowwe afkomend van landbou-, huislike- en industriële afloop. Afvalwater behandelingsaanlegte verwyder ook nie al die chemiese besoedelstowwe doeltreffend nie. Verskeie metodes word gebruik om water skoon te maak, een van die mees gewildste is filtrasie. In hierdie studie is die doeltreffenheid van twee gebruikspunt-filters bepaal.

Die eerste filter, naamlik die Swoxid prototipe, bestaan uit keramiek gefunksionaliseerde titanium dioksied (TiO_2) wat geaktiveer word deur ultraviolet (UV) lig. Tydens hierdie aktivering word hidroksiel radikale gegenereer wat bakterieë inaktiveer en chemiese besoedelstowwe kan afbreek.

Die doeltreffenheid van die Swoxid prototipe om water te suiwer, wat besmet is met patogene, is bepaal. Water van die Plankenburg rivier is gefiltreer deur die prototipe, met en sonder blootstelling aan sonlig. Die bakterieë wat getoets is, was koliforme, fekale koliforme, heterotrofiese bakterieë, *Enterococcus* spp., *Salmonella* spp. en *Shigella* spp. In meeste van die toetslopies waar die Swoxid blootgestel was aan sonlig, was daar totale verwydering van bakterieë, behalwe koliforme. In hierdie spesifieke geval, is geen bakterieë waargeneem vir die eerste paar dae nie, alhoewel op die vierde en sesde dag, is her-groei waargeneem. Hierdie resultate stel voor dat die bakterieë in stasionêre fase was en eers na vier tot ses dae weer lewensvatbaar was. In meeste van die toetslopies, was die resultate met son- of sonder son blootgestelling, vergelykbaar. Hierdie resultate stel voor dat die verwydering van bakterieë hoofsaaklik deur filtrasie plaasgevind het en dat die radikale wat gegenereer is nie die bakterieë geinaktiveer het nie. Die tweede Swoxid prototipe was doeltreffend om meeste van die bakterieë te verwyder, egter, daar was groei van die heterotrofiese bakterieë en die koliforme. Die resultate van die tweede Swoxid prototipe is vergelykbaar met die UV kontrol wat voorstel dat die verwydering van die bakterieë is hoofsaklik weens aan die blootstelling van die bakterieë aan UV en nie aan die radikale nie.

Skandeer elektron mikroskopie is gebruik om die effek van radikale op die membrane van *Staphylococcus aureus* Xen 36 waar te neem. Die resultate wys dat die lipied bi-laag van bakteriële membrane geoksideer was wat gelei het tot die formasie van krake op die oppervlak van die sel-oppervlak.

Die Swoxid prototipe was ook effektief om sekere mikro-besoedelstowwe te verwyder, beide uit steriele gedistilleerde water, voorberei met spesifieke mikro-besoedelstowwe, asook uit rivierwater. Die resultate is vergelyk met dié van ‘n kommersiële UV-filter en met heelwat van die mikro-besoedelstowwe was die resultate vergelykbaar. Dit kan dus wees dat die verwydering hoofsaaklik as gevolg was van UV bombardering, en nie deur die werking van die radikale nie. Toekomstige verbeterings en veranderings aan die Swoxid prototipe wat voorgestel word sluit in die verhoging in die kwantiteit van die TiO_2 wat gebruik word om die membraan te funksionaliseer.

Die tweede filter membraan prototipe het bestaan uit poly(D,L-melksuur) (PLA) nanovesel membrane gefunksionaliseerd met ‘n antimikrobiese oplossing (biosied) met hoofsaaklik koper (Cu(II)) en sink (Zn(II)) ione. Die voorgestelde meganismes van Cu(II) and Zn(II) sluit in die versteuring van die membraan van mikroorganismes, verlaging in die membraanpotensiaal en binding aan swael-bevattende proteïne en DNA. Tien variasies van die membraan is vervaardig wat ingesluit het lae, medium en hoë biosied lading en lae en hoë digtheid membrane.

Eerstens is uitloging eksperimente uitgevoer deur induktiewe-gekoppeld massa spektrometrie (ICPMS) te gebruik om te bepaal of die kwantititeit van Cu (II) en Zn(II) ione wat uitloog laer is as die regulasies gestipuleer deur die Verenigde State Omgewings Beskermings Agentskap (USEPA) en Suid-Afrikaanse standaarde vir drinkwater. Die resultate het getoon dat die konsentrasies van beide Cu(II) en Zn(II) ione laer was as die bepaalde regulasies van USEPA en Suid-Afrika. Die resultate het ook gewys dat daar, uit die membrane met ‘n hoër digtheid, meer Cu(II) en Zn(II) ione uitgeloog het as die laer digtheid membrane. Daar het ook meer Zn(II) ione uitgeloog as Cu(II) ione, wat voorstel dat die Cu(II) ione sterker gebind was aan die PLA vesels. Die membrane is blootgestel aan *Escherichia coli* Xen 14 en Staphylococcus aureus Xen 36 en die afname in bioluminesensie is waargeneem oor tyd deur die XENOGEN VIVO VISION ‘In Vivo Imaging Lumina System’ (IVIS) te gebruik. Die membrane met die hoogste biosied lading en hoë membraan digtheid was die meeste effektief om die metabolisme van die bakterieë te inaktiveer. Daarom is daar in die opvolgende eksperimente steriele gedistilleerde water, geïnokuleer met 10^8 selle/mL *E. coli* Xen 14 en *S. aureus* Xen 36 deur die antimikrobiese nanovesel membrane gefiltreer om die verlaging van die bakterieë te bepaal. Alhoewel, die membraan met die hoogste biosied lading, dightheid en vesel diameter het slegs gelei tot ‘n 1.5 log verlaging. Die ontwerp van die nanoveselmembraan is verander na een laag PLA nanovesels, bedek met ‘n tweede laag wat die PLA en biosied ingesluit het. Drie variasies is vervaardig wat membrane met drie verskillende digthede ingesluit het en kontroles met dieselfde digthede, maar sonder funksionalisering met die biosied. Bakterieë is slegs verwyder deur twee hoë

digtheid membrane op mekaar te plaas, met die hoogste verlaging van 5 log met die insluiting van drie hoë digtheid membrane. Die nanoveselmembrane is ook gevisualiseer voor en na filtrasie deur skandeer elektron mikroskopie (SEM) te gebruik. Dit is bepaal dat die filtrasie geen noemenswaardige effek op die struktuur van die nanoveselmembrane gehad het nie. Daar is ook gevind dat die membraan porie-grootte groter was as bakterieë, wat die onvoldoende filtrasie resultate verduidelik. Dit is ook deur elektron dispersiewe spectroskopie (EDS) bepaal dat die Cu(II) ione moontlik sterkter gebind het aan die membraan as die Zn(II) aangesien die konsentrasie van die Cu(II) ione hoër was op die membraan as die Zn(II) ione na filtrasie.

Die strukture van die bakteriële oppervlak was ook beskadig, veral dié wat in kontak was met die geimmobiliseerde metaalione op die membraan. Die ontwerp van die antimikrobiese nanoveselmembraan kan verbeter word deur die membraandigtheid te verhoog om kleiner porie-grootte te verseker. Daarbenewens, kan die membrane op so ‘n manier vervaardig word dat die Cu(II) ione meer doeltreffend uitloog.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACTM Acetaminophen

Ag Silver

Au Gold

BEATC Benzyl Triethylammonium Chloride

BLPD Biocide-containing Low Planar Density

BHPD Biocide-containing High Planar Density

CAF Central Analytical Facility

CAFO Concentrated Animal Feeding Operations

CBZ Carbamazepine

CLSM Confocal Laser Scanning Microscopy

Cu(II) Copper

DiBAC4(3) (Bis-(1,3-Dibutylbarbituric Acid)Trimethine Oxonol)

EDC Endocrine Disrupting Compound

EDS Electron Dispersive Spectroscopy

EtOH Ethanol

H_2O Water

HMDS Hexamethyldisilazine

ICP-MS Inductively-Coupled Plasma Mass Spectrometry

LB Luria Broth

LC-MS Liquid Chromatography Mass Spectrometry

MBR Membrane Bioreactor

MDMA 3,4-methylenedioxymethamphetamine

MeOH Methanol

MF	Microfiltration
MOA	Mechanism of Action
NCHPD	Negative Control High Planar Density
NF	Nanofiltration
$O^{\cdot}$	Oxygen Radical
$OH^{\cdot}$	Hydroxyl Radical
PC	Polycarbonate
Pd	Palladium
PI	Propidium Iodide
PLA	Poly Lactic Acid
PVA	Poly Vinyl Acetate
POU	Point Of Use
RO	Reverse Osmosis
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
SNC	Stellenbosch Nanofiber Company
SPE	Solid Phase Extraction
TiO_2	Titanium Dioxide
SMX	Sulfamethoxazole
TEM	Transmission Electron Microscopy
TSB	Tryptic Soy Broth
UF	Ultrafiltration
USEPA	United States Environmental Protection Agency
UV	Ultraviolet

VGSRC Voltage Gated Sodium Receptor Channels

WHO World Health Organisation

WWTP Wastewater Treatment Plant

Zn(II) Zinc

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CHAPTER 1: Introduction and Objectives

General Introduction and Objectives

1.1. Introduction

Freshwater is an essential resource for all life on earth, yet, it is scarce and numerous areas across the globe lack a clean supply (WHO 2012). Due to the global water crisis, an estimated 663 million are left without access to a source of clean water and 2.4 billion people do not have access to proper sanitation facilities (WHO/UNICEF, 2015). As many individuals do not have access to a centralised water source, they make use of surface waters. The use thereof may cause outbreaks of waterborne diseases such as cholera, typhoid fever, salmonellosis, shigellosis and giardiasis amongst others (Adam, 2001; Arnone & Walling, 2007; Betley, Miller & Mekalanos, 1986). Approximately 13 million people, mostly from developing countries, die from waterborne diseases annually (Arnone & Walling, 2007). Therefore, surface waters need to be adequately treated before consumption.

Furthermore, numerous bodies of water are contaminated by chemical pollutants from numerous sources such as industrial, domestic, and agricultural waste due to direct discharge from these sources and the inadequate treatment of waste water by waste water treatment plants (WWTPs) (Genthe et al., 2013; Kim & Zoh, 2016). In some cases, the quantity of a certain chemical may be higher in the treated effluent than in the influent (Archer et al., 2017). Examples of chemical pollutants include pesticides, aromatic hydrocarbons, phthalate plasticizers, certain polychlorinated biphenyls, dioxins, furans, alkyl phenols, synthetic steroids, alkyl phenol ethoxylates, polychlorinated biphenyls, polybrominated compounds, steroid sex hormones, phthalates and phytoestrogens (Dargnat et al., 2009; Liu, Kanjo & Mizutani, 2009; De Alda & Barcelo, 2000; Roslev et al., 2007). Many of these chemicals pose a health risk to wildlife and potentially humans by disrupting the endocrine system and are therefore known as endocrine disrupting compounds (EDCs). Other chemicals introduced into water systems from wastewater may reduce fertility, cause miscarriages and increase the chance of developing various cancers (Robins et al., 2011; Soto & Sonnenschein, 2010). Considering the fact that WWTPs are not the most efficient means of eliminating chemical pollutants from wastewater, alternative solutions are required.

There are numerous methods that can be implemented to purify water, namely thermal treatment, boiling, solar radiation, alum and iron coagulation, ozonation, charcoal, activated carbon, UV disinfection, ion exchange disinfection and chemical treatment (Agrawal & Bhalwar, 2009; AWWA, 1999; Faust & Aly, 1998; Joyce et al., 1996; Long, 1998; Naranjo, & Chaidez, 1997; Randtke, 1998; Sobsey, 1989; Sobsey & Leland, 2003). However, one of the methods commonly used to circumvent the consumption of contaminated water is the use of

point-of-use (POU) filters. Filtration is based on the principle of applying pressure and vacuum to a porous membrane system so that water will pass through and separate microbial contaminants and suspended particulates in the process (Ramakrishna et al., 2010). The advantage of filtration is that unlike chlorination and the chemical treatment of water, it does not result in the formation of harmful by-products (Zularisam, Ismail & Salmim, 2006). There are different types of POU filters namely colloidal-silver impregnated ceramic filters, slow sand filters, activated carbon filters, granular beds, sediment filters and the LifeStraw filter (Huisman, 1974; Kallman, Oyanedel-Craver & Smith, 2011; Sagara, 2000; Snyder et al., 1995; Time, 2005; Williams, 1992).

In this study, validation tests were performed on a pilot scale POU filter that has been recently developed to treat water containing microbial and chemical contamination. The Swoxid prototype consists of a TiO_2 based ceramic membrane that relies on solar radiation for functionality. Therefore, in addition to mechanical filtration, the device makes use of thermal inactivation and oxidation to inactivate microorganisms. Oxidation occurs upon exposure of the membrane to solar radiation where the TiO_2 acts as a photo-catalyst and generates hydroxide radicals. These radical species then inactivate microorganisms and chemical pollutants (Ireland et al., 1993). In fact, TiO_2 catalysed oxidation has been shown to degrade organic pollutants originating from industrial waste and the combustion of fossil fuels. These chemicals include azo dyes, Acid Blue 40, ethylene, methyl vinyl ketone, acenaphthene, anthracene, fluorene and naphthalene, among many others. (Antharjanam, Philip & Suresh, 2003; Dass, Muneer & Gopidas, 1994, Mammadov et al., 1992; Muneer et al., 1992, Muneer, Phillip & Das, 1997).

The second part of the study focused on determining the parameters of functionalized nanofibers to act as antimicrobial water filtration membranes in a POU filter. These nanofibers were industrially synthesised with poly (D,L-lactic acid) (PLA), a hydrophobic and biodegradable polymer, and functionalized with copper (Cu) and zinc (Zn) ions as the antimicrobial agents. Studies have shown that nanofibrous membranes functionalised with metals are very effective against bacteria in hospital wastewater and show potential of being applied in trickling filter systems (Daels et al., 2010; Daels et al., 2011). Metals are proposed to have multiple targets on bacterial cells making them effective biocides. For example, metal cations destabilise bacterial cell membranes and create pores within the membranes (Xie & Lang, 2016). Furthermore, they hinder the enzymes of the electron transport chain. Once inside, they create reactive oxygen species that react with and destroy DNA, proteins, lipids and other macromolecules (Freinbichler et al., 2012; Warnes, 2010; Yoshida, Furuta & Niki, 1993).

1.2. Problem Statement

Many individuals lack access to a clean water supply thus forcing them to make use of surface waters, which are often contaminated with pathogens. However, these same individuals often don't have effective methods of purifying water. As a result they contract waterborne diseases and are exposed to micropollutants from the surface waters that they consume.

1.3. Hypothesis

The hypotheses for this study are that the Swoxid prototype will disinfect contaminated water and denature the structure of selected chemical pollutants and that the antimicrobial nanofiber membranes will effectively remove and kill bacteria from contaminated water.

1.4. Aim and Objectives

The main aim of this study was to investigate the efficiency of the Swoxid prototype and the antimicrobial nanofiber membranes in the removal of microbial and chemical contamination from water. The objectives of this study were therefore:

1. To determine the efficiency with which the Swoxid prototype can improve the microbiological quality of the Plankenburg River water. This was investigated by observing the reduction in the numbers of bacteria found in the Plankenburg River water after filtration.
2. To determine the efficiency with which the Swoxid prototype improves the chemical quality of the Plankenburg River water. This was investigated by performing ultra-performance liquid chromatography mass spectrometry (UPLC-MS) analysis to observe the reduction in the levels of the persistent micropollutants carbamazepine (CBZ) and sulfamethoxazole (SMX) in water samples as well as an array of micropollutants in the Plankenburg River water after filtration.
3. To determine the optimum biocide loading, fiber diameter and density of the nanofibers in the antimicrobial nanofiber membrane for filtration.
4. To characterize the antimicrobial nanofiber membranes and the structure of bacteria post exposure to the antimicrobial nanofiber membrane using scanning electron microscopy (SEM) and electron dispersive spectroscopy (EDS).

1.5. Thesis Layout

Chapter 1

Chapter 1 contains a brief introduction as well as the objectives of this study.

Chapter 2

Chapter 2 includes a literature review on various topics namely the water crisis globally and in South Africa, EDCs, particularly CBZ and SMX, pathogenic bacteria in water sources, water filtration, the different kinds of point of use filters, nanofibers in water filtration, the different methods of producing nanofibers and the mechanisms of action of TiO_2, Cu and Zn.

Chapter 3

The efficiency of the Swoxid prototype in removing bacteria was determined using filtration tests and plate counting. Furthermore, ultra performance liquid chromatography tandem mass spectrometry (UPLC - MS/MS) experiments were performed to determine the efficiency of the Swoxid prototype in removing micropollutants.

For the antimicrobial nanofiber membrane, the optimal membrane was yet to be selected for the study; therefore, the Stellenbosch Nanofiber Company (SNC) manufactured ten varieties of the membranes in the first design and three in the second design. This chapter describes the various tests that were conducted to determine the most efficient membrane. These tests included leaching experiments, membrane characterization and determining the effect on the metabolism and viability of the antimicrobial membranes against gram positive and gram negative bacteria.

This chapter concludes with the concluding remarks and recommendations for future research.

CHAPTER 2:

Literature Review

2.1. Introduction: Water Situation in South Africa and Globally

Water is an essential resource used for various applications and is shared by 7 billion people. Despite the huge demand for water, there is less than 1 % of fresh usable water available on our planet (Dimick, 2014). Not surprisingly, there are numerous people living without access to clean water. For example, the World Health Organisation (WHO) estimated that approximately 884 million people live without access to a water source that provides clean water of 20 L per person daily within 1 km of the person's home (WHO, 2008). The Consultative Group on International Agricultural Research estimated that 2.7 billion people will live in water scarce regions by 2025 (Radjenovic et al., 2008). The water crisis is further exacerbated by the deaths that occur as a result of the lack of access to clean water or rather the consumption of contaminated water. In 2001, it was estimated that approximately 13 million people die from waterborne infections annually, with most of these deaths occurring in developing countries (Arnone & Walling, 2007). However, in 2006 the number was reduced to 1.8 million deaths where 99.8 % of these deaths occurred in developing countries and 90 % of these deaths were children (Nat, Bloomfield & Jones, 2006).

South Africa is an example of a water stressed country. It has an average annual rainfall of 450 mm which is 230 mm below the global annual rainfall (http://www.dwa.gov.za/IO/Docs/CMA/CMA%20GB%20Training%20Manuals/gbtrainingmanualchapter1.pdf). The water crisis in our country is attributed to the fact that the rain falls in an uneven spatial distribution and is seasonal. Additionally, our rivers have a low stream and our major towns and cities are located far from larger bodies of water. As a result, water has to be transferred on a large scale across catchments. The total surface water available in South Africa is approximately 49 200 million m^3 annually and is the main source of water supply in the country (http://www.dwa.gov.za/IO/Docs/CMA/CMA%20GB%20Training%20Manuals/gbtrainingmanualchapter1.pdf). However, only a maximum of 11 000 m^3/a of usable water can be extracted from surface waters and has to be used by the rural, urban, mining, industrial, power generation, afforestation sectors and irrigation as indicated in Figure 2.1. (http://www.dwa.gov.za/IO/Docs/CMA/CMA%20GB%20Training%20Manuals/gbtrainingmanualchapter1.pdf).

Despite the water scarcity in our country and contrary to most African countries, 92.5 % of households in South Africa are supplied with piped municipal water. The provinces in the Western Cape and Gauteng appear to have the highest percentage of households with access to municipal water with percentages of 93.3 % and 91 % respectively. On the other

hand, the provinces with the lowest percentages of households with access to municipal water are Limpopo (54 %) and Mpumalanga (65.8 %). Furthermore, only 1.2 % of households in South Africa use the bucket toilet system to dispose of their waste. (http://www.statssa.gov.za/?p=9145).

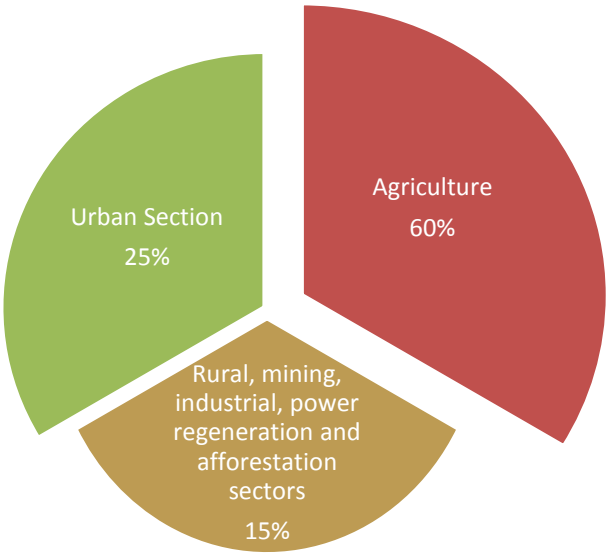


Figure 2.1: Percentage allocation of available surface water to the different sectors

Despite its scarcity and importance, water is sullied by various factors. In the United States of America, the cause of pollution in large bodies of water is contamination with animal faecal matter and the inadequately treated water from water treatment plants (Levy et al., 1998). A major source of animal faecal contamination is concentrated animal feeding operations (CAFOs) (USEPA, 2004). The faecal matter produced by these CAFOs is estimated to be 335 million tons of dry matter per year (Kellogg et al., 2000; USDA 2010). Water pollution by sewage effluent, agricultural, and informal residential runoff is also a problem in South Africa as was documented for the Rietvlei nature reserve wetland area by Oberholster et al., (2008) the Umgeni River by Sibanda et al., (2015) and the Klip River wetland by McCarthy et al., (2007) to name a few examples (Oberholster, Botha & Cloete, 2008; Sibanda, Selvarajan & Tekere, 2015; McCarthy et al., 2007). Other sources of contamination include industrial effluent and chemical contamination from WWTPs as discussed below.

2.2. Endocrine Disrupting Micropollutants

Another major challenge globally is the contamination of water sources with chemical compounds referred to as micropollutants. These compounds end up and accumulate in aquatic systems through the washing off of personal care products, bodily excretion through the urinary and biliary system, leaching from landfills, drain water, agricultural and industrial wastewater (Archer et al., 2017; Radjenovic et al., 2008; Ternes, 1998; Vethaak et al., 2005; Voutsa et al., 2006; Ying, Kookana & Ru, 2002). Although these compounds occur at trace concentrations in water systems, some of them are capable of eliciting harmful effects on the endocrine systems of animals and humans and are thus referred to as endocrine disrupting compounds (EDCs) (Dargnat et al., 2009; Liu, Kanjo & Mizutani, 2009). EDCs include pesticides, aromatic hydrocarbons, phthalate plasticizers, certain polychlorinated biphenyls, dioxins, furans, alkyl phenols, synthetic steroids, alkyl phenol ethoxylates, polychlorinated biphenyls, bisphenol A, pharmaceutical products, polybrominated compounds, steroid sex hormones, phthalates and phytoestrogens (Dargnat et al., 2009; Liu, Kanjo & Mizutani, 2009; Lopez & Barcelo, 2000; Roslev et al., 2007). EDCs cause their endocrine disrupting functions by imitating or antagonizing hormones by binding to hormone receptors (Burger & Moolman, 2006; Jiao & Cheng, 2008; Sumpter & Johnson, 2005).

WWTPs are used to treat water contaminated with these organic pollutants; however, some of these compounds persist in treated effluent (Archer et al., 2017; Kasprzyk-Horden, Dinsdale & Guwy, & 2009; Verlicchi, Aukidy & Zambello, 2012). A study by Archer et al., (2017) showed that in some cases, for example in the removal of dm-Citalopram and morphine by a WWTP in Gauteng, South Africa and diclofenac, carbamazepine, erythromycin, and sulfamethoxazole by other WWTPs, there is more of a certain micropollutant in the effluent than in the influent after processing. This indicates that the removal of EDCs by WWTPs is not always efficient (Archer et al., 2017; Luo et al., 2014). An explanation for the observation of certain micropollutants occurring in larger quantities in the effluent than in the influent is the possible deconjugation of the metabolites of the micropollutant back to the parent compound as most of them that are consumed are conjugated within the body before excretion. The presence of EDCs in water systems poses a potential health threat in that there is the risk of exposure to these compounds. For example, due to the fact that our country suffers from water scarcity, some parts of the country reuse treated effluent from WWTPs for irrigation, sport field, agriculture and other industrial purposes (Daso et al., 2011). Two persistent micropollutants that are the focus in our research group are described below.

2.2.1. Carbamazepine (CBZ)

CBZ is commercially marketed under the names Tergretol, Carbatrol, Epitol and Equetro (https://www.drugs.com/carbamazepine.html). It is used for the treatment of epilepsy, neuropathic pain, trigeminal neuralgia, bipolar depression and mania (Katzung, & Trevor, 2015; Valdés & Huerta, 2016). The compound belongs to the benzodiazepines class of drugs and consists of two benzene rings that are attached to an azepine functional group attached to an amide group (Figure 2.2) (Katzung & Trevor, 2015). CBZ exerts its anti-epileptic effect by blocking the voltage gated sodium channels. Moreover, this is achieved by inhibiting the release at the glutamergic synapse thus stabilising the inactivated state of the voltage gated sodium receptor channels (VGSRC) (Katzung & Trevor, 2015; Rogawski & Löscher, 2004). Under normal conditions, the drug is a weak inhibitor of the VGSRC; however, its inhibitory effect is exacerbated when the membrane is depolarised (Rogawski & Löscher, 2004). VGSRC are involved in the rising phase of action potentials. When neurons depolarize and reach the action potential threshold, the channels open by undergoing a conformational change (Rogawski & Löscher, 2004). When open, the voltage-gated sodium channels allow sodium ions in; however, a few milliseconds afterwards, the channel become inactive and prevents the influx of sodium ions (Rogawski & Löscher, 2004). The sodium channels in the brain can rapidly pass through these phases causing neurons to fire high-frequency action potentials which are necessary for an epileptic episode (Rogawski & Löscher, 2004). The blocking of voltage-gated sodium channels serves to prevent the spread of abnormal firing of the neurons to distant sites, thus preventing seizures (Rogawski & Löscher, 2004). After consumption, CBZ is almost entirely absorbed from the gastro-intestinal tract. The majority of the drug recovered is in the urine (72 %) and mainly consists of hydroxylated and conjugated metabolites whereas only 2 % of the drug remains as the parent compound. The CBZ that is not absorbed (28 %) is recovered from the faeces, where 14 % of the drug is in the form of its metabolites and the rest is in the form of the parent compound (Cunningham et al., 2010). The side effects of long term CBZ consumption are drowsiness, rashes and the decrease in the processing of information (Brodie, Richens & Yeun, 1995; Lee et al., 2011).

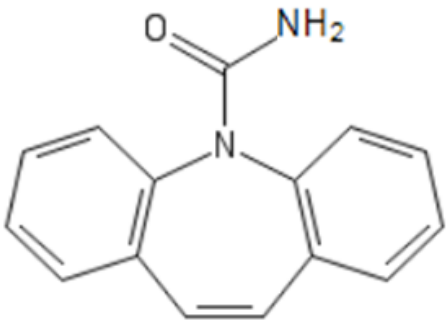


Figure 2.2: The structure of CBZ (drawn by the author on PubChem)

CBZ is an example of a recalcitrant micropollutant as the structure of the compound is so stable that it is not easily degraded during biological processing at WWTPs (Figure 2.2) (Katzung & Trevor, 2015; Zhang, Geissen & Gal, 2008). The compound has been shown to have a removal efficiency of less than 10 % at WWTPs as well as laboratory and pilot scale membrane bioreactor systems (Hai et al., 2011; Valdés & Huerta, 2016). The efficiency of removal of the compound, however, appears to be enhanced by treatment under anoxic conditions (Hai et al., 2011). Despite its stability, the compound is subject to photolysis. Andreozzi et al. (2002) demonstrated that the compound can absorb solar UV radiation. The group went further by exposing an aqueous solution containing 8.0×10^{-6} mol/dm^3 CBZ to solar irradiation. The results of the experiment were that the concentration of CBZ decreased with an increase of the time of solar irradiation by approximately 25 % over 70 hours (Andreozzi et al., 2002).

The low removal efficiency of CBZ is a problem because the compound is an endocrine disruptor. For example, it is well known that antiepileptic drugs may affect thyroid function which forms part of the endocrine system. CBZ is no exception as it alters thyroid function by reducing the thyroid hormone serum levels (Vainionpaä et al., 2004). Thyroid hormones are essential for metabolism, normal development of the vertebrate in mammals as well as cell proliferation and differentiation during neonatal development (Nunez, 1984). Thyroid hormones are also required for normal brain activity throughout the adult life of mammals (Joffe, & Sokolov, 1994). A reduction of thyroid hormone during the developmental phases of mammals can cause undesirable abnormalities (Bernal & Nunez, 1995).

A study by Drewes et al. (2002) showed that in the tertiary effluent of the North West Water Reclamation Plant, which is found in the city of Mesa, Arizona, CBZ has occurred at concentrations between 0.155 µg/L and 0.22 µg/L. In the tertiary effluent of the Scottsdale Water Campus, which is also in Arizona, the compound has been found to occur at 0.445 µg/L. However, subsequent treatment of the effluent with reverse osmosis (RO) eliminated

the pharmaceutical compound, along with the others from the filtrate (Drewes, Heberer & Reddersen, 2002). Drewes et al. (2002) also discovered that the CBZ occurs at concentrations of 0.455 and 0.61 µg/L in groundwater monitoring wells of Arizona near Tucson (Drewes, Heberer & Reddersen, 2002). In South America, by the Suquiá River, the levels of carbamazepine can reach up to 0.113 µg/L (Valdés & Amé, 2014). In Germany, particularly by the River Rhine, Kuehn et al. (2000) discovered that the compound occurs at concentrations between 0.2 – 0.55 µg/L (Kuehn & Mueller, 2000). In South Africa, particularly the Umtangeni area in Kwa-Zulu Natal, the compound was also found at low levels between 0.38 – 1.65 µg/L across various points on the Umgeni River, which is very high in comparison to the other regions reported above (Matongo et al., 2015).

Although the concentrations of CBZ across the waters in the various regions appear low, the drug has been reported to accumulate in periphyton, algae, zooplankton, invertebrates, fish and birds (Valdés & Huerta, 2016). For example, Garcia et al. (2012) have shown that CBZ can accumulate at concentrations of 1.03 ± 0.51 ng/g wet wt., 0.77 ± 0.15 ng/g wet wt., and 693.07 ± 228.6 ng/L in the white muscle, liver and plasma of the tilapia fish, respectively (Garcia et al., 2012). However, the research that has been conducted thus far has demonstrated that the drug is neither acutely toxic to the micro- and macroorgansims mentioned above. For example, acute toxicity tests by Ferrari et al. (2003) demonstrated that at concentrations below 25 µg/L, the compound did not have much of an effect on fish, bacteria and crustaceans. Furthermore, the compound was not detected in the algae *Ankistrodesmus braunii* or in the medium after the ecotoxological test, suggesting that perhaps the compound was metabolised by the algae (Ferrari et al., 2003). A study by Kim et al 2007. demonstrated that CBZ had an EC50 value of 52.5 mg/mL on the bacterium *Vibrio fischeri* after five min of exposure. Additionally, the EC50 value of CBZ for the crustacean *Daphnia magna* was 76.3 mg/mL after 96 hours of exposure, which is relatively high (Kim et al., 2007). The group also showed that CBZ is neither acutely nor chronically toxic to fish (*Oryzias latipes* used in their study) as the EC50 of the compound was 35.4 mg/mL. This is further supported by the results of Ferrari et al. (2003) with the fish *Danio rerio* where the No Observed Effect Concentration and the Lowest Effect Observed Concentration were 25mg/mL and 50 mg/mL, respectively. However, in the same study by Ferrari et al. (2003) chronic toxicity tests performed on the crustacean *Ceriodaphnia dubia* showed that the compound maintained high toxicity at low concentrations (Ferrari et al., 2003). Therefore, in terms of chronic exposure, literature appears to be contradictory regarding the toxicity of CBZ on crustaceans but show that it has no or low toxicity towards fish. Despite the lack of toxicity to fish, CBZ does have an effect on the human endocrine

system. Therefore, the bioaccumulation of the drug in fish may pose a hazard as fish is consumed by humans.

2.2.2. Sulfamethoxazole (SMX)

SMX has been observed at microgram per litre levels in sewage effluent and surface waters (Lam & Mabury, 2004). This is due to the fact that SMX has a removal efficiency of between -26 % and 64 % from conventional WWTPs (Liu et al., 2018). SMXs belong to the first class of antibiotics that is commonly combined with trimethoprim to treat gastroenteritis, *Pneumocystis carinii* pneumonia, bronchitis, Shigellosis, diarrhoea and urinary tract infections (Dantas et al., 2008; Eliopoulos & Huovinen, 2001; https://aidsinfo.nih.gov/drugs/401/sulfamethoxazole---trimethoprim/0/patient). The mechanism of action (MOA) of the drug is to inhibit the synthesis of dihydrofolic acid which is required for the production of folate and thus SMX is a broad spectrum drug. The structure of SMX consists of a benzene-sulfonamide attached to an oxazol and the compound is readily degradable (Das et al., 2015; Clara et al., 2005; Tadkaew et al., 2011). The compound has a very low solubility in water (610 mg/L) and has an octanol water partition coefficient of 0.89 (https://pubchem.ncbi.nlm.nih.gov/compound/Sulfamethoxazole#section=Vapor-Pressure; https://www.drugbank.ca/drugs/DB01015).

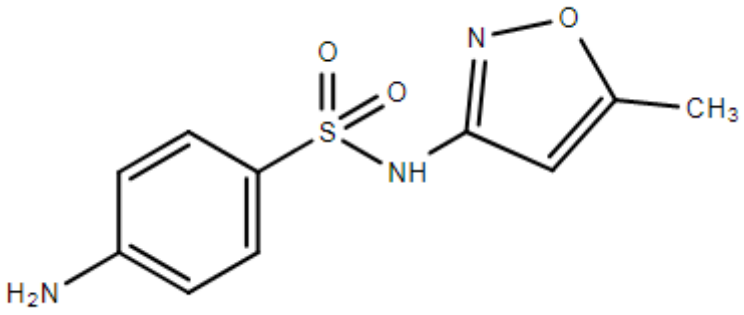


Figure 2.3: The structure of SMX (drawn by author on Chemspider).

SMX and its metabolites are not known to be cyto- or genotoxic; however the persistent levels of these compounds in bodies of water may contribute to antimicrobial resistance and a shift in microbial communities for bacteria in contact with these compounds (Chen et al., 2016; Richard et al., 2014).

Despite poor removal efficiency from conventional waste water treatment, the compound can be removed using advanced oxidation, adsorption and membrane technology. In fact, the

compound is removed at an efficiency of approximately 66-67 % under treatment with a membrane bioreactor (MBR) (Hai et al., 2011). Therefore, it would appear that alternative forms of removal, especially MBR treatment, are more efficient in removing SMX than conventional WWTPs.

2.3. Pathogenic Microorganisms in Water Sources

Many individuals lack access to clean water facilities globally, consequently, they make use of surface waters for their everyday activities. Nevertheless, most surface waters are contaminated with waterborne pathogens such as bacteria, protozoa and viruses, thereby making the continuous use of these waters a serious health threat (Pandey et al., 2014). Although surface water is the common source of infections caused by waterborne pathogens, these microorganisms can also be contracted through the consumption of fish from the contaminated surface waters, as well as through skin contact (Arnone & Walling, 2007). It has been reported that the waterborne pathogens of greatest concern have the following qualities: firstly, they are able to replicate outside the human host. Secondly, they can survive in the environment for long periods and still maintain their abilities to be infectious. Thirdly, they are resistant to all types of water treatment, and finally, they can be released into the environment at high numbers, but are able to infect humans at low numbers (Rosen, 2000).

Prevalent pathogenic bacteria include *Campylobacter* spp., *Escherichia coli* O157:H7, *Legionella pneumophilia, Leptospira interrogans, Salmonella* spp., *Shigella* spp. and *Vibrio cholerae* and *Yersinia entercolitica.*

2.3.1. Pathogenic Waterborne Bacteria

The genus of *Campylobacter* was established in 1963 (Sebald & Veron, 1963). *Campylobacter* spp. are a group of gram negative bacteria that can either be spiral-, rod- or curved-shaped. Furthermore, they may have single, bipolar or no flagella (Man, 2011). The bacteria do not form spores and are chemoorganotrophs that use amino acids and citric acid cycle intermediates as their energy sources and most species use aerobic respiration (Vandamme et al., 2005). They can be found in the faeces of domestic and wild animals; however, other sources include poultry, cattle, sheep and pigs and in some cases domestic pets (Fitzgerald & Nachamkin, 2015). *Campylobacter* spp. cause campylobacteriosis, inflammatory bowel disease, gastroenteritis and may cause Barrett's esophagus, irritable

bowel syndrome and colorectal cancer, among others (Kaakoush et al., 2015; Man, 2011). Campylobacteriosis leads to fever, gastroenteritis, vomiting, headaches, abdominal pain and bloody or watery diarrhoea (EFSA, 2011). The diseases associated with *Campylobacter* spp. are summarised in Table 1. Sources of *Campylobacter* are water, poultry and other food products contaminated with the bacteria (Little, 2010).

Table 2.1: *Campylobacter* spp. and their associated diseases

***Campylobacter* spp.**	**Associated Disease/ Illness/Condition**	**Reference**
C. coli	Bacteraemia, diarrhoea and gastroenteritis	Black et al., 1988; Blaser, 1997
C. concisus	Barrett's esophagus, Crohn's disease, gastroenteritis, gastroesophageal reflux disease, periodontal disease and ulcerative colitis	Blackett et al., 2013; Kaakoush et al., 2015; Macfarlane, 2007; Mahendran, 2011; Zhang, Geissen & Gal, 2008
C. curvus	Bronchial abscess and liver abscess	Han, Tarrand & Rice, 2005
C. fetus	Bacteraemia and meningitis	Kaakoush et al., 2015; Man, 2011
C. gracilis	Brain abscess and Crohn's disease	Zhang, Geissen & Gal, 2008; De Vries, Arents & Manson, 2008
C. hominis	Crohn's disease	Zhang, Geissen & Gal, 2008
C. jejuni	Bacteraemia, cholecystitis, Gastroenteritis, diarrhoea, Guillain-Barré syndrome (GBS) and Miller Fisher syndrome, meningitis	Kaakoush et al., 2015; Man, 2011; Vaughan-Shaw et al., 2010
C. lari	Gastroenteritis	Kaakoush et al., 2015
C. rectus	Breast and vertebrate abscess, chest wall infection, Crohn's disease and oral inflammation	De Vries, Arents & Manson, 2008; Macuch & Tanner, 2000; Han, Tarrand & Rice, 2005; Spiegel & Telford,

		1984; Zhang, Geissen & Gal, 2008
C. showae	Colorectal cancer, Crohn's disease, intraorbital abscess and periodontal diseases	Kaakoush et al., 2015; Warren et al., 2013; Wu et al., 2013; Zhang, Geissen & Gal, 2008
C. upsaliensis	Gastroenteritis	Kaakoush et al., 2015
C. ureolyticus	Gastroenteritis and ulcerative colitis	Mukhopadyha et al., 2011

E. coli O157:H7 is a gram negative pathogenic strain of *E. coli* which was discovered after two outbreaks of bloody diarrhoea in 1982. The bacterium belongs to a bigger group of pathogenic *E. coli* that also cause bloody diarrhoea namely enterohemorrhagic *E. coli*. However, *E. coli* O157:H7 is the most common and most studied member of the group (Griffin & Tauxe, 1991). Furthermore, according to the US Centres for Disease Control and Prevention (CDC), *E. coli* O157:H7 is one the top five pathogens that causes hospitalization (Zhang et al., 2017). The CDC estimated that in the US alone, the pathogen was responsible for ten thousand illnesses, thousands of hospitalizations and hundreds of deaths annually (Huang et al., 2011). The bacterium occurs in the faeces of cattle; however, infection with the bacterium can occur through eating food or drinking water contaminated with the bacterium (Arnone & Walling, 2007; Gelting et al., 2011; Herman, Ayers & Lynch, 2008). *E. coli* O157:H7 can cause haemolytic uremic syndrome, haemorrhagic colitis and diarrhoea (Johnson, Lior & Bezanson, 1983; Pai et al., 1984). Haemolytic uremic syndrome is the more concerning of the side effects in that it leads to damaged blood cells, kidney failure and even death (Buchanan & Doyle, 1997).

Legionella pneumophillia is a gram negative bacillus bacterium that occurs within aquatic environments (Edelstein & Lück, 2015). The bacterium is the causative agent of Legionnaire's disease which is a severe type of pneumonia; however, only a few out of many people exposed to the bacterium actually develop the disease. Furthermore, the disease is more likely to develop in immunocompromised individuals (Phin et al., 2014).

Leptospira interrogans is an obligate aerobic and motile bacterium. Furthermore, it is tightly coiled in morphology and belongs to the order *Spirochaetales* (Faine et al., 1999; Levett,

2001). The bacterium causes leptospirosis which can lead to flu and fever-like symptoms, headaches, severe renal and hepatic danger and jaundice (Faine et al., 1999). The bacterium colonizes the renal tubule of mammals and is excreted in urine (Faine et al., 1999). Transmission of *L. interrogans* to humans occurs through contact with contaminated urine, animal carriers, soil containing the bacterium and the consumption of contaminated water (Faine et al., 1999; Farr, 1995).

Salmonella spp. are a group of gram negative rods that are facultatively anaerobic (D'Aoust & Maurer, 2007). They are carried and passed through food contaminated with the bacterium such as fish, poultry, eggs and milk products (Arnone & Walling, 2007; Herikstad, Motarjemi & Tauxe, 2002). *Salmonella* spp. can survive in water bodies for a long time and are introduced into water sources by disposing human, animal and hospital waste in surrounding water bodies as has been reported in Ouagadougou (Katukiza et al., 2013; Wright, 1989). The contamination of water is a problem for humans because water contaminated with faeces is used to irrigate crop, which is another source of exposure and infection with the bacterium (Islam et al., 2004; Kusumangirum, Suliantari, & Dewanti-Hariyadi, 2012; Ongeng et al., 2011). *Salmonella* spp. are important causes of diarrhoea and are divided into typhoidal (cause typhoid fever) and non-typhoidal *Salmonella* spp. (NTS). Typhoidal *Salmonella* spp. include *S. typhi* and *S. paratyphi* (Gordon, 2008). The symptoms of typhoid fever include a high fever, diarrhoea and the formation of ulcers on the small intestine (Arnone & Walling, 2007). The NTS are known to cause salmonellosis, which leads to diarrhoea (Arnone & Walling, 2007; Gordon, 2008). NTS is distributed globally, both in developed and developing countries (Gordon, 2008). In sub-Saharan Africa, NTS is one of the leading causes of bacterial bloodstream infections (Dione et al., 2011; Feasey et al., 2012, Reddy, Shaw & Crump, 2010).

Along with *E. coli* O157:H7, *Campylobacter* and *Salmonella enterica*, *Shigella* is the most common foodborne pathogen. Infection with the bacterium occurs through contact with someone who is already infected with *Shigella* or through the consumption of contaminated food or water. The bacterium causes shigellosis, which leads to diarrhoea and bacillary dysentery as symptoms (Arnone & Walling, 2007; WHO, 2005).

Vibrio cholerae is a gram negative bacterium that mostly occurs in natural aquatic habitats and forms associations with zooplankton (Lipp, Huq & Colwell, 2002). The bacterium is the causative agent of cholera which is characterized by severely runny diarrhoea (Betley, Miller & Mekalanos, 1986). The MOA of *V. cholerae* entails colonization of the small intestine once inside a human host and producing the enterotoxin, cholera toxin (Norris, 1974; Rabbani & Greenough, 1990). Cases of cholera are less likely to occur in developed areas as these

areas have access to clean water. Instead, cases of the disease occur in areas of crowded housing and where there is a lack of a supply of clean water. Ali et al. (2015) estimated that approximately 1.4 billion people are at risk of developing cholera in endemic countries, which were mostly developing countries. Moreover, 2.8 million cases of cholera in these countries occur yearly and 87 000 individuals in non-endemic countries develop the disease. The research group estimated that 91 000 individuals die annually of the disease in endemic countries and that 25 000 individuals die annually in non-endemic countries. Furthermore, the disease is predicted to mostly affect children less than 5 years old (Ali et al., 2015).

Yersinia entercolitica is a gram negative rod which can take on the form of a coccobacilli or elongated bacillus morphology (Bibel & Chen, 1976; Bottone & Mollaret, 1977). At 25ºC, the bacterium is pertrichously flagellated whereas at 37ºC it has no flagella and is non-motile (Bottone & Mollaret, 1977). The bacterium occurs in the gastrointestinal tract of a wide range of animals from mammals, birds and cold blooded animals (Hurvel, 1989; Fredriksson-Ahomaa, Stolle & Korkeala, 2006). *Y. entercolitica* consists of pathogenic and non-pathogenic strains. The bacterium is responsible for causing yersiniosis, the main symptom of which is diarrhoea, however, the pathogenic strain *Y. enterocolitica* serogroup O:8 has been implicated in the diseases bacteraemia, meningitis and panopthalmitis. Other strains have been implicated in enteritis, enterocolitis, acute mesenteric lymphadentis, terminal ileitis and septicemia (Sonnenwirth, 1970; Bottone, 1999; Blei & Puder, 1993; Fredriksson-Ahomaa, Stolle & Korkeala, 2006). Infection with the bacterium occurs through the ingestion of contaminated pork, unpasteurized milk, dairy products or water (Arnone & Walling, 2007; Bottone, 1999; Fredriksson-Ahomaa, Stolle & Korkeala, 2006).

2.3.2. Pathogenic Waterborne Viruses

Adenoviruses are double-stranded DNA viruses that are 70 nm in diameter with a genome size of 36 000 base pairs (Horwitz, 2001). Furthermore, adenoviruses lack an envelope and may occur in raw sewage (Enriquez, Hurst & Gerba, 1995; Horwitz, 1996; Martin et al., 1994; Ginsberg, 2013). Adenoviruses cause conjunctivitis and haemorrhagic cystitis. Moreover, they infect the heart and the respiratory and gastrointestinal systems. Adenoviruses 40 and 41 are prevalent causes of gastroenteritis in children. (Horwitz, 1996; Martin et al., 1994; Ginsberg, 2013).

Astroviruses are a group of single-stranded positive RNA viruses that are 28-30 nm in diameter and belong to the family *Astroviridae* (Matsui & Greenberg, 1996; Monroe et al., 1993; Bosch, Pintó & Guix, 2014). The viruses obtained their name from their star-like

appearance as observed with electron microscopy (Madeley & Cosgrove, 1975). Astroviruses mainly infect young children and elderly individuals and are transmitted through the consumption of sewage polluted shellfish and water from contaminated streams (Cubitt, 1991; Kurtz & Lee, 1987). As with several other waterborne pathogens, astroviruses are associated with gastroenteritis and diarrhoea (Kurtz & Lee, 1987; Madeley & Cosgrove, 1975; Bosch, Pintó & Guix, 2014). Furthermore, the viruses can cause vomiting (Arnone & Walling, 2007; Bosch, Pintó & Guix, 2014).

Caliciviruses are also non-enveloped single-stranded RNA viruses, which belong to the family *Caliciviridae* (Hansman et al., 2007; Kroneman et al., 2013). They are divided into several genogroups, which include NoV GI, GII, GIV, SaV GI, GII, GIV, and GV among others. The aforementioned list of genogroups is all human pathogens. Caliciviruses can cause gastroenteritis, vomiting, diarrhoea, abdominal pain, nausea, fever and headache (Patel et al., 2008; Rock et al., 2002).

Enteroviruses are RNA viruses that lack an envelope and belong to the *Picornaviridae* family. Enteroviruses consist of polioviruses, Coxsackie group A, group B, human rhinoviruses, non-polioviruses and ECHO. Furthermore, they are divided into four genetic species, namely EV-A, EV-B, EV-C and EV-D (Ashkenazi & Melnick, 1962; van der Sanden, Koopmans & van der Avoort, 2013). Enteroviruses are resistant to several antiseptic agents and heat inactivation when stabilized by magnesium salts or divalent cations (Wallis & Melnick, 1961; Wallis & Melnick, 1962). Additionally, coxsackie viruses are resistant to antibiotics, chemotherapeutic agents and disinfectants such as ethanol (EtOH) and ether (Wallis, & Melnick, 1962). Usually, infection with enteroviruses does not cause any symptoms apart from gastrointestinal and respiratory illnesses in some cases. Immunocompromised individuals, infants, children and those lacking a humoral immune system; however, may suffer from paralysis/poliomyelitis, meningitis, hand-foot-and-mouth disease (HFMD) and cardiac disease (Chen & Shih, 2011; De Palma et al., 2009; McKinlay, Pevear & Rossmann, 1992; Norder et al., 2011; Rotbart, 1999). The sources of enteroviruses are water contaminated with faecal matter and sea food contaminated with the viruses, amongst others (Atmar et al., 1995; Green, 1998).

Hepatitis A is an RNA virus that lacks an envelope and belongs to the *Picornaviridae* family (Emini et al., 1985). Furthermore, the virus is similar in structure to astroviruses and noroviruses (Deboosere et al., 2010). Hepatitis A causes hepatitis, jaundice and fever. The virus can be obtained through the consumption of contaminated raw produce, dairy, shellfish and water (Arnone & Walling, 2007; Centers for Disease Control and Prevention, 1993;

Dalton et al., 1996; Deboosere et al., 2010). Alternatively, the virus is transmitted through person-to-person contact (Deboosere et al., 2010).

Hepatitis E is a positive strand RNA virus that has a genome size of 7200 bp. Furthermore, the genome of Hepatitis E is capped and polyadenylated (Emerson & Purcell, 2003). The virus belongs to the genus *Hepevirus* and to the family *Hepeviridae*. The virus causes jaundice, fever, and acute hepatitis and may lead to severe liver disease (Arnone & Walling, 2007; Emmerson; Panda, Thakral & Rehman, 2007). The virus can be obtained from pigs or person-to-person contact (Arnone & Walling, 2007).

The reovirus is a double-stranded RNA virus and is divided into ten segments, which are further divided into three size classes, namely small, medium and large (Bellamy et al., 1967; Gomatos & Tamm, 1963; Tyler & Oldstone, 2013). They mainly infect children and cause vomiting, diarrhoea, gastrointestinal and respiratory illnesses (Tyler & Fields, 1996; Tyler & Oldstone, 2013).

The rotavirus contains eleven double-stranded RNA particles and is divided into four serotypes namely G1P[8], G2P[4], G3P[8], and G4P[8] (Gouvea et al., 1990; Santos & Hoshino, 2005). The virus is associated with gastroenteritis, vomiting and is considered the leading cause of diarrhoea-related illnesses and death among infants and young children (Arnone & Walling, 2007; Guardado et al., 2004; Kane et al., 2004; O'Ryan et al., 2001; Salinas, 2004; Velazquez et al., 2004).

2.3.3. Pathogenic Waterborne Protozoa

Cryptosporidium parvum is a spore forming, single-cell intestinal parasite. The pathogen is the causative agent of cryptosporidiosis which is characterized by watery diarrhoea, abdominal cramping, nausea, vomiting and fever (Arnone & Walling, 2007; Current, 1983; Navin & Juranek, 1984; Carey, Lee & Trevors, 2004). The pathogenic protozoan also causes gastrointestinal illnesses and can infect both immunocompromised and immunocompetent individuals (Current, 1983; Jokipii & Jokipii, 1986; Navin & Juranek, 1984; Wolfson et al., 1985). Infection with the protozoan occurs from drinking water contaminated with faecal matter containing the protozoan (Current & Navin, 1986; D'Antonio et al., 1985; Gallaher, 1989; Hayes et al., 1989; Carey, Lee & Trevors, 2004). Additionally, the protozoan is resistant to most types of disinfectant (Angus et al., 1982, Campbell et al., 1982; Finch, 1993; Madore, 1987; Carey, Lee & Trevors, 2004).

Cyclospora spp. are spherical intestinal parasites that are 8-10 µm in size. The protozoan can infect both immunocompromised and immunocompetent individuals and is the causative agent of cyclosporiasis (Arnone & Walling, 2007; Ortega et al., 1993). The disease is associated with prolonged diarrhoea with nausea, weight loss and anorexia (Arnone & Walling, 2007; Naranjo, Sterling & Gilman, 1989; Ortega et al., 1993). The pathogen may be transmitted by consumption of contaminated water and vegetables (Arnone & Walling, 2007; Ortega et al., 1997).

Entamoeba histolytica, which was officially named in 1903, is an intestinal parasite with high infection rates in tropical and subtropical areas (Faust & Russel, 1964; Jackson, 1998). The parasite causes invasive amoebiasis which is characterized by prolonged diarrhoea with bleeding, abscesses of the liver and small intestine (Arnone & Walling, 2007).

Giardia lamblia is a flagellated unicellular intestinal parasite and is the causative agent of giardiasis which is characterized by diarrhoea, nausea and indigestion (Arnone & Walling, 2007; Barwick et al., 2000). The parasite has two major stages in its life cycle. When in the environment, giardia cysts remain resilient by being inert. Upon exposure to the acidic environment of the stomach, particularly in the proximal small intestine, the cysts develop into trophozoites which replicate and causes the symptoms associated with giardiasis. After being exposed to the biliary fluid, some of the trophozoites form cysts again in the jejunum and are excreted with the faeces (Adam, 2001). The cycle is completed upon infection of a new host which occurs when water and food products contaminated with faecal matter are consumed (Adam, 2001; Barwick et al., 2000).

Naegleria fowleri is an amoebaflagellate that causes primary amoebae meningoencephalitis. The disease usually affects children and young adults and can leads to the inflammation of the brain and the meninges (Arnone & Walling, 2007). The pathogen can be acquired from swimming in or consuming water contaminated with the parasite (Barnett et al., 1996).

Estimating the approximate numbers of these microorganisms is a challenge as the testing for these pathogens is expensive. Resultantly, the indicator organisms are tested for to get an estimation of the persistence of these enteric pathogens (Crane, Westerman & Overcash, 1980). The numbers of *Cryptosporidium* and *Giardia lambia* cysts correlate to the level of faecal contamination of water (Rosen, 2000).

2.4. Water Purification

Water is contaminated by various sources such as nutrient, chemical and microbiological pollution. Moreover, it is also sullied by domestic, agricultural and industrial wastewater (Wang & Chen et al., 2011). The water crisis across the globe is severe as access to clean water is a luxury that many do not have. To circumvent this problem of contaminated water and the diseases associated with it, several ways to purify water exist. These methods include boiling, chlorination, thermal treatment with solar radiation, ozonation, UV disinfection, alum and iron coagulation, sedimentation, activated carbon, ion exchange disinfection and filtration.

2.4.1. Alum and iron coagulation

Conventionally, solids in water may be removed through sedimentation followed by filtration (Faust & Aly, 1998). However, there are often small particles that remain that may settle too slowly and pass through with the filtrate during filtration (Faust & Aly, 1998). Coagulation entails the process of these particles clumping together so that they may be more readily removed (Randtke, 1998). However, it is difficult for these particles to naturally clump together as they often have a negative charge (Randtke, 1998). The role of a coagulant, such as iron or aluminium is to coat these negatively charged particles and neutralize their charges as the coagulants have a positive charge (Faust & Aly, 1998; Randtke, 1998). Once the charges are neutralized, it is easier for the particles to coagulate and to be removed through sedimentation and filtration. Alum and iron coagulants are commonly used to clear the turbidity in raw water (Matilainen, Lindqvist & Tuhkanen, 2005).

2.4.2. Charcoal and activated carbon

Charcoal and activated carbon are mainly used to remove organic pollutants from water; however they do adsorb microbes (AWWA, 1999). The disadvantage of the use of charcoal and activated carbon is that dissolved organic matter quickly occupies the adsorption sites and provides a surface for bacteria to colonize and form biofilms. To prevent the formation of bacterial colonization and reproduction, functionalisation of the activated carbon with silver can be employed; however this may be expensive (Agrawal & Bhalwar, 2009).

2.4.3. Chemical Treatment: Chlorination

The treatment of water with chlorine is very effective against bacteria except mycobacteria (Sobsey, 1989). Contact with a few mg/mL of the reagent for 30 min causes a 99.99 % reduction in growth of enteric bacteria and some viruses. However, the reagent is ineffective against protozoa such as *C. parvum* and *G. lambia*, and viruses such as Hepatitis A (Angus et al., 1982; AWWA, 1999; Campbell et al., 1982; Finch, 1993; Madore, 1987; Mbithi, Springthorpe & Sattar, 1990). Furthermore, the efficiency of the reagent is reduced in turbid waters (Agrawal & Bhalwar, 2009). An additional disadvantage of chlorination is that the chemical reacts with the residual natural organic matter within the treated water. Examples of by-products from water treated with chlorine are trihalomethanes and haloacetics among others. These by-products are potentially carcinogenic and in some instances, can cause miscarriages and damage to the nervous system (Swietlik et al., 2004; Zularisam, Ismail & Salim, 2006).

2.4.4. Ion exchange disinfection

This method employs tri-iodide or penta-iodide exchange resins to inactivate waterborne viruses, protozoa and bacteria (Naranjo, & Chaidez, 1997). The mode of action of iodine entails oxidising the cell constituents of microorganisms and inactivating proteins through iodination (Willey, Sherwood & Woolverton, 2011).

2.4.5. Ozonation

Treatment with ozone is also a very effective method to disinfect water. Ozone acts as an oxidising agent which can inactivate bacteria 3.125 times faster than chlorine (Long, 1998). Other microorganisms inactivated by ozone are algae and viruses (Camel & Bermond, 1998; Glaze, 1987). In addition to the inactivation of bacteria, ozonation can also serve to eliminate foul taste, odour and micropollutants through oxidation. Ozonation can also be used to decolour heavily polluted waters (Camel & Bermond, 1998; Gottschalk, Libra & Saupe, 2000; Hoigné, 1998). The disadvantage to ozonation is that ozone is very reactive and forms chemical by-products with the organic matter in the water (van Leeuwen, 2000; Hyung Kim & Yu, 2005).

2.4.6. Thermal treatment and boiling

Heating to pasteurization temperatures, namely 60ºC for 10 min or alternatively 55ºC for several hours can inactivate most pathogens, such as viruses, bacteria and the protozoa *C. parvum*, *G. lamblia* and *E. histolytica*. However, boiling is the preferred method of killing pathogens as it is more effective against all classes of waterborne pathogens, even those that occur in highly turbid waters (Sobsey & Leland, 2003). The disadvantage of boiling, however, is that the requirement of electricity or fuel that may not be affordable or accessible to individuals that dwell in areas of heavily polluted waters (Sagara, 2000).

2.4.7. Thermal treatment with solar radiation

Similar to the solar water disinfection process (SODIS) system, some households sterilize their water by using transparent plastic bottles that are painted black on one side and exposing the filled bottles to the sun. The principle behind thermal treatment with solar radiation is to expose the water to sunlight for several hours so that the water may be heated to 55ºC. This consequently leads to the inactivation of the waterborne pathogens present in the water inside the bottle (Joyce et al., 1996).

2.4.8. UV Disinfection

Disinfecting water with UV light may occur through exposing water to a UV lamp or to direct sunlight in transparent bottles. UV can inactivate bacteria and chlorine-resistant protozoa such as *C. parvum* oocysts and *G. lamblia* cysts (Agrawal & Bhalwar, 2009). UV radiation, particularly around the wavelength 260 nm causes thymine-thymine dimerization of DNA. This prevents proper replication and transcription of DNA and RNA respectively, inevitably killing the bacteria (Willey, Sherwood & Woolverton, 2011). A drawback to this method of water purification is that UV does not penetrate water well (Willey, Sherwood & Woolverton, 2011). Furthermore, UV treatment is ineffective in sterilizing turbid water as the dissolved organic matter absorbs UV or shields the bacteria thus decreasing the quantity of UV that the bacteria are exposed to (Agrawal & Bhalwar, 2009).

2.5. Water Filtration

In the context of this study, filtration is a process that entails the removal of suspended particulates from water by the application of pressure and vacuum to a porous membrane system to push the water through (Ramakrishna et al., 2010). The process offers an efficient and sustainable method of purifying water. Furthermore, filtration provides superior water quality in that in the example of RO, it includes the removal of organic pollutants, particles, and inorganic compounds (Zularisam, Ismail & Salmim, 2006). Additional advantages of filtration over conventional water treatment methods are that it has a small footprint, compact module, is environmentally friendly and can handle wide fluctuations in feed quality (Zularisam, Ismail & Salmim, 2006).

The process relies primarily on the principle of size exclusion of different components and the application of pressure to a membrane (Vickers, & Freeman, 2005; Wang, & Chen et al., 2011). For example, some filtration membranes can separate macromolecular components such as starch and proteins whereas other membranes separate extremely small particles such as monovalent ions. Filtration membranes operate in two modes namely cross-end and dead-flow, which are depicted in Figures 2.4. A and B. In dead-flow, the feed is forced vertically through the membrane and the rejected matter increases in the feed (Figure 2.4. A). Cross-flow filtration entails passing the feed horizontally through the membrane (Figure 2.4 B) (Ramakrishna et al., 2010). There are different types of membrane filtration for the range of sizes, namely RO, nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF) which are discussed below and summarised in Table 2 (Wang & Chen et al., 2011).

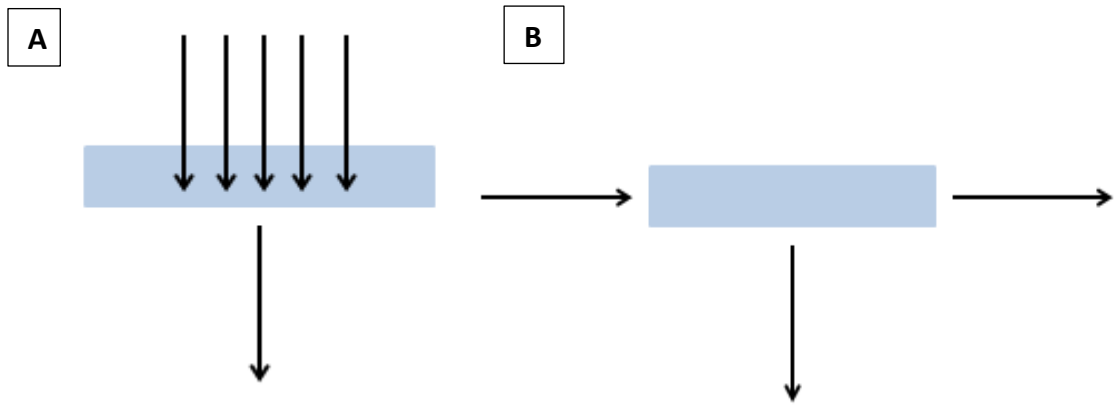


Figure 2.4. A: Cross-end flow**. B:** Dead-end flow (Adapted from Ruiz-García, A., Melián-Martel, N., & Nuez, I; 2017).

2.5.1. Microfiltration (MF)

The MF process has the largest pore sizes in comparison to the other types of pressure-driven filtration processes and is used as a particulate filter (Marshall, Munro, & Trägårdh, 1993). Microfiltration membranes can be made of poly (acrylonitrile), poly (vinylidene fluoride), cellulose-acetate, cellulose nitrate blends, nylons, and poly tetra-fluoroethylene or polyacrylonitrile-poly vinyl chloride polymers (Baker, 2004). MF makes use of low pressure of between 0.2-2 bars and can sieve out particles within the range of 0.04 – 0.2 µm (Pearce, 2011; Ramakrishna et al., 2010). As such, MF is used for the separation of microorganisms such as bacteria, algae and protozoa as well as matter such as proteins, emulsions, colloids and suspended solids (Pearce, 2011). In industry, MF is used for filtering out bacteria from wastewater, separation of water and oil emulsions, the sterilization of beer and wine, the clarification of fruit juices and fermentation broth and for the recovery of biomass (Echavarria et al., 2011). MF is also often used as a pre-treatment method for RO and NF (Pearce, 2011; Vickers & Freeman, 2005).

Despite its various applications, MF membranes are susceptible to fouling by the matter that it is used to separate (Khulbe et al., 2000). Fouling can be described as the accumulation of the matter to be separated on the membrane and within its pores through microbial or solute adhesion and gel layer formation (Marshall, Munro & Trägårdh, 1993). Ultimately this affects the efficiency of the membrane (Khulbe et al., 2000; Marshall, Munro & Trägårdh, 1993). It has been suggested that the fouling of microfiltration membranes occur in three phases. The first phase is considered to be caused by colloid and bacterial fouling or the compaction of the membrane. During the second phase, the flux decreases and a concentration gradient of the retained proteins form near the membranes. The third phase entails a continued decrease in the flux of filtration and membrane fouling occurs (Fane & Sep, 1983). The filtration of proteins is also hindered by fouling of the MF membranes and also occurs in a three step process. Firstly, the proteins rapidly deposit on the surface of the membrane and at the pores causing an increase in the resistance of the membrane. Secondly, a second deposition occurs on top of the first layer and further causes an increase in membrane resistance. Lastly, the pores of the membrane are inevitably covered (Marshall, Munro & Trägårdh, 1993).

Although MF is used to filter out microorganisms and the abovementioned matter, the filtration process cannot sieve out viruses, ions, organic and inorganic compounds (Vickers & Freeman, 2005).

2.5.2. Ultrafiltration (UF)

Like MF, UF is another example of a particulate filter that uses a porous membrane. The development of UF membranes occurred in Germany in the 1920s and was subsequently used in industry (AWWA, 2007; Pearce, 2011). The early UF membranes could filter out particles with 10 – 30 kDa. Fine UF membranes were used for wastewater treatment. (Pearce, 2011). Modern UF membranes can remove particles within the size range 0.002 – 0.02 µm and are used to remove viruses, colloids, pyrogen, particulates and bacteria (Maher, 2012; Yonge, 2012). Furthermore, UF membranes are also made of the same polymers as microfiltration membranes (Baker, 2004). UF is used in milk processing, the clarification of fruit juices and wastewater treatment (Cassano et al., 2008; Galaverna et al., 2008; Pearce, 2011; Ramakrishna et al, 2010; Timmer & van der Horst, 1998).

Similar to MF, UF membranes are also susceptible to fouling. Therefore, to prevent biofouling, ultrafiltration membranes are treated with chloramines or chlorine (which is used to treat membranes that are tolerant to chlorine such as those made of polysulfone) (Bartels et al., 2005; Park et al., 2008).

2.5.3. Nanofiltration (NF)

NF was developed in the late 1970s as another version of RO (AWWA, 2007). The membranes are made of cellulose acetate blends or polysulfone or polyamides and are designed to have a molecular weight cut off between 300 and a 1000 (Baker, 2004). Unlike MF and UF, NF membranes are semi-permeable and not porous (Hong & Elimelech, 1997). The pressure used by NF is in the range of 4-8 bars (Hong & Elimelech, 1997). NF occurs in three stages namely pre-treatment, membrane filtration and post-treatment (Pearce, 2011). Pre-treatment often involves the addition of acid and scale inhibitor to prevent soluble salts from precipitating. This is followed by filtration in a 5-20 µm cartridge to prevent fouling of the membrane. Post-treatment entails aeration, degasification, adjustment of pH, fluoridation, disinfection and the addition of anti-corrosive agents. NF process is used for the removal of natural organic matter, disinfection by-products, hardness, colour, inorganic and volatile compounds (Hong & Elimelech, 1997).

2.5.4. Reverse Osmosis (RO)

The development of RO membranes occurred in the 1960s for desalination (Pearce, 2011). Similar to NF, RO occurs in the three stages mentioned above (AWWA, 2007). The principle of osmosis entails the migration of solvent from a less concentrated solution to a more concentrated solution across a semi-permeable membrane. In RO, pressure is applied to the more concentrated solution to prevent osmosis from occurring. Instead, solvent from the more concentrated solution migrates to the less concentrated solution (Maher, 2012). RO employs membranes with pore sizes below 0.001 µm and operating pressures between 20-80 bars. Additionally, membranes used for RO are made of the same polymers used for NF (Baker, 2004).

RO is used for the removal of solutes, salt, metal ions and other dissolved substances except volatile organics (Sagara, 2000). Due to its ability to remove the abovementioned dissolved substances, RO is applied in the following fields: desalination, biotechnology, textile, pulp and paper industry, dairy and mine wastewater and the beverage industry, among others (Häyrynen et al., 2008; Juang et al., 2008; Pearce, 2011; Vourch et al., 2008; Zhang et al., 2008). Separation of solutes occurs as a result of size exclusion, charge exclusion, physical and chemical interactions between the solutes and the membrane (Bellona & Drewes, 2005; Mondal, Hsiao & Wickramasinghe, 2008; Radjenovic et al., 2008). The membranes most commercially available are the spiral-wound and hollow fiber membranes, which have an extremely high packing density, allowing for elevated levels of permeate production. The disadvantage; however, is that hollow fiber membranes are more susceptible to fouling than spiral-wound membranes (Gabelich et al., 2005).

Often, the feed water that is to be filtered is pre-treated by first subjecting it to microfiltration and ultrafiltration. This is done to prevent fouling with colloidal matter (Malaeb & Ayoub, 2011). However, RO water membranes are also susceptible to chemical fouling (Pomerantz et al., 2006). This is known as scaling and it occurs when high levels of calcium, silica, phosphate, chlorine, chloramines, carbonate and other ions deposit onto the membrane (Pomerantz et al., 2006). However, scaling can be managed by the application of antiscalants, reversing the feed flow and reducing the pH and recovery rate (Bartman et al., 2009; Pomerantz et al., 2006).

Table 2.2: Summary of the different types of filtration processes

	Microfiltration	**Ultrafiltration**	**Nanofiltration**	**Reverse Osmosis**
Particle(s) removed	Colloids, bacteria, algae, protozoa, proteins, emulsions, suspended solids	Colloids, viruses, bacteria, particulates	Dissolved organic pollutants, divalent ions	Solutes, salt, metal ions, monovalent species and other dissolved substances
Operating pressure (bars)	0.2-2	1-5	4.8-8.2	20-80
Size excluded (μm)	>0.04-0.2	>0.002-0.02	>0.01	>0.001
Type of Material	Poly(acrylonitrile), poly(vinylidene fluoride), cellulose-acetate, cellulose nitrate blends, nylons, and poly (tetra-fluoroethylene) and poly(acrylonitrile)-poly(vinyl chloride)	Poly(acrylonitrile), poly(vinylidene fluoride), cellulose-acetate, cellulose nitrate blends, nylons, and poly (tetra-fluoroethylene) and poly(acrylonitrile)-poly(vinyl chloride)	Cellulose acetate blends or polysulfone and polyamides	Cellulose acetate blends or polysulfone and polyamides
Disadvantages	• Susceptibility to fouling which leads to a decline in flux	• Susceptible to fouling • Unable to provide enhanced selectivity and concentration polarization	• Very expensive • Susceptible to fouling and oxidative chemicals such as chlorine • Uses higher energy than MF and UF processes • Water requires pre-treatment	• Susceptible to fouling • Performance of the membranes decrease as a result of pressure compaction

Advantages	• Provides improved water quality • Decreased maintenance • Filters out bacteria, algae, protozoa, suspended particulate matter, colloidal matter and proteins	• Makes use of low operating pressure • Provides improved water quality • Filters out bacteria, viruses, pyrogens and colloidal matter	• Does not make use of chemicals • Removes viruses and organic matter • Reduces the turbidity of water • Removes natural organic matter, disinfection by-products, hardness, colour, inorganic and volatile compounds	• Highly efficient in the selective rejection of minerals • Able to conduct separation at room temperature • Has a high permeability for water • Able to remove solutes from water

2.6. Point of Use Filters

Although effective, the filtration systems mentioned above are rather costly and are mostly applied in centralised water treatment systems (Pianta et al., 2000; Sagara, 2000). There are numerous individuals that are limited to making use of contaminated ground or surface water that cannot afford or do not have access to the filtration systems mentioned above. In response to this problem, various POU filters have been developed as a cost-effective and portable alternative for the purification of water (Oyanedel-Craver & Smith, 2008). There are various kinds of point of use filters, with several examples given below:

2.6.1. Activated Carbon Filters

Activated carbon is conventionally incorporated into filters in its granular or powder form. It has been shown to be very effective in removing organic pollutants due to its porous nature; however, it is less effective in removing bacteria from water sources. For example, some studies have shown that the bacterial counts in the effluent are more than in the influent (Bell et al., 1984; Wallis, Stagg & Melnick, 1974). However, Snyder et al. (1995) demonstrated that the treatment of ground water with powdered activated charcoal improved the water

quality, providing contrasting results (Snyder et al., 1995). One of the disadvantages to activated carbon filters is that they are easily colonized by heterotrophic microorganisms (Molloy et al., 2007).

2.6.2. Colloidal-Silver Impregnated Ceramic

The use of ceramic filters dates back to the late 1980s and early 1990s. The filters were primarily used in third world areas where local labour is used to manufacture them. Made in various types of shapes, ceramic filters are primarily made of clay and other materials such as sawdust, flour or rice husks. The mixture is then subjected to a filter press, subsequently air dried and then fired in a kiln to form the final product. The burning process combusts the sawdust, flour and rusks to make the filters porous and thus permeable to water (Kallman, Oyanedel-Craver & Smith, 2011). For colloidal-silver impregnated ceramic filters, the filter is painted with or immersed in a colloidal silver solution to confer antimicrobial properties after processing through the kiln. For some of the ceramic filters, antifouling can be maintained by regularly cleaning the filters with a brush (Kallman, Oyanedel-Craver & Smith, 2011; Oyanedel-Craver, & Smith, 2008; http://www.wsp.org/UserFiles/file/926200724252_eap_cambodia_filter.pdf).

The mechanism by which colloidal-silver impregnated filters purify water may include a combination of filtration, sorption to the clay and inactivation by the silver (Oyanedel-Craver, & Smith, 2008). However, ceramic filters on their own without the colloidal silver have been shown to be very effective but include silver to enhance efficiency (Kallman, Oyanedel-Craver & Smith, 2011). The advantages of using colloidal-silver impregnated ceramics are that they are made from readily available materials; can be used in different climates and is socially accepted in many areas; does not confer an undesirable taste to the water; are cost effective and not only remove pathogens but reduce the turbidity of contaminated water (Oyanedel-Craver, & Smith, 2008). The disadvantage of ceramic filters, however, is that they do not remove chemical pollutants (Kallman, Oyanedel-Craver & Smith, 2011; Oyanedel-Craver, & Smith, 2008; http://www.wsp.org/UserFiles/file/926200724252_eap_cambodia_filter.pdf).

2.6.3. Life Straw

Life straw is a highly efficient, portable water filter in the shape of a straw. The technology was introduced to the market in 2005 and designed for the purification of contaminated water

by individuals who lack access to clean water, particularly those living in areas of developing countries that lack piped municipal water or those that live near disaster stricken areas (http://www.lifestraw.com/our-story/). The filter functions like a normal straw in that as water is pulled up, bacteria are removed. This is achieved by the microscopic pores within the hollow fibre membrane of the straw that trap contaminants as water is drawn up the straw (https://www.lifestraw.com/pages/how-our-products-work). The filter can purify at least 700 litres of water. Moreover, the filter removes 99 % of bacteria and viruses (Time, 2005).

2.6.4. Sediment Filter

Sediment filters are primarily used to remove large particulate matter that occurs in water. They consist of screens, meshes, tightly packed fibers or a porous matrix that excludes large particulate matter by size. Sediment filters are often used to pre-treat water before subsequent treatment with UF or RO. Sediment filters are limited in that they have a filtration capacity and once it is reached, the particulate matter found in water may seep through with the effluent. An additional disadvantage of sediment filters is that microorganisms can grow on the filtration medium of sediment filters (Sagara, 2000).

2.6.5. Slow Sand Filtration

The technique of slow sand filtration has been used for centuries and provides an inexpensive method of purifying water. There are two types of slow sand filtration, namely pressure and gravity influenced sand filtration. A pressure filter comes in the form of a closed vessel, which is filled with sand or other granular material and the water is forced through the sand under a pressure system. Pressure systems are more commonly used in industrial settings; however some households have pressure sand filters installed (Jenkins, Tiwari & Darby, 2011; Huisman, 1974).

A gravity filter consists of an open container made of concrete partially filled with clean sand. Water is purified by passing through the sand and is in the process filtered through the sand via gravity. Gravity filters are divided into slow and rapid filters. The slow gravity filters make use of fine sand and require less cleaning than rapid filters (Haig et al., 2011). Rapid filters on the other hand work 20 – 50 times faster than the slow filters and make use of coarse material. On the surface of some sand filters is a filter skin that is mainly organic in nature and consists of protozoa, plankton, diatoms, rotifers, algae and bacteria. These microorganisms are there to break down and digest organic matter that occurs in raw water,

therefore removing organic contamination. Once the water passes through the filter skin, it makes contact with the sand and bacteria and viruses attach to the sand through mass attraction or electrical forces (Bar-Zeev et al., 2011; Huisman, 1974, Aslan & Cakici, 2007).

A laboratory scale study making use of sand filters demonstrated that they are effective at removing bacteria and viruses. A study by Jenkins et al. (2011) showed that the filter led to a 63 – 99 % removal of faecal coliforms and *E. coli*, and a 1.14 log reduction of echoviruses. Furthermore, in the study, the filter led to a log reduction higher than 5 for the protozoa *G. lamblia* and 99.8 % removal of *Crytosporidium* oocysts (Jenkins, Tiwari & Darby, 2011).

The advantage of slow sand filtration is that it is cost effective as it can be assembled through locally available materials. The disadvantage of slow sand filtration is that it is a very slow process and depending on how heavily pollute the water is, it has to be used in conjunction with another form of water purification such as chlorination (Jenkins, Tiwari & Darby, 2011; Huisman, 1974).

2.6.6. Spool Filters

A spool filter consists of a core surrounded by fiber strings that are 15 -20 µm in diameter. The fibers are teased to form a pattern when wound around the core. Proper brushing of the fibers provides a greater surface area for adsorption. The fibres may also be coated with activated carbon to adsorb chemicals. Spool filters remove particulate matter by straining, which describes the process of removing or trapping particulate matter that is too large to penetrate the pores of a sieve (Sagara, 2000; Williams, 1992).

2.7. Nanofibers

2.7.1. Application of Nanofibers in Water Filtration

Due to their ideal properties, nanofibers are used in various applications namely, protective clothing, wound dressing, drug delivery, artificial organs, tissue engineering scaffolds, catalysis, enzyme carriers, affinity membranes and filtration among others (Ahn et al., 2006; Gopal et al., 2006; Jia et al., 2002; Kedem et al., 2005; Ma, Kotaki & Ramakrishna, 2005; Pham, Sharma & Mikos, 2006). These properties include a high surface area to volume ratio, high specific surface area, small pore size, good interconnectivity of the pores, adaptable membrane thickness and low basis weight. (Barhate & Ramakrishna, 2007; Botes & Cloete, 2010; Feng, et al., 2013; Ramakrishna et al., 2010).

In addition to these uses, the application of nanofibers in water filtration has been investigated. In this scenario, nanofibers can be woven or assembled to form the semi-permeable membranes mentioned above for the filtration of water. Studies have already shown that nanofibrous membranes have high flux rates and low transmembrane pressures (Baker, 2004). Additionally, nanofibrous membranes offer a higher permeability than conventional filtration membranes and have high filter efficiencies due to the small diameters of the nanofibers (Daels et al., 2010; Thavasi, Singh & Ramakrishna 2008).

Ramakrishna et al 2010. showed that electrospun nanofibers can be applied as water filtration membranes to remove microparticles at different concentrations, performed similar to MF membranes (Ramakrishna et al., 2010). In another study Nylon 6 nanofibers were electrospun to form a membrane through which demineralized water was filtered in a dead end system (Daels et al. 2010). The pressure range used was 0.03 – 0.15 bars, which is very close to the pressure range used for microfiltration. The group found that the membrane had a high clean water permeability value (6651 $m^{-2} \cdot h^{-1} \cdot bar^{-1}$) which allows for the filtration of large volumes of water (Daels et al., 2010). This membrane was also used in a bioreactor and an activated sludge membrane bioreactor (AS-MBR) where it was submerged in a mixture of synthetic water and activated sludge. The membrane had a very good removal efficiency for the turbidity (99 %), total suspended solids (99 %), chemical oxygen demand (94 %) and ammonium. The drawback to this setup, however, was that fouling occurred very rapidly during the filtration process, which suggested that the membrane may not be efficient in filtering water heavily polluted with particulate matter (Daels et al., 2010). To study the filtration efficiency of the membrane in the absence of fouling, the group added the cationic polymer MPE50 to the solution. The polymer hinders biofouling by adsorbing onto microbial flocs and neutralizing the negative surface charge. Although the MPE50 reduced fouling, it decreased the total removal efficiency. An alternative strategy to reduce fouling, which was applied in the same study, was to apply the membrane in a trickling filter system. The removal efficiencies for the different abiotic factor of the trickling filter membrane bioreactor (TF-MBR) were comparable to those of the AS-MBR, except for the removal of the ammonium. An additional advantage of TF-MBR is that it can run for a period of over 80 days (Daels et al., 2010). Therefore, it appears that the application of nanofibrous membranes in trickling filter systems seems to be the most optimal for the filtration of heavily polluted water.

To improve the efficiency of purifying water, nanofibrous membranes may also be functionalized with an antimicrobial. In a different study by Daels et al., a nanofibrous membrane of polyamide was functionalised with silver nanoparticles, poly [(dimethylimino) (2-hydroxy-1.3-propanedily) Chloride] (WSCP), 2,2-dibromo-3-nitrilopropionamide (DBNPA),

thiocyanic acid,(2- benzothiazolylthio)methyl ester (Busan 72) and bronopol as the antimicrobials (Daels et al., 2011). In the study, the non-functionalised membranes managed to remove 2.2 $\log_{10}$ CFU/100 mL of bacteria in hospital wastewater whereas the functionalised with the antimicrobials had removal efficiencies of between 3.2 - 5.6 $\log_{10}$ CFU/100 mL (with the membrane functionalised with WSCP performing the best) (Daels et al., 2011).

To further demonstrate the fact that functionalization of nanofibers enhances the efficiency with which they inactivate and remove bacteria, a separate study by Yao et al. (2008) demonstrated that treated polyurethane (PU) nanofibers led to a 99.9 % inactivation of *Staphylococcus aureus* and *E. coli* after contact with the bacteria for 4 h. For functionalization, the nanofibers were exposed to argon plasma to incorporate oxide and peroxide functional groups. Subsequently, the PU nanofibers were irradiated with UV to generate poly (4-vinylpyridine) into the PU fibers. Then, the PU nanofibers were functionalized with hexylbromide transmitting antimicrobial properties to the nanofibers (Yao et al., 2008). Despite the efficacy of the nanofibers, the application of such a treatment system would be impractical in that consumers will seldom wait 4 h for the treatment of water before consumption.

An additional study that investigated the application of functionalized nanofibers in water treatment was conducted with polycarbonate (PC) nanofibers functionalized with benzyl triethylammonium chloride (BTEAC). The study showed that the BTEAC functionalized polycarbonate nanofibers led to complete inhibition of *S. aureus* ATCC6538, *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 4352. In addition to remarkable antimicrobial efficiency, the nanofibers led to a 99.97 % filtration efficiency by removing particles bigger than 0.3 µm in size (Kim et al., 2007).

Although the prospect of using nanofibers in water filtration appears promising, the electrospinning of nanofiber membranes on a large scale is not that reproducible and controllable yet. Additionally, the process of producing nanofiber membranes is rather costly. Further research should focus on these improving reproducibility and methods to reduce production cost (Daels et al., 2011).

2.7.2. Fabrication of Nanofibers

The methods used to fabricate nanofibers include the modular melt blowing technique, drawing, multicomponent fibre spinning, self-assembly of biomolecules and more commonly electrospinning (Botes & Cloete, 2010; Matsumura, Uemura & Mihara, 2004; Ward, 2005).

The drawing technique produces very long and thin fibers one by one. During the drawing process, a polymer solution is pulled from a microdroplet or micropipette and the solvent evaporates. After the pulling, the polymer solidifies into a fiber. The substrates for drawing should be able to undergo intense deformation and should be able to withstand the stresses applied during the pulling. (Ondarcuhu & Joachim, 1998).

The melt blowing technique was developed in the 1950s at the Naval Research Laboratory (McCulloch, 1999). The technique relies on a die developed by Wente with several orifices and slots (Wente, 1956). The intention behind developing the technique was to create sub-micron fibers that could clear radioactive particles in the upper atmosphere (McCulloch, 1999). However, a research group at Exxon modified the design and were the first to produce melt blown microfibers on a commercial scale (Harding, Keller & Buntin, 1974; McCulloh, 1999). During the melt blowing procedure, a polymer solution is forced out of an orifice die through the application of air to the system. Subsequently, the polymer is drawn down with a jet of hot air and fibers are formed and deposited on a rotating collector to form a web (Bresee & Ko, 2003). The melt spinning technique is similar to the melt blown and entails forcing out a polymer solution and then drawing it down. During the drawing process, the polymer solidifies to form strong fibres however the minimum diameter of the fibers is 10 µm (Grafe).
The advantage of the technique is that it provides larger quantities of fiber at a lower cost than electrospinning (Barhate & Ramakrishna, 2007). However, there are disadvantages to the modular melt blowing technique. Firstly, the fabrics produced by the technique are weak and require a support or layering of multiple fabrics. This increases the cost of the fabrics making them expensive (Ward, 2005). Secondly, the modular melt blowing technique cannot produce fibers as fine as those produced by electrospinning (Barhate & Ramakrishna, 2007).

Electrospinning is the most versatile of the methods to produce nanofibers. Using the technique, structures ranging from nonporous polymer coatings to macroporous fibrous structures can be produced. However, the technique is uneconomic in that it requires recovery of the scale solvent from the dilute air stream on a massive scale (Ward, 2005). Electrospinning entails the application of an electric field to a polymer solution which creates a jet that inevitably forms fibers and collects onto a surface. The diameter of the fibers

produced by electrospinning may be less than 3 nm and depends on the concentration of the polymer solution. The less concentrated a solution, the thinner the fibers will be (Huang et al., 2003; Jaroszczyk et al., 2005).

There are two types of electrospinning processes: one that makes use of a needle and a needleless setup. The first patent for a process similar to needle based electrospinning was issued in 1902 and 1934, however, at the time, the application thereof did not appear useful. It was only until the mid-1990s when the potential that nanofibers have in various fields was discovered that electrospinning became a popular method of processing polymers (Barhate & Ramakrishna, 2007; Cooley, 1902; Formhals, 1934; Reneker & Chun, 1996). Electrospinning is compatible with thermoplastic polymers and for the needle-based process, the basic set up required is a high voltage power supply, a syringe container, a needle nozzle and a counter electrode collector (Li & Xia, 2004; Liang et al., 2007). For the electrospinning process, a polymer solution is prepared by dissolving a polymer in the desired solvent. The solution is then inserted into a capillary tube (Huang et al., 2003). The electrospinning process entails applying a high electric field formed between the needle outlet and the electrode on the opposite side to the needle outlet. From the needle outlet, the polymeric solution is ejected and when a droplet of the solution is exposed to the electric field, it assumes a cone-like shake referred to as “Taylor Cone” (Li & Xia, 2004; Taylor, 1969). When the electric force reaches a critical value, the polymeric solution is ejected and forms a jet. The presence of the electric field and the repulsion of the charges within stretch the jet into a long filament (Niu & Lin, 2012). Eventually, when the electric force surpasses the surface tension of the polymer solution, the solvent evaporates and the filamentous polymer solidifies into fibers, which are ejected and deposited onto the collector (Huang et al., 2003). The electrospinning process is summarised in Figure 2.5.

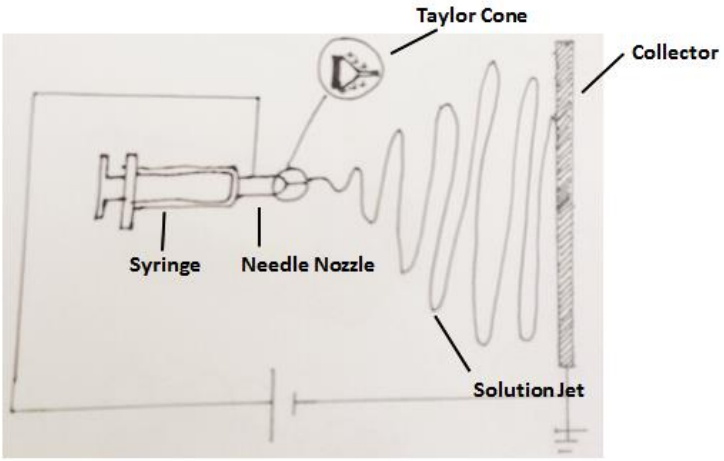


Figure 2.5: The electrospinning process adapted from Niu & Lin, 2012

The patent for a procedure describing needleless based electrospinning was released in 1979, many years later than that for the needle-based electrospinning process. The procedure entails using electrostatic force to spray a polymeric solution, which is processed by a ring spinneret and collects onto a support to form a fleece (Simm, 1979). The fibers produced by this procedure could reach sizes below 1 µm; however, several years later, the procedure was customised for nanofibers (Simm, 1979; Niu & Lin, 2012). Since then, other patents released have made use of rotating spinnerets in the production of fibers. For example, in 2004, another needleless-based electrospinning procedure was released. The process makes use of magnetism to create spikes from the solution and proceeds by the conventional electrospinning process (Yarin & Zussman, 2004). The following year, a rotating roller was invented to generate fibers for the mass spinning of nanofibers (Jirsak et al., 2005). Further advances in the field of needless-based electrospinning include the employment of air bubbles to initiate the electrospinning in 2007 and the use of a conical wire coil to generate fibers in 2009, which proves to be a more efficient method of generating nanofibers than the needle-based electrospinning (Liu & He 2007; Wang & Niu et al., 2009). Other types of fiber generators developed later on include spinnerets such as metal plate, splashing spinneret, ball, disc, coil, beaded chain, rotary cone, cylinder and bowl edge (Figure 2.6) (Lu et al., 2010; Tang, Zeng & Wang, 2010; Thoppey et al., 2010; Thoppey et al., 2011; Wu, 2010).

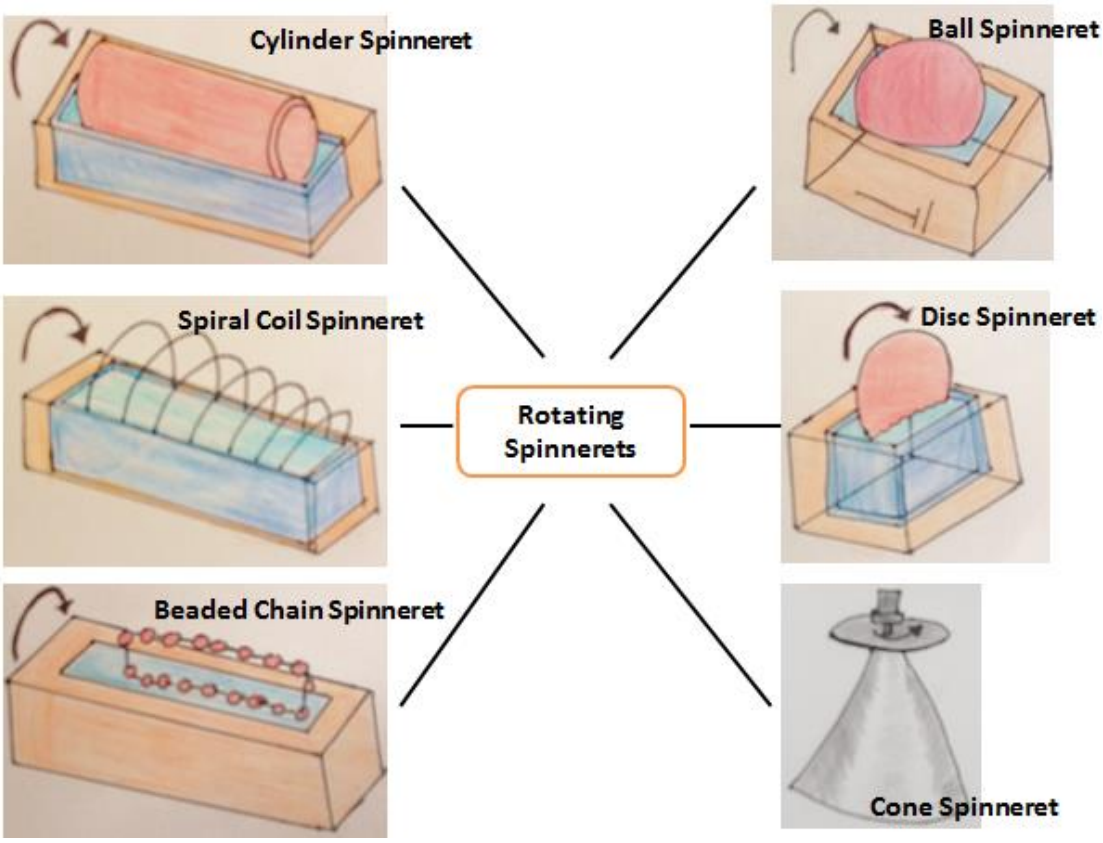


Figure 2.6: The different types of rotating spinnerets as adapted from Niu & Lin, 2012.

Electrospinning with spinnerets involves placing the apparatus into the polymer solution and rotating it. This forms a layer of the polymer solution around the spinneret from which conical spikes arise. Thereafter, a high voltage is applied and Taylor cones are formed from the gathering of the electric force by the spikes. Eventually, these cones are then stretched out to form jets, which then form fibers (Niu & Lin, 2012). As an alternative to rotation, stationary spinnerets may be used where a magnetic field, gravity or high pressure gas flow is used to disturb the polymer solution and form fibers by stretching the perturbations of the solution (Liu Y; Yarin & Zussman, 2004).

The process of rotating spinnerets described above is similar to the technique used by SNC, known as the ball electrospinning technology. The technique entails placing multiple balls in the polymer solution and rotating the balls from which jets are formed and collected on a surface – where the nanofiber is formed (https://sncfibers.com/). The advantage of the rotating spinnerets such as those used in the ball spinning technique compared to conventional electrospinning is that it allows for a high throughput as the production of jets is not limited by the number of needles.

2.8. Mechanism of Action of Agents of Functionalization

2.8.1 Titanium Dioxide

Titanium (Ti) can be used to kill bacteria when it is coupled to dioxide (O_2). TiO_2 is an ideal photocatalyst as it is physically and chemically stable, cost-effective, easily available, non-toxic and highly reactive (Sung-Suh et al., 2004). Furthermore, it has a high refractive index and UV absorption capacity, excellent incident photoelectric conversion efficiency and dielectric constant, good photocatalytic activity, photostability, and long-time corrosion resistance. It does, however, have three main disadvantages. Firstly it has a large bandgap namely 3.0 eV for rutile and 3.2 eV for anatase TiO_2, respectively. This is not ideal as the photon absorption of semiconductors largely depends on their bandgap energy. Due to the large bandgap, visible light cannot be used to excite TiO_2. Secondly, TiO_2 has a high recombination rate of electron-hole pairs which has a negative effect on the photocatalytic activity of TiO_2 and results in a low quantum yield and a limited photo oxidation rate. Thirdly, TiO_2 also has a weak separation efficiency of photocarriers which leads to low photocatalytic activity (Huang, Yan & Zhao, 2016).

For TiO_2 to exert its biocidal effect on bacteria, UV light is required. When UV light is applied to TiO_2, its electrons are excited and move from the valence band to the conduction band. During this process, water becomes oxidised and forms hydroxyl ($OH^{\cdot}$) and oxygen ($O^{\cdot}$) radicals. Thereafter, the $O^{\cdot}$ radicals becomes reduced and the $OH^{\cdot}$ radical degrades organic pollutants or microbes and forms carbon dioxide (CO_2 and water (H_2O) (Feng et al., 2014). Some of the compounds that can be degraded from TiO_2 catalysed oxidation include azo dyes, Acid Blue 40, ethylene, methyl vinyl ketone, acenaphthene, anthracene, fluorene and naphthalene, among many others. (Antharjanam, Philip & Suresh, 2003; Dass, Muneer & Gopidas, 1994, Mammadov et al., 1992; Muneer et al., 1992, Muneer, Phillip & Das, 1997).

2.8.2 Copper and Zinc

Functionalisation entails altering a material to give it enhanced physical, chemical or biological properties (Wang, 2012). Some ideal physicochemical properties that functionalisation is used to achieve include corrosion protection, thermal protection, hydrophilic or hydrophobic properties, antifouling or antimicrobial properties and as retardant (Pehkonen & Yuan, 2018). The physicochemical property of most interest in functionalisation is the antimicrobial activity. This can be achieved by coupling an antimicrobial to a material such as nanofibers enhancing the capability with which a nanofiber filter or any other type of

filter removes and inactivates pathogens (Niu & Lin, 2012). Often the antimicrobial of choice is silver nanoparticles; however, other metals may be used as well, such as Cu and Zn.

Cu has throughout history been used in the treatment of wounds and the purification of water (Dollwet & Sorenson, 1985). In small quantities, it is an essential element to bacteria and other organisms as it is involved in numerous metabolic processes. These metabolic processes include oxidative phosphorylation, photosynthesis and the management of free radicals. Cu is used by the enzymes cytochrome oxidase, superoxide dismutase, NADH dehydrogenase 2, aromatase oxidase and deoxy-D-arabino-heptulosonate-7-phosphate synthase (Ladomersky & Petris, 2015; Rensing, & Grass, 2003). The element undergoes cyclic oxidation and reduction to form Cu(II) and Cu(I) enabling it to act as an electron acceptor and donor, respectively. These ions then co-ordinate with functional groups that occur on the amino acids within proteins, namely hydrides, alkyl groups, sulphates, thiols, phosphines and thioethers (Ladomersky & Petris, 2015).

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + OH^- + OH \quad (1)$$

$$Cu(II)\ O_2^- \rightarrow Cu(I) + O_2 \quad (2)$$

However, larger quantities of the element are toxic in that, under anaerobic conditions, they generate reactive oxygen species (ROS) such as the hydroxyl radical through the Fenton and Haber Weiss reaction (Liochev & Fridovich, 2002). Briefly, the Cu(I) ion donates an electron to hydrogen peroxide forming Cu(II) and a hydroxyl radical. This mainly occurs in the periplasm where Cu is mainly enriched (Macomber, Rensing & Imlay, 2007). Alternatively, Cu(II) is reduced to Cu(I) by an oxygen radical. The hydroxyl radical has a high enough reduction potential to oxidise most types of macromolecules such as proteins, lipids and DNA, which causes further damage to cells as a whole (Freinbichler et al., 2012; Yoshida, Furuta & Niki, 1993). Cu ions can also generate superoxide radicals (Kimura & Nishioka, 1997). Like Cu, Zn is also capable of generating hydroxyl radicals (Raghupathi, Koodali & Manna, 2011).

Excessive quantities of Cu ions under aerobic conditions can deplete the sulfhydryls on cysteine and glutamate residues by acting as oxidising agents for disulfide bond formation. The reaction generates hydrogen peroxide which may generate harmful hydroxyl radicals (Macomber & Imlay, 2009).

$$2\ Cu(II) + 2RSH \rightarrow 2\ Cu(I) + RSSR + 2\ H^+ \quad (3)$$

$$2\ Cu(I) + 2\ H^+ + O_2 \rightarrow 2\ Cu(II) + H_2O_2 \quad (4)$$

Due to the fact that Cu ions can promote disulfide bond formation, they can lead to incorrect disulfide bond formation in the periplasm. In a study by Hiniker et al. (2005) the genes encoding DsbC and DsbD were mutated in *E. coli* cells (Hiniker, Collet & Bardwell, 2005). The genes encode periplasmic thiol-disulfide oxidoreductases that correct non-native disulfide bond formations (Bader et al., 2000; Zapun et al., 1995). After mutation of these genes, the *dsbC*$^{-1}$ and *dsbD*$^{-1}$ mutants were exposed to varying concentrations of Cu and were found to be Cu sensitive when compared to the wildtype. When the mutant strains were transfected with plasmids encoding *dsbC* and *dsbD*, Cu tolerance was restored (Hiniker, Collet & Bardwell, 2005).

Other mechanisms of toxicity include competing with iron in iron-sulfur clusters of proteins or with other metal ions (Macomber & Imlay, 2009). Additionally, internal excessive exposure leads to the degradation of DNA (Warnes, 2010). A study by Pramanik et al. (2012) used the reporter gene assay to demonstrate that Cu damages DNA. Briefly, *DH5α* cells transformed with pUC19 were treated with an LD_{50} dose of CuI nanoparticles. Some Cu nanoparticles release the Cu as ions (Pallavicini et al., 2010). The pUC19 plasmid carries the *lacZ* gene which encodes the enzyme β-galactosidase. X-gal acts as a chromogenic substrate for β-galactosidase and emits a blue colour when hydrolysed. The cells failed to hydrolyse the X-gal as there was a lack of blue colonies observed, indicating that *lacZ* was not transcribed. This probably resulted from damage to the pUC19 plasmid (Pramanik et al., 2012). It is hypothesised that Cu-induced DNA damage results from the generation of ROS. In the study by Pramanik et al., (2012) an *in vitro* assay demonstrated that when pUC19 is incubated with CuI, ROS is formed which leads to the formation of a nick in the plasmid as observed through gel electrophoresis (Pramanik et al., 2012). However, a study by Macomber et al., (2007) showed that *E. coli* cells that had their Cu homeostasis genes mutated (namely *copA*, cueO and *cusCFBA*) were not sensitive to H_2O_2 killing after exposure to Cu. Additionally, they observed that Cu decreased the rate at which H_2O_2 damaged the DNA of the mutant cells (Macomber, Rensing & Imlay, 2007).

When bacteria are exposed to excessive Cu levels externally, their membranes are damaged rapidly and they lose the integrity of the cell (Santo et al., 2011). Atomic force microscopy images taken by Pramanik et al. (2012) showed that the membranes of *Bacillus subtilis* and pUC19 were completely disrupted. This demonstrates that the Cu is effective against both gram negative and gram positive bacteria and that the MOA is the same for both types of bacteria (Pramanik et al., 2012).

Bacteria also have mechanisms in place to tolerate excess levels of Cu. These mechanisms make use of proteins that are involved in maintaining Cu homeostasis and are given in Table

2.1. The mechanisms of Cu homeostasis and transport are best described for *E. coli* and *Enterococcus hirae* and will be discussed in this review (Ladomersky & Petris, 2015; Solios 2003). The key genes for Cu homeostasis in *E. hirae* are *copA*, *copB*, *copY* and *copZ*. The genes *copA* and *copB* encode an ATPase Cu influx and efflux pump, respectively (Odermatt et al., 1992). The genes *copY* and *copZ*, on the other hand, encode a Cu responsive repressor and a Cu chaperone, respectively (Figure 2.7). When intracellular Cu levels are excessive, copZ binds to the Cu and donates it to copB for exportation and to copY. Conventionally, copY is bound to two *cop* boxes that are located in front of the *cop* operon. However, in the presence of excess Cu, when copZ donates Cu(I) to copY, the repressor translocates off the *cop* boxes allowing transcription of the *cop* operon to occur (Figure 2.7) (Solioz et al., 2010; Solioz & Soyanov, 2003). The cop operon enables *E. hirae* to survive in Cu levels of up to 8 mM (Solioz et al., 2010; Solioz & Soyanov, 2003). Cu homeostasis in *E. hirae* is summarised in Figure 2.7.

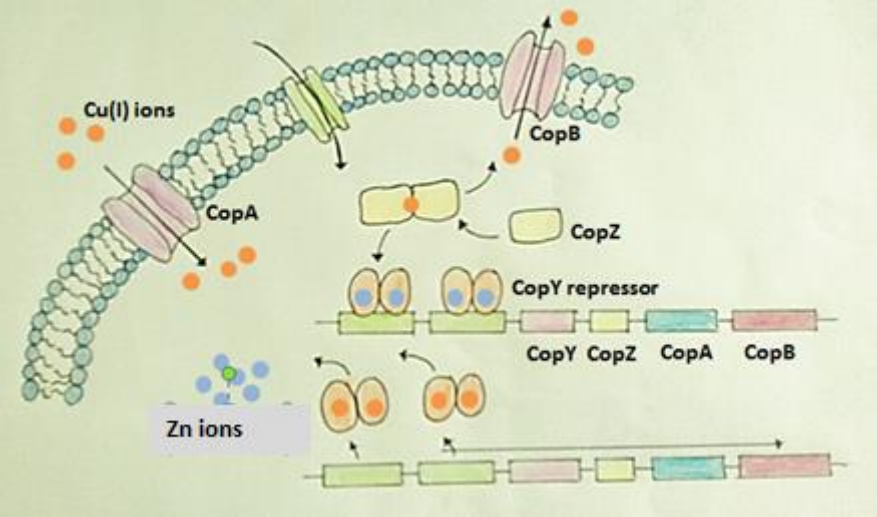


Figure 2.7: Cu homeostasis in *E. hirae*. CopZ binds to excess Cu(I) in the cytoplasm and transfers it to CopB to export it. Additionally, copZ donates Cu(I) to the CopY repressor displacing it from being bound to the *cop* operon so that the operon will be induced (adapted from Solioz et al., 2010).

In *E. coli*, excess levels of intracellular Cu are sensed by the transcription factors CueR and CusR (Rensing et al., 2000; Stoyanov, Hobman & Brown, 2001). Once the intracellular Cu levels reach 10^{-21} M, CueR induces the expression of the Cu tolerance genes (Changela et al., 2003). One of the gene products that are transcribed are metallochaperones such as CusF, CopZ of *Enterococcus hirae* and CupA of lactobacilli and streptococci which first transport the intracellular Cu ions to exporter proteins (Fu et al., 2013). In *E. coli*, CusF binds

to periplasmic Cu(I) and transfers the ion to CusABC (Egler et al., 2005; Su, Long & Yu, 2010). The tripartite complex CusABC is partly embedded in the inner membrane (CusA) but spans the periplasm and is also embedded in the outer membrane (CusC). The complex is responsible for extruding excess periplasmic Cu(I) (Figure 2.8) (Long, 1998; Frank, Munson, Su). The transcription of CusF and CusABC is regulated by CusR, which is phosphorylated by CusS to induce transcription of these genes when periplasmic levels of Cu(I) are high (Rensing et al., 2000).

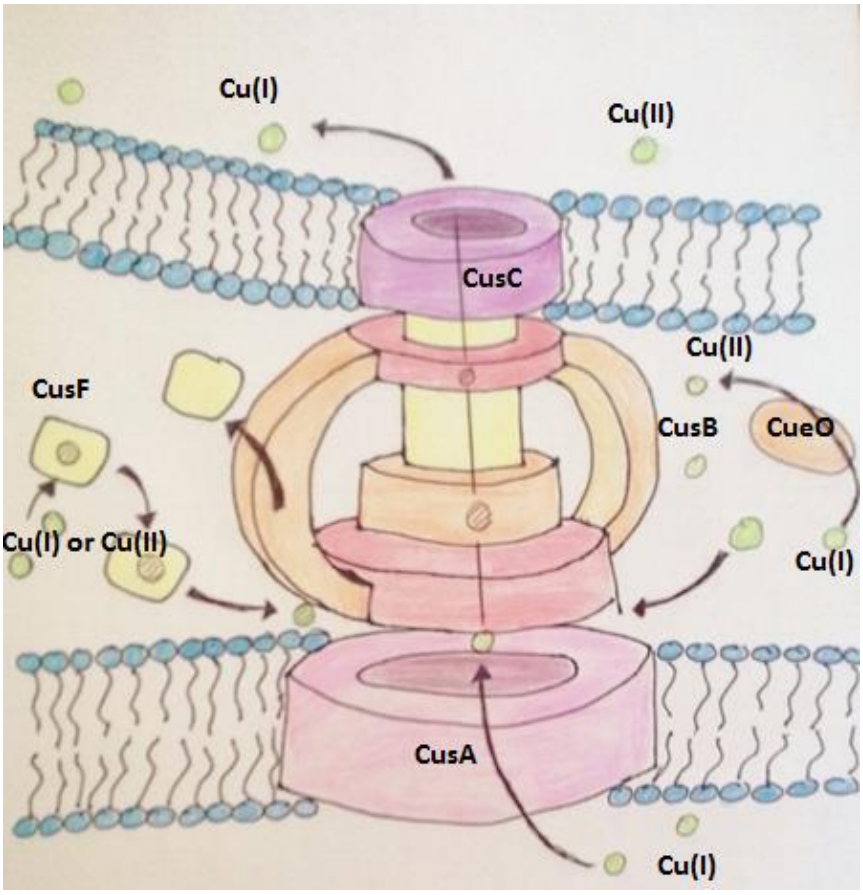


Figure 2.8: Cu extrusion Mechanism in *E. coli* as adapted from Rensing & Grass., 2003.

An important exporter protein for the extrusion of intracellular Cu levels is CopA. The gene *copA* encodes a P-type ATPase that pumps out excess Cu(I) from the cytoplasm into the periplasm and maintains Cu level homeostasis in *E.coli* (Rensing et al., 2000). Other types of P-type ATPases are CtpV of *Mycobacterium tuberculosis*, CopA1 and CopA2 of *Pseudomonas aeruginosa* and GolT of *S. typhimurium* (Ladomersky, & Petris, 2015). These proteins hydrolyse ATP to pump Cu(I) out. The tripartite complex, CusABC is an additional Cu(I) exporter by functioning as a proton antiporter which enables the tolerance of relatively high concentrations of Cu under anaerobic conditions (Outten et al., 2003). As Cu(I) is more

toxic than Cu(II), it is oxidised by CueO, a multi-Cu oxidase located in the periplasm (Grass & Rensing, 2001).

Table 2.3. Genes involved in mediating homeostasis of Cu(I) levels and confer resistance in *E. coli*

Name of Gene	Gene Product	Function	Location in/on the bacteria cell	References
copA	copA	P-type ATPase that pumps Cu(I) from the cytoplasm into the periplasm and maintains homeostasis	Within the inner membrane	Rensing, 2000
cusABC	CusABC	Tripartite proton/cation antiporter	Tripartite complex is embedded in the inner membrane (through CusA) and spans across the distance (CusB) between the inner membrane to outer membrane (CusC)	Long, 1998; Frank, 2001; Frank, 2003, Munson, 2000; Su 2011
cusF	CusF	Metallochaperone that binds periplasmic Cu(I) for extrusion by the CusABC efflux pump to prevents toxic levels of Cu(I) in the periplasm	Periplasm	Egler et al., 2005; Su, Long & Yu, 2010
cusR	CusR	Regulates the transcription of cusCFBA	Intracellular	Rensing, 2003
cusS	CusS	Histidine kinase that senses Cu(I) within the periplasm and phosphorylates CusR	Within the inner membrane	Rensing, 2003
yakK	CueO	Oxidises Cu(I) to Cu(II)	Periplasm	Grass & Rensing, 2001
Ybbl	CueR	Senses intracellular Cu and activates the transcription of copA and CueO		Rensing, 2003; Stoyanov, Hobman & Brown, 2001

As Zn is also metal, it is highly likely that its MOA against bacteria is the same as that of Cu and other metals. This is supported by the fact that like Cu, Zn oxide nanoparticles generates ROS, which was demonstrated in a study by Raghupathi et al., (2011) (Raghupathi, Koodali & Manna, 2011). These findings are supported by the observations of a group of Gedanken who saw the formation of the hydroxyl-radical and superoxide radicals in a suspension of ZnO examined by electron spin resonance spectroscopy (Berry et al., 2005; Zhang & Jiang et al., 2010). A study by Padmavathy et al., (2008) showed that the surface of ZnO nanoparticles disturbed both the cell wall and cell membrane of *E.coli* (Padmavathy & Vijayaraghavan, 2008). A study by Brayner et al., (2006) also demonstrated that ZnO damaged the cell membrane of *E.coli*, increased membrane permeability and led to the accumulation and cellular internalization of the ZnO particles inside the cell (Brayner et al., 2006).

2.9. Conclusion

Clean water is a scarce resource that often individuals from poorer communities lack access to. This often leads to these individuals making use of river or other forms of surface water and thus exposing themselves to waterborne diseases caused by pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp. and *Vibrio cholerae,* to name a few.

There are several methods of purifying water such as chlorination, ozonation, thermal treatment and UV disinfection; however, filtration remains one of the most widely applied methods as it is easy and often cost-effective to use. There are four types of filtration that are mainly used on an industrial scale namely microfiltration, ultrafiltration, nanofiltration and RO. However, individuals living in poorer communities cannot afford these filtration systems and instead use POU filters such as slow sand filters and ceramic filters.

In this thesis, two filters were tested namely the Swoxid prototype and an antimicrobial nanofiber membrane. The former consists of nanofibers ceramic and makes use of TiO_2 and UV light. The latter uses Cu(II) and Zn(II) to inactivate bacteria.

CHAPTER 3 –

Experimental Chapter: Determining the Efficacy of Two Point-of-Use Filter Prototypes

3.1 Introduction

Filtration plays an important role in the purification of water and is applied in various forms. The conventional set up for a membrane filtration unit consists of an inlet source, feed tank, and feed pump that is connected to a rigid flat sheet or a cylindrical membrane, which is further connected to a pressure transducer or pressure regulator and flow meter (Mullett, Fornarelli & Ralph, 2014; Sweity, 2011). Membranes used for filtration can be made of the polymers poly (acrylonitrile), poly (vinylidene fluoride), cellulose-acetate, cellulose nitrate blends, nylons, polysulfone, polyamide, poly tetra-fluoroethylene or polyacrylonitrile-poly vinyl chloride polymers (Baker, 2004). The feature that these polymers have in common is that they provide the mechanical strength required for the pressure applied across the membrane during the filtration process, which range between 0.2 – 80 bars (Baker, 2004; Baker, 2012; Hong & Elimelech, 1997; Maher, 2012; Pearce, 2011; Ramakrishna et al., 2010; Yonge, 2012). Furthermore, they are also selected on their ability to withstand varying ranges of pH used during the filtration process (Baker, 2012).

Whereas membrane filtration processes are widely used as fixed installations at wastewater and drinking water treatment plants or industrial factories, the advantage of POU filters are that they are portable (Baker, 2012; Mullett, Fornarelli & Ralph, 2014; Sweity, 2011). Ideally, they do not require the use of chemicals; they are more accessible than other methods of purifying water and in most cases lower in cost. Moreover, they do not necessarily have to consist of polymers or require the mechanical strength to withstand the high pressures used in membrane filtration systems. In this study, two POU filters were investigated. The first filter, namely the Swoxid prototype consisted of ceramic functionalised with titanium dioxide (TiO_2). The second filter consisted of an electrospun poly (D,L-lactic acid) (PLA) membrane functionalised with copper (Cu) and zinc (Zn). Both filters are designed to be used by individuals residing in poorer communities and do not have access to clean municipal water.

The advantages of using ceramic for filtration, as described in Chapter 2 are that ceramic is made from readily available materials, it can be used in different climates and is socially accepted in many areas, it does not confer an undesirable taste to the water, ceramic-based filters are cost effective and not only remove pathogens but reduce the turbidity of contaminated water (Kallman, Oyanedel-Craver & Smith, 2011; Oyanedel-Craver, & Smith, 2008; http://www.wsp.org/UserFiles/file/926200724252_eap_cambodia_filter.pdf). Additionally, the Swoxid filter is able to retain particles as small as 100 nm - the approximate size of the influenza virus (Vajda et al., 2016).

The advantages of using nanofibers for filtration are that they offer high permeability, high surface area to surface volume ratio, the ability to form highly porous membranes, as well as

the adaptability of fibre diameter and extremely small pore size (~0.3 µm), which allows for the exclusion of waterborne pathogens bigger than 0.3 µm (Botes & Cloete, 2010).

The efficiency with which nanofibers remove microbial pathogens may be enhanced through functionalisation, such as encoating a membrane with an antimicrobial agent (Niu & Lin, 2012). For example, in a study by Bjorge et al. (2010) a nanofiber membrane was fabricated and tested against coliform bacteria. The membrane reduced the growth of the coliform bacteria to by 2 log however, functionalisation of the membrane with silver nanoparticles resulted in a 4 log – 6 log reduction (Bjorge et al., 2010).

In the case of the Swoxid prototype, functionalisation is achieved by coating the ceramic with TiO_2, which when activated by UV light, kills microorganisms and degrade micropollutants through reactive oxygen species (ROS) production (Antharjanam, Philip & Suresh, 2003; Dass, Muneer & Gopidas, 1994, Mammadov et al., 1992; Muneer et al., 1992, Muneer, Phillip & Das, 1997). Ultra-performance liquid chromatography mass spectrometry (UPLC-MS) was used to assess its efficiency to remove micropollutants.

In the case of the antimicrobial nanofiber membrane, functionalisation is achieved by spinning the PLA with a solution containing Cu(II) and Zn(II) which serve to inactivate bacteria and other microorganisms that come in contact with the surface of the membrane through the mechanisms described in Chapters 1 and 2.

The consecutive set of experiments entailed finding the optimal antimicrobial nanofiber membrane design, with emphasis on density and thickness as well as determining the ultra-structure using SEM. This was followed by leaching experiments using inductively coupled plasma mass spectroscopy (ICP-MS) were performed to determine whether the quantity of Cu(II) and Zn(II) that leach into the filtrate falls below the recommended limit for drinking water as stipulated by the South African National Standards for Drinking Water and the United States Environmental Protection Agency (USEPA). The research focussed on determining the efficiency of the Swoxid prototype and the antimicrobial nanofiber membrane as filters by using various techniques namely, plate counting, bioluminescence imaging and scanning electron microscopy (SEM). Additionally, the efficiency with which the filter can degrade micropollutants was determined using UPLC-MS.

3.2. Materials and Methods

3.2.1. Swoxid Prototypes

The First Swoxid Prototype

The first Swoxid prototype, which was provided by Dr Endre Horvath from the École Polytechnique Fédérale De Lausanne, consisted of a ceramic membrane functionalised with TiO_2 and enclosed in a glass frame. UV light from the sun reacts with the TiO_2 to generate radicals which theoretically inactivate bacteria. In addition to oxidation, the functionality of the device relied on mechanical filtration and thermal inactivation. Gravity flow was used to facilitate filtration (Figure 3.1).

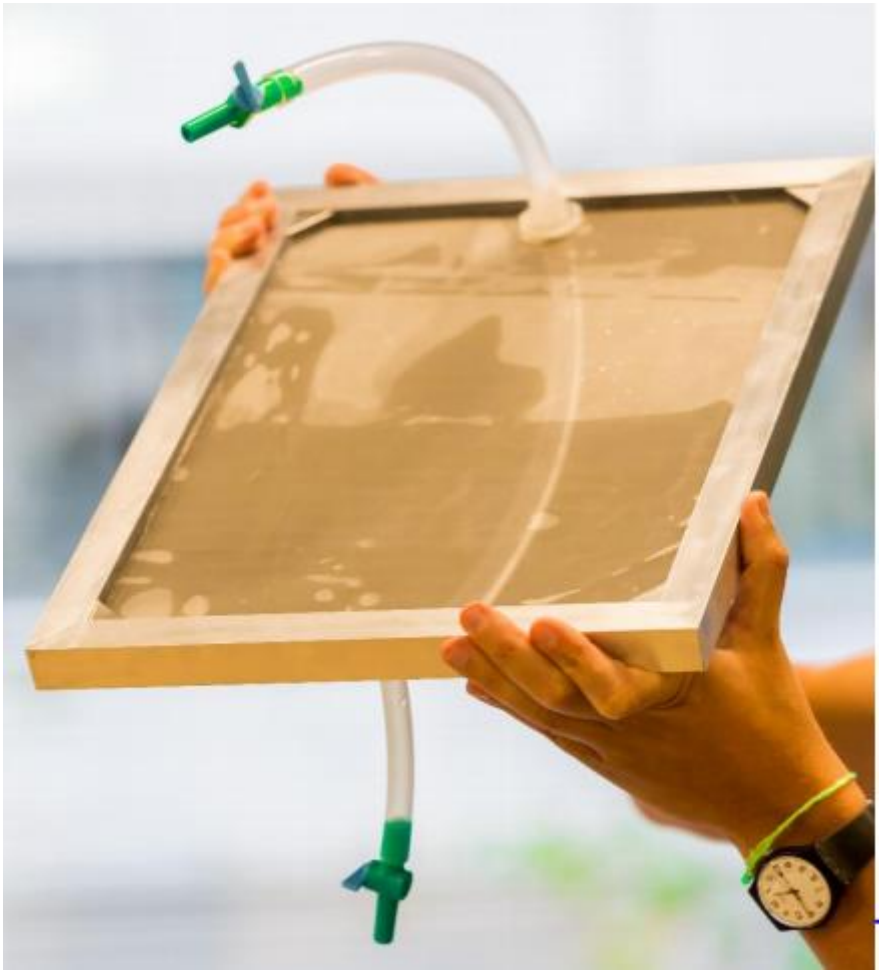


Figure 3.1. Image of the first Swoxid prototype (image provided by Dr. Endre Horvath from École Polytechnique Fédérale La Lausanne)

The Second Swoxid Prototype

Adjustments were made to the initial Swoxid prototype. Instead of two glass panes covering a ceramic membrane, the prototype was changed to a plastic box with a ceramic frame inside functionalised with TiO_2 and it does not act as a filter. Instead of radiation from the sun, it relied on a UV lamp (wavelength 273 nm) to generate radicals and a pump (Aspen; flow rate of 104 mL/min) to pass water through the prototype. Therefore, unlike the first prototype, the second prototype required electricity. A commercial Eheim UV Purifier (used at a wavelength of 273 nm) without TiO_2 functionalization was used as a control. The second prototype was used instead of the first as it was considered that it may be more effective in terms of filtration rate and antimicrobial activity. This was expected on the basis that an Aspen pump was used to pass water through instead of relying on gravitational flow. Moreover, it was expected that the antimicrobial activity will be improved as the UV lamp provides a direct source of UV without the variation that occurs with sunlight.

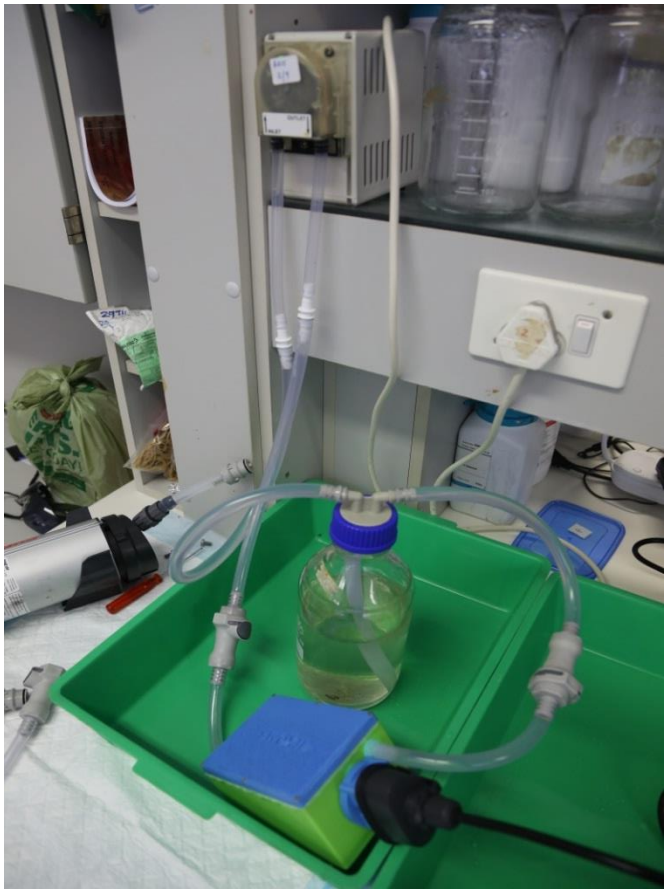


Figure 3.2. Image of the second Swoxid prototype (image provided by Dr. Endre Horvath from École Polytechnique Fédérale La Lausanne)

3.2.1.1 Determining the efficiency of the Swoxid prototype filters to inactivate bacteria in water.

Water was sampled from the Plankenburg River in 3 L sterile flasks in September 2018 and immediately transported to the laboratory. The sampled water was filtered through the Swoxid prototype under conditions of sun exposure and within the lab (under no UV exposure to the sun as a control). The water from the Plankenburg River (influent) and effluent were plated out on *Salmonella-Shigella* agar (Merck), mEndo agar (Merck), m-Faecal Coliform agar (Merck), *Enterococcus* selective agar (Sigma Aldrich) and nutrient agar (Merck) within a few hours from sampling for the selection of *Salmonella* and *Shigella* spp, coliform bacteria, faecal coliforms, *Enterococcus* spp. and heterotrophic bacteria. These bacteria were selected because *Salmonella* and *Shigella* spp are pathogenic bacteria and the rest of the bacteria are indicators of faecal contamination or of other enteric pathogens. The filtrate was incubated at 4ºC and over the course of 6 days; it was plated on the different selective media every two days.

To determine the effect that exposure to the sun has on bacterial numbers, the sampled water was exposed to the sun for a day in a transparent 2 L Schott bottle and the sampled water was plated out on *Salmonella-Shigella* agar, mEndo agar, m-Faecal Coliform agar, *Enterococcus* selective agar and nutrient agar.

Statistical Analyses

The results were plotted on graphs generated by Graph Pad Prism and for the statistical analysis of the results; two-tailed unpaired t tests were performed at the 95 % confidence interval.

3.2.1.2. Effect of Exposure on Bacterial Cell Ultra-structure

Glass stubs (10 mm in diameter) were kindly provided by École Polytechnique Fédérale La Lausanne. Two stubs were coated with the Swoxid membrane on the surface whereas the surfaces of two polypropylene squares were used as controls. TSB medium (10 mL) was inoculated with *S. aureus* Xen 36, which was cultivated overnight at 37ºC and adjusted to 10^7 CFU/mL. Thereafter, the culture was harvested at 6330 x g for 2 min and the pellet was resuspended in 0.9 % sodium chloride (from Sigma Aldrich) and 50 µL aliquots of the suspension were spotted onto the glass stubs. One of each of the coated stubs and

polypropylene squares was exposed to UV(B) (within the range of 280-315 nm) using a lamp provided by Phillips for 30 min whereas the other two were not. Thereafter, the stubs and the pieces were suspended in 2.5 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for fixation to the surface of the stubs. For dehydration, the stubs were suspended in 0.1 M sodium cacodylate buffer twice for 15 min each. Thereafter, the stubs were washed with a series of EtOH concentrations, namely 50 %, 70 % and 90 % for 10 min each. This was followed by two washes in 100 % EtOH for 10 min each at room temperature and an additional two washes in 100 % hexamethyldisilazine (HMDS) for 20 min each. Thereafter the HMDS was removed and air-dried overnight.

The following day, the glass stubs were mounted onto a rectangular metal frame using double-stick, carbon conductive tape. Thereafter, the samples were coated with 50-100 Angstrom Au using a Leica sputter coater. The samples were then viewed under the Zeiss Merlin SEM. The working conditions used to obtain images were a working distance of 4.6 mm, an accelerating voltage of 3 kV and a probe current of 150 pA. Furthermore, the samples were viewed at magnifications ranging between 10 000 to 30 000X magnification and the images were processed using the software SMARTSEM.

3.2.1.3. Efficiency of Micropollutant Removal

One litre of RO water spiked with sulfamethoxazole (SMX) and carbamazepine (CMZ) to a representative environmental concentration of 4 µg/L; each was pumped through the Eheim UV purifier and the second Swoxid prototype for 20 min (at a wavelength of 273 nm for both conditions), respectively, using an Aspen pump at a rate of 104 mL/min. After each run, a 100 mL aliquot of each sample was taken and spiked with 1000 ppb of carbamazepine d10 and sulfamethoxazole 13C6 (Sigma Aldrich). The preparatory phase entailed solid phase extraction (SPE) where 4 mL of methanol (MeOH) was applied through the Oasis cartridges under gravity. The aliquots were applied to the respective Oasis cartridges and the vacuum was run at a rate of 5 mL/min. Thereafter, 4 mL of MeOH was run through the Oasis cartridges under vacuum and the fragments were collected in labelled 10 mL test tubes. Then, the samples were dried under nitrogen and resuspended with 500 µL MeOH. A standard curve of 1 ppb, 5 ppb, 10 ppb, 25 ppb, 50 ppb, 100 ppb, 200 ppb, 500 ppb and 750 ppb was created in MeOH for CMZ and SMX. Subsequently, carbamazepine d10 and sulfamethoxazole 13C6 for each compound was added to the standard. The same procedure was followed for the Plankenburg River water samples using a different set of internal standards.

Thereafter, UPLC (Waters AQUITY) was performed to analyse the concentration of the samples. For the chromatographic analysis, de-ionised water (MilliQ) containing 0.1 % formic acid (Mobile phase-A) and 100 % HPLC-grade methanol (Mobile phase-B) was used to separate the target analytes. The initial conditions consisted of 100 % mobile phase-A which was run for 0.2 min and subsequently reduced to 10 % mobile phase-A over 6.8 mins and finally to 0 % mobile phase-A over 0.1 mins. Thereafter, to re-equilibrate the system, the conditions were returned to 100 % mobile phase-A over 0.4 mins and kept for 2.5 mins. The total run time was 10 min. A reversed-phase BEH C18 column (Waters AQUITY, 1.7µm pore size, 2.1 x 100mm) which contained a 0.2µm in-line column filter was used. The column temperature was kept at 50°C. The flow rate of the mobile phases was set at 0.4 ml/min and a sample injection volume of 2 µL was used. The UPLC was coupled with a triple quadrupole mass spectrometer (Xevo TQ-MS, Waters AQUITY) equipped with an electron spray ionisation source. All the analytes were determined using a positive ionisation mode (ESI+). Nitrogen was used as both nebulising and desolvation gas, and argon was used as the collision gas.

The LC-MS data was acquired by using a multiple reaction monitoring (MRM) mode using two fragment ions for each compound where possible. The optimised MS/MS parameters for the micropollutants analysed in the river samples are shown in Table 3.1 and the application of the respective micropollutants in Table 3.2. To obtain a linear reference standard calibration curve for each target analyte, a 10-point concentration range ranging from 1 ppb to 750 ppb in MeOH the solvent in which the water samples were re-constituted. The integration of the analyte standard curves and surface water sample concentrations was determined using the TargetLynx software (Version 4.1, Waters). The graphs were generated using GraphPad Prism.

Table 3.1: Information about the chromatographic retention times and mass spectrometry parameters used in the LC-MS method to estimate the micropollutant concentrations.

Contaminant of Emerging Concern (CEC)	RT (min)	Parent m/z	Daughter 1			Daughter 2			Corresponding Internal Standard
			m/z	CV (V)	CE (eV)	m/z	CV (V)	CE (eV)	
Acetaminophen (ACTM)	2.14	152.0	110.0	20	25	93.0	20	25	Methamphetamine-d5
Benzotriazole (BZT)	3.24	120.0	65.0	30	20	92.0	30	15	Naproxen-d3
Caffeine	3.01	195.0	138.0	38	15	110.0	38	23	Methamphetamine-d5
Carbamazepine (CBZ)	5.21	237.0	194.0	20	25	179.0	40	38	Carbamazepine-d10
Cocaine	3.43	304.0	182.0	40	20	82.0	40	30	Cocaine-d3
Codeine	2.30	300.0	215.0	40	25	152.0	40	40	Cocaine-d3
Diclofenac	6.66	296.0	250.0	15	15	215.0	15	15	Naproxen-d3
3,4-methylenedioxymethamphetamine (MDMA)	2.61	194.1	163.1	20	25	105.1	20	25	MDMA-d5
Methamphetamine (METH)	2.70	150.0	91.0	25	20	119.0	25	10	Methamphetamine-d5
Methaqualone	5.40	251.1	132.0	30	30	91.0	30	35	Methamphetamine-d5
Sulfamethoxazole (SMX)	3.33	254.0	156.0	20	25	147.0	20	25	Sulfamethoxazole-13C6

Table 3.2: Application of the micropollutants analysed.

Contaminant of Emerging Concern (CEC)	**Application**	**Reference**
Acetaminophen (ACTM)	Analgesic and antipyretic drug	Litovitz et al., 2002
Benzotriazole	Corrosion inhibitor	Cotton & Scholes, 1967
Caffeine	Central Nervous System Stimulant	Nehlig, Daval & Debry, 1992
Carbamazepine	Anti-epileptic Drug	Katzung & Trevor, 2015
Cocaine	Recreational Drug	https://www.drugabuse.gov/publications/drugfacts/cocaine
Codeine	Analgesic drug	Eckhardt et al., 1998
Diclofenac	Anti-inflammatory drug	Schwaiger, 2004
3,4-methylenedioxymethamphetamine (MDMA)	Recreational Drug	Kikura et al., 1997
Methamphetamine (METH)	Used in the treatment of attention deficit hyperactivity disorder	Sim et al., 2001
Methaqualone	Recreational Drug	Ewart & Priest, 1967
Sulfamethoxazole	Antibiotic	Eliopoulos & Huovinen, 2001

3.2.2. Antimicrobial Nanofiber Membrane

Due to the fact that biocide loading, density of the nanofiber membrane and fibre diameter may influence the efficiency of the antimicrobial nanofiber membrane, the Stellenbosch

Nanofiber Company (SNC) has fabricated ten different varieties of membranes, which differed according to these properties. The membranes consisted of a teabag paper layer that served as the substrate and was overlaid with an electrospun nanofiber membrane consisting of poly(D,L-lactic acid) (PLA), Polysorbate 80 (reagent grade surfactant) and a biocide (BioClear). The membranes were cut into discs (47 mm in diameter) to fit into a Millipore filter device (Figure 3.2). The combination of variables is listed in Table 3.3. The membranes were produced at a limited quantity; therefore some experiments could not be repeated. Various tests were conducted with *Escherichia coli* Xen 14 and *Staphylococcus aureus* Xen 36 as the test microorganisms to determine which of the 10 varieties showed the highest efficiency in removing bacteria. The bacteria were selected as they both have bioluminescent markers inserted and are widely used in the indication of water contamination.

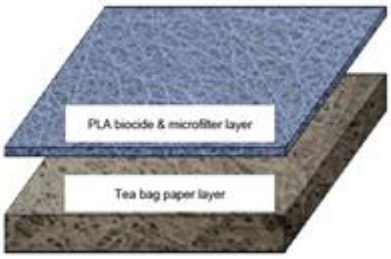


Figure 3.3: Image of the structure of the first batch of membranes (image provided by SNC).

Table 3.3: The ten different varieties of the filter membranes according to biocide loading, density and average fibre diameter

Abbreviation	Biocide Concentration **w/w %**	Density **gsm**	Average Fibre Diameter **nm**
LLL	Low: 2.4	Low: 0.3 ± 1	Low: ± 603
LLH	Low: 2.4	Low: 0.3 ± 1	High: ± 942
LHL	Low: 2.4	High: 1 ± 5	Low: ± 733
LHH	Low: 2.4	High: 1 ± 5	High: ± 933
MLL	Medium: 15	Low: 0.3 ± 1	Low: ± 643
MHL	Medium: 15	High: 1 ± 5	Low: ± 682
HLL	High: 50	Low: 0.3 ± 1	Low: ± 733
HLH	High: 50	Low: 0.3 ± 1	High: ± 896
HHL	High: 50	High: 1 ± 5	Low: ± 682
HHH	High: 50	High: 1 ± 5	High: ± 915

3.2.2.1. Determining the quantity of Cu and Zn that leaches into the filtrate

A flow through filtration system (Millipore) was sterilised at 121°C for 15 min before one membrane of each sample was placed into the system. A litre of sterile RO water was filtered through each type of membrane. Thereafter, 50 mL aliquots were taken from each filtrate and HNO_3 was added to each aliquot to the final concentration of 0.1 % v/v. The ten aliquots were analysed for the presence of Cu and Zn cations using ICP-MS at the central analytical facility (CAF) of Stellenbosch University.

In a separate experiment, one of the biocide high planar density (BHPD) membranes was swirled in 1 L of RO water and 10 mL aliquots were taken over specific time intervals namely 30s, 1 min, 2 min, 3 min, 4 min, 5, min, 10 min, 20 min, 30 min, 40 min, 50 min and 60 min. The aliquots were analysed for Cu and Zn cations again using ICP-MS at CAF.

3.2.2.2. Determining the shortest contact time for the inactivation of the metabolism of *E.coli* Xen 14 and *S.aureus* Xen 36

Ten mL of Luria broth (LB) (Biolab, Merck) and tryptic soy broth (TSB) (Biolab, Merck) were inoculated with *E. coli* Xen 14 and *S. aureus* Xen 36, respectively and the two bacterial cultures were cultivated overnight at 37^0C. After cultivating the bacteria overnight, the cells were subsequently pelleted by centrifuging at 6330 x g for 2 min and the pellet was resuspended in saline water to remove any nutrients from the media. Bacterial suspensions containing 10^7 CFU/mL cells were prepared using the Spectroquant Pharo 300 (Merck) to measure the cell density. Thereafter, 1 mL of the culture was added onto the membrane and the change in bioluminescence was recorded over the course of 60 min using the XENOGEN VIVO VISION In Vivo Imaging Lumina System (IVIS) Spectrum In Vivo Imaging System (Caliper Life Science). The data was processed using the Living Image ® 3.1 Software. The experiments were only performed once as the number of membranes was limited due to manufacturing costs.

3.2.2.3. Determining the effect that exposure to the high planar density, biocide-containing membranes have on the metabolic activity of bacteria

Liquid cultures of *E. coli* Xen 14 and *S. aureus* Xen 36 were diluted to correspond to OD_{600} values of 1.00 and the dilutions were further diluted in the range 10^{-2} – 10^{-10}. The dilutions were then plated out to determine the cell numbers, after which 1 mL of the standardised cultures was transferred to separate HHH and HHL membranes. After 1 min, the membranes were rinsed with saline (0.9 % NaCl containing RO water, NaCl provided by Sigma) and a 100 µL aliquot of the rinsate was transferred to a separate eppendorf tube. Thereafter, the membrane was submerged into the rinsate and vortexed for an additional 4 min and another aliquot was taken and transferred to an eppendorf tube. This was repeated after 5 and 10 min and the aliquots were diluted in the range 10^{-2} – 10^{-10}. Subsequently, the dilutions were plated out on TSB (Sigma Aldrich) plates (for the dilutions containing *S.*

aureus Xen 36) and LB (Merck) plates (for the dilutions containing *E. coli* Xen 14). The plates were then incubated overnight at 37^0C.

3.2.2.4. Determining the efficiency of the antimicrobial nanofiber membranes to filter and inactivate bacteria in water

Optimization was planned, with due consideration of the cost and availability of materials. Therefore, a series of modifications was performed sequentially.

First Trial

One litre of saline (0.9 % NaCl from Sigma Aldrich) water spiked with approximately 10^7 CFU/mL *E.coli* Xen 14 or *S. aureus* Xen 36 was filtered through millipore manifolds containing the HHH, HHL and the respective negative control membranes. The HHL and HHH membranes were selected as they showed the best results in the bioluminescence experiments. If these two membranes are unable to reduce bacterial growth considerably through filtration and contact inactivation, it is unlikely that the other variants will be able to either. An aliquot of the spiked litres of water was taken and diluted before filtration. The dilutions were subsequently plated out on LB agar for the *E.coli* Xen 14 and TSB agar for the *S. aureus* Xen 36. Thereafter, the spiked water was filtered through the membrane-containing manifolds at a rate of 0. 625 L/min using the millipore manifold. The filtrate was then diluted and the dilutions were plated out on to the respective plates. The plates were subsequently incubated at 37ºC overnight.

Second Trial

The same procedure performed in the first trial was followed in the second trial. However, instead of performing filtration with just one membrane, four membranes were stacked on top of another, particularly two HHH membranes on top of two HHL membranes with the side of the nanofibers facing each other. The rate of filtration with the two membranes combined was 0.0419 L/min using the millipore manifold.

Third Trial

The initial results indicated that the membranes were not efficient in retaining bacteria. It was therefore proposed to design and manufacture new membranes with a higher nanofiber density that would also result in a nanofiber network with smaller pore sizes. A second batch of membranes was fabricated that consisted of the teabag paper as substrate, the middle layer of varying densities of PLA covered by the top PLA layer containing the biocide (Figure 3.3 and Table 3.4). As in the case with the initial batch of membranes, the quantity of these membranes were limited; therefore most of the experiments could not be performed in duplicate or triplicate or with the respective controls.

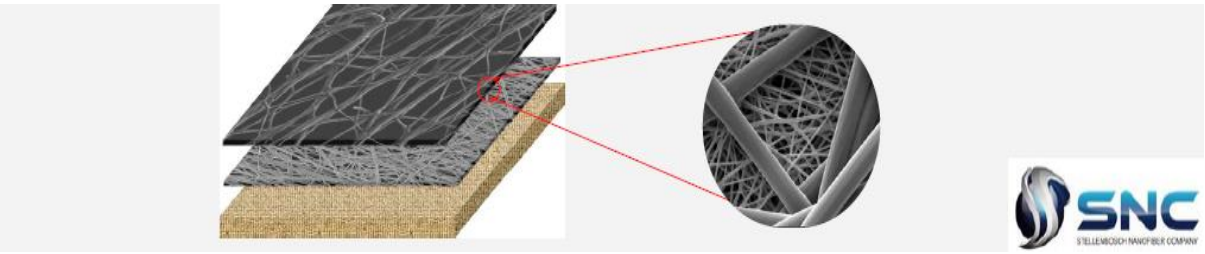


Figure 3.4: The structure and the layering of the high planar density membranes (image provided by SNC)

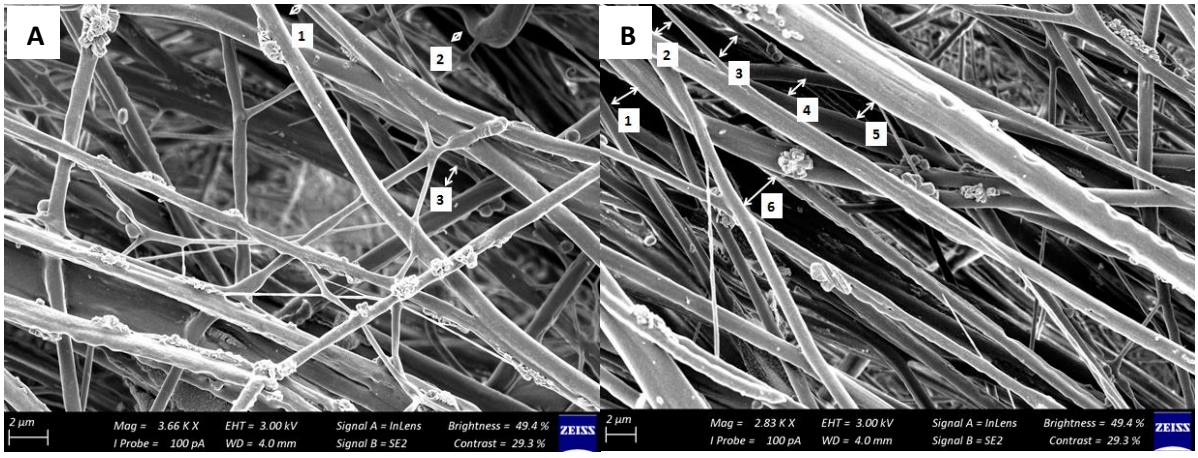


Figure 3.5: A and B. SEM image of 1 BHPD membrane at different magnifications.

Figures 3.5 A and B depict one BHPD membrane In the figure, pore #1 is 0.707 µm, pore #2 is 0.740 µm and pore #3 is 1.109 µm in width. In Figure 3.5 B, pore #1 is 1.800 µm, pore #2 is 1.240 µm, pore #3 is 1.321 µm, pore #4 is 0.964 µm, pore #5 is 1.081 µm and pore #6 is 2.583 µm in width. The diameters of the pores were not intentionally selected but were a result of the ball spinning process.

Table 3.4: Parameters of the second batch of membranes

Variation	Format	Planar Density **gsm**
1	Middle PLA[a] layer: low planar density	5
	Top biocide/PLA layer	5
2	PLA[a] layer: low planar density (variation 1 control)	5
3	Middle PLA[a] layer: medium planar density	10
	Top biocide/PLA layer	5
4	PLA[a] layer: medium planar density (variation 3 control)	10
5	Middle PLA[a] layer: high planar density	15
	Top biocide/PLA layer	5
6	PLA[a] layer: high planar density (variation 5 control)	15

Filtration tests were performed with the high planar density membranes. This approach was followed as it can be assumed that if the membranes with the highest planar density are ineffective in inactivating the bacteria, then it is likely that the other membranes with the lower planar density will be even more ineffective. Consecutive filtration experiments were performed with the BHPD and negative control high planar density (NCHPD) membranes for both *E.coli* Xen 14 and *S. aureus* Xen 36 as described for the first trial using a millipore manifold.

Fourth Trial

Filtration experiments were performed with two BHPD membranes (variation 5) and saline (0.9 % NaCl from Sigma Aldrich) spiked with 10^7 CFU/mL *S. aureus* Xen 36. The filtration rate was 0.010 L/min and the millipore manifold was used.

Fifth Trial

Water spiked with 10^7 CFU/mL *S. aureus* Xen 36 was filtered through two high planar density membranes, particularly a BHPD placed on top of a NCHPD membrane (variation 6) at a filtration rate of 0.062 L/min.

Sixth Trial

Water spiked with 10^7 CFU/mL *S. aureus* Xen 36 was filtered through a combination of one BHPD (variation 5) and two NCHPD (variation 6) membranes a filtration rate of 0.010 L/min.

Statistical analysis

The results were plotted on graphs generated by Graph Pad Prism and for the statistical analysis of the results; two-tailed unpaired t tests were performed with a 95 % confidence interval.

3.2.2.5. SEM Observation of the High Planar Density Membranes Pre- and Post Filtration

After filtration, the membranes used in the fifth and sixth trials were separated and fixed with 2.5 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for 2 hours. The membranes were subsequently washed with 0.1 M phosphate buffer, pH 7.4 followed by sterile RO water and were left to air dry. Once dry, a square section of the membranes was cut out and attached to an aluminium stub by sticking to a double sided carbon tape. The carbon tape allows for good conductivity between the sample and SEM stub to occur. A square section of an unused BHPD membrane was also included. Once the samples were attached onto the stubs, they were subjected to gold (Au) sputtering under vacuum for 2.5 min using an Edwards sputter coater to increase conductivity. The samples were then imaged using a Zeiss Merlin SEM at CAF. The working conditions used to obtain images were a working distance of 9.5 mm, an accelerating voltage of 10 kV and a probe current of 11 nA. Furthermore, the samples were viewed at magnifications ranging between 100 and 17 000X magnification and the images were processed using the software SMARTSEM.

Additionally, EDS was performed on the membranes to confirm the incorporation of the Cu and Zn along with the other metals. The preparation for the membranes were similar to that

performed for SEM imaging, however, instead of sputtering the samples with Au from the Edwards sputter, the membranes were subject to carbon coating using the quorum evaporating coater. The reason for this is because the Edwards sputter coater also incorporates Cu atoms in addition to Au during the process, which would enhance the signal detected for Cu as Cu(II) is already present on the membranes. However, the Quorum Evaporating Coater coats the membrane samples with carbon. The images were analysed using the software AZTEC 3.0.

3.3. Results and Discussion

3.3.1. Determination of the efficiency with which the Swoxid membrane inactivated bacteria in water

First Swoxid Prototype

There was a significant decrease in the number of heterotrophic bacteria (P values are < 0.0075 at most) after 2 days for the controlled conditions (not exposed to sunlight) with the first Swoxid prototype (Figure 3.6 A); however, there was not a significant decrease in the cell numbers after filtration compared to the cell numbers before filtration, and there was an increase in bacterial numbers between days 2 and 6. This is an indication that despite the pore size of 100 – 500 nm of the first Swoxid prototype, the membrane did not retain the bacteria, which continued to grow in the filtrate over the course of time. The second set of results, namely the experimental conditions with exposure to sun (Figure 3.6 B) depict a log reduction of approximately 1.25 following filtration; however, an increase in growth was observed after 2 days, and a slight decrease between days 4 and 6, which can be explained by the bacteria entering a stationary phase and dying off. The observation of growth after filtration can also be explained by the fact that bacteria that occur in nutrient-limited conditions (as found in river water) are more resistant to disinfection than bacteria grown in nutrient-rich conditions.

According to literature, the heterotrophic bacteria are not known to be resistant to oxidation, however, the lack of efficacy for the prototype under experimental conditions could be explained by the fact that not enough radicals are generated and that they act over a short distance.

The filter led to a 4.63 log reduction of the coliforms under controlled conditions and the colony count was lower than 25 CFU/mL. Moreover, the log reduction before filtration and after filtration is statistically significant as the P value is lower than 0.05 (Figure 3.7 A). There

was minimal to no growth on the second day; however, the bacteria that passed into the filtrate grew on the fourth day to an approximate $\log_{10}$ CFU/mL of 5. However, on the sixth day, a reduction in the bacterial count was observed, which as mentioned above can be explained by the bacteria entering a stationary phase and dying off. A log reduction of 4.61 was obtained for the coliform bacteria after filtration with exposure to sunlight; however, on the second day growth is observed to the $\log_{10}$ CFU/mL of approximately 2.2 (Figure 3.7 B). This can be explained by the fact that the bacteria that were too few to count on day 0 grew in number to $\log_{10}$ CFU/mL of 2.2.

Figure 3.8 A and B depicts filtration of the river water under controlled conditions (without exposure to sunlight) and experimental conditions for the enteric bacteria and faecal coliforms, respectively. Here, complete removal of bacteria is observed under both conditions without the growth of bacteria over the course of 6 days.

Similar results were observed with the *Enterococcus* species where complete removal was observed after filtration and no growth was observed over the course of 6 days under both conditions as depicted in Figure 3.9 A and B.

For the *Salmonella* and *Shigella* spp. results under controlled conditions where the membrane was not exposed to sunlight (Figure 3.10 A), there was a log reduction of 2.83 after filtration; however there was a significant amount of growth on day 2 and 4 and no growth on day 6. This again can be explained by the cells that entered the filtrate multiplying until day 2 and entering a stationary phase afterwards. Under experimental conditions; however, there was complete removal of bacteria without any growth over the course of 6 days.

Enterococcus spp., faecal coliform and enteric bacteria were absent in the filtrate in both controlled and experimental conditions; however, this was not the case for the results with the heterotrophic bacteria and the coliforms. Although the coliforms did not grow in the filtrate for the first 2 days of the controlled conditions and the first day of the experimental conditions, growth of the coliforms occurred after 4 and 2 days, respectively. This might suggest that the bacteria mentioned above are generally too big to enter through the pores of the Swoxid prototype membrane which are between 100-500 nm, while those that were not inactivated persisted (passed through) as ultra-micro bacteria. The other observation that these results suggest is that the quantity of the ROS generated was insufficient to efficiently inactivate the heterotrophic bacteria and the coliforms.

The results of the filtration are depicted in Figures 3.6 – 19. In the Figures, ns refers to results that are non significant, * refers to results that are significant and the asterisks signs ** and *** indicate results that are very significant.

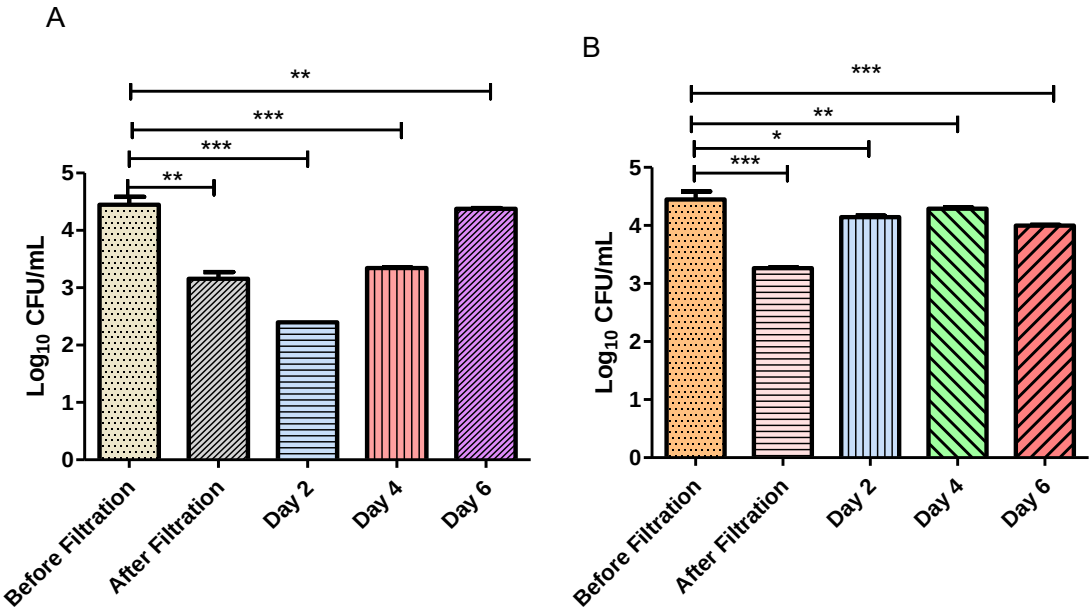


Figure 3.6: A: Numbers of heterotrophic bacteria in the Plankenburg River water before and after filtration with the first Swoxid prototype under controlled conditions (without exposure to the sun) over 6 days. **B**: Numbers of heterotrophic bacteria in the Plankenburg River water before and after filtration with the first Swoxid prototype under experimental conditions (with exposure to sunlight) over 6 days.

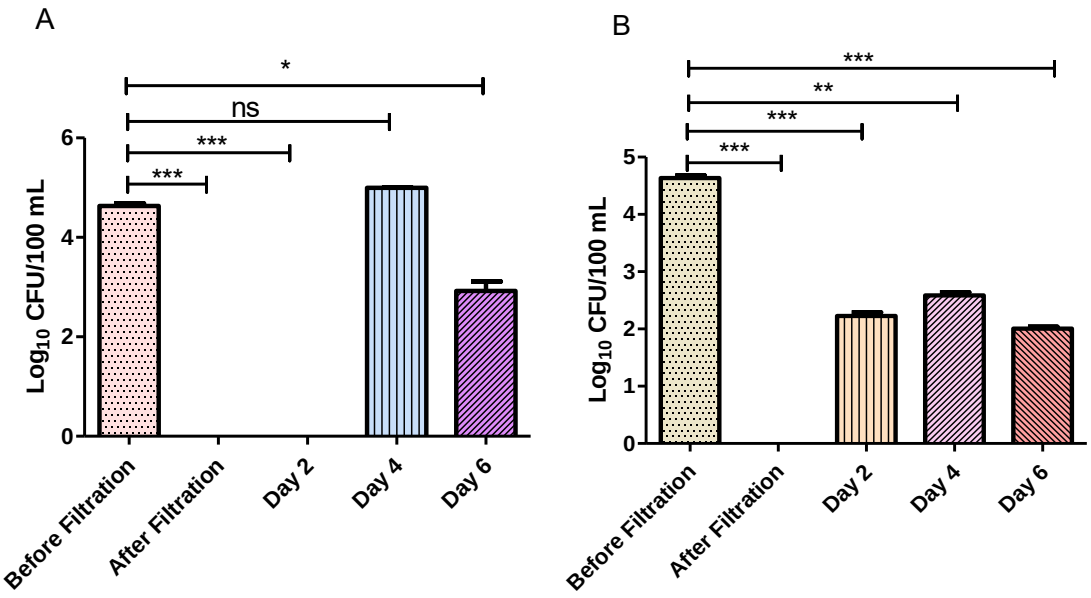


Figure 3.7 A: Number of coliforms in the Plankenburg river water before and after filtration with the first Swoxid prototype under controlled conditions over 6 days. **B**: Number of coliforms in the Plankenburg river water before and after filtration with the first Swoxid prototype under experimental conditions over 6 days.

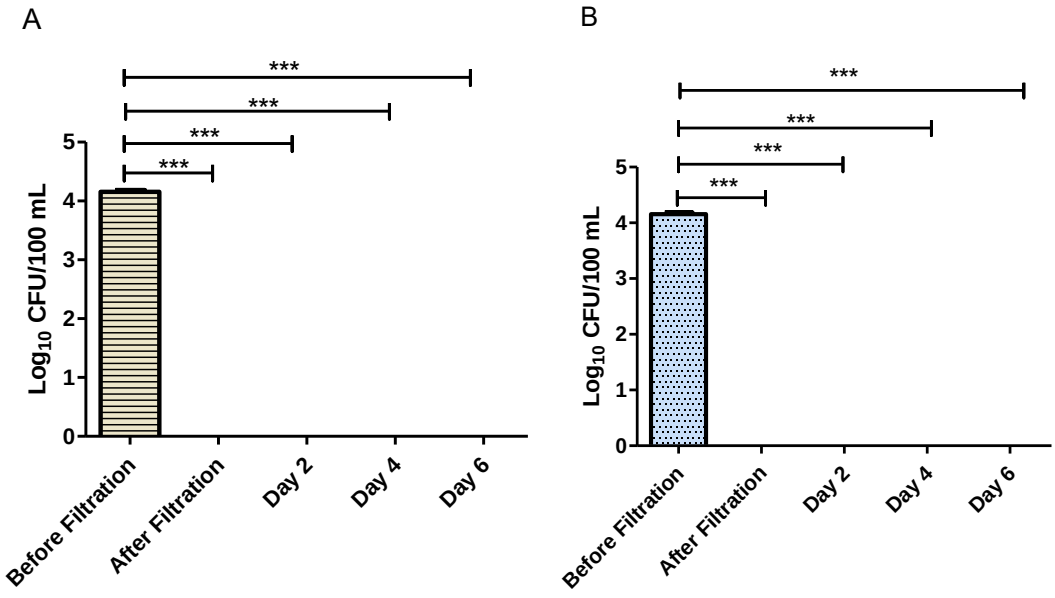


Figure 3.8 A: Number of enteric bacteria and faecal coliforms in the Plankenburg river water before and after filtration with the first Swoxid prototype under controlled conditions over 6 days. **B**: Number of enteric bacteria and faecal coliforms in the Plankenburg river water before and after filtration with the first Swoxid prototype under controlled conditions over 6 days

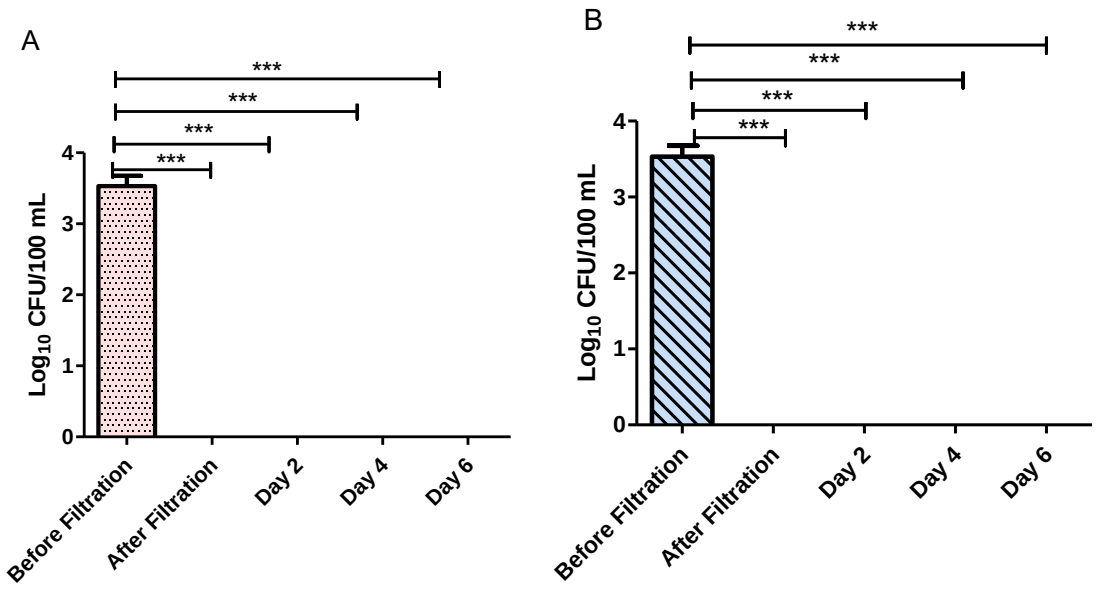


Figure 3.9 A: Number of *Enterococcus* spp. in the Plankenburg river water before and after filtration with the first Swoxid prototype under controlled conditions over 6 days. **B**: Number of *Enterococcus* spp. in the Plankenburg river water before and after filtration with the first Swoxid prototype under experimental conditions over 6 days.

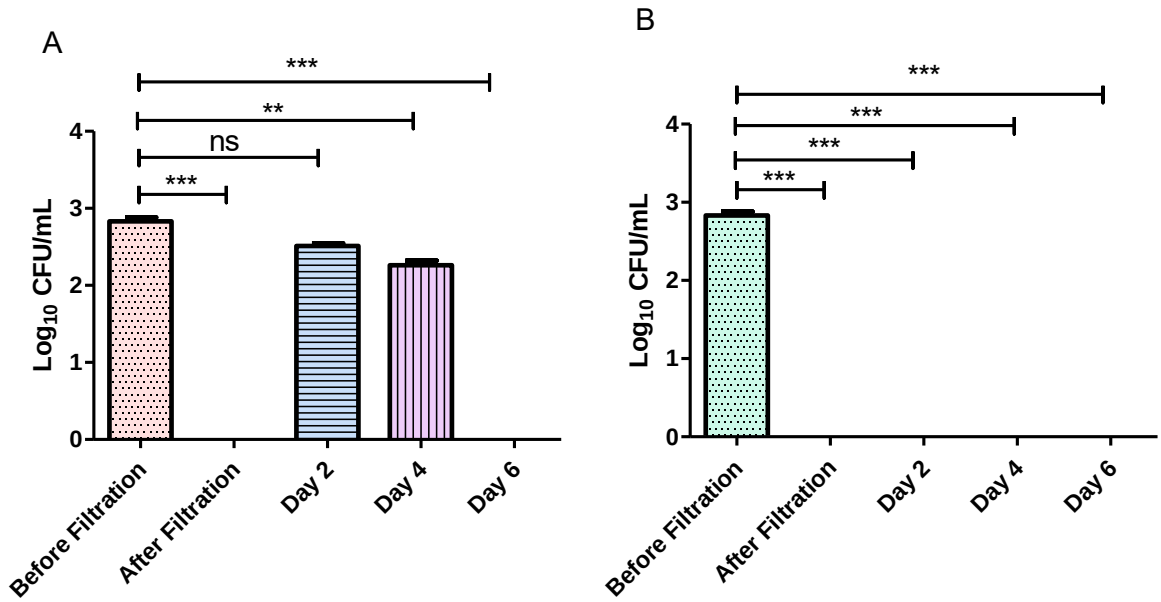


Figure 3.10 A: Number of *Salmonella* and *Shigella* spp. in the Plankenburg river water before and after filtration with the first Swoxid prototype under controlled conditions over 6 days. **B**: Number of *Salmonella* and *Shigella* spp. in the Plankenburg river water before and after filtration with the first Swoxid prototype experimental controlled conditions over 6 days.

It appears that exposure to sun also had an influence on the bacterial numbers even before the water was filtered (Figures 3.11 A – B). For example, in Figure 3.11 A, a 1.38 log reduction was observed for the heterotrophic bacteria and complete inactivation was observed for *Salmonella* and *Shigella* spp. In Figure 3.11 B, we see complete inactivation of the coliforms, faecal coliforms, enteric bacteria and *Enterococcus* species.

In the Figures 3.11 A and B, NA refers to nutrient agar, SS refers to *Salmonella Shigella* agar and mFC refers to faecal coliforms.

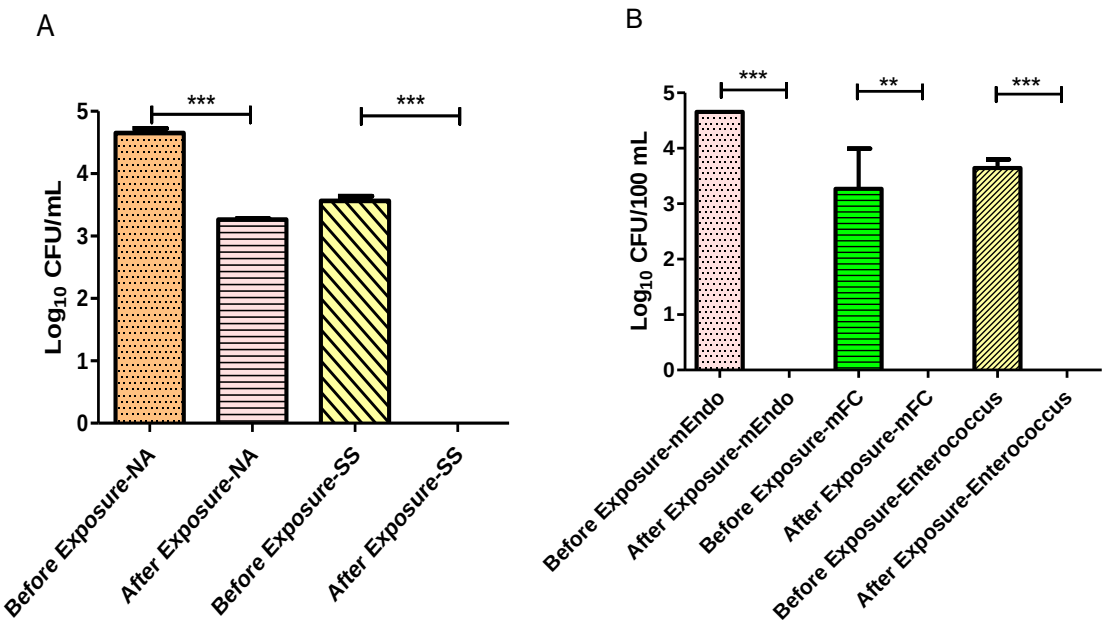


Figure 3.11 A: Numbers of bacteria in Plankenburg river water before and after exposure to the sun in the influent vessel for 1 day on the different types of media. **B**: Numbers of bacteria in Plankenburg River water before and after exposure to the sun in the influent vessel for 1 day on the different types of media.

Second Swoxid Prototype

The heterotrophic bacteria, coliforms, faecal coliforms, *Enterococcus* spp., *Salmonella* and *Shigella* spp. appeared to have been completely inactivated by the second Swoxid prototype within 20 min (Figures 3.12, 3.14, 3.16, 3.17 and 3.18, respectively). However the Eheim UV purifier (control) also achieved complete inactivation of the above mentioned bacteria within 20 min (except for the faecal coliforms). This indicates the efficacy of UV radiation in the inactivation of bacteria in the system, and that inactivation by ROS was not significant.

Although no growth of bacteria was observed for the treatment with the second Swoxid prototype and the Eheim UV purifier over the course treatment with the Swoxid for 60 minutes, growth of the heterotrophic bacteria and coliforms was observed after two days (Figures 3.13 and 3.15) and the differences were statistically significant (P0.0001 for the heterotrophic bacteria). In fact, the growth of bacteria on the second day for the coliforms exceeded the initial bacterial count. This indicates that complete inactivation did not occur after the treatment of the Plankenburg River water with the Swoxid prototype or with the Eheim UV purifier. Instead there were some bacteria that survived but did not grow after immediate treatment with the Swoxid prototype and the Eheim UV purifier but grew over the course of the two days. The remarkable increase in growth could be explained by the possibility of a high organic content in the river water providing the necessary nutrients for the coliforms to grow.

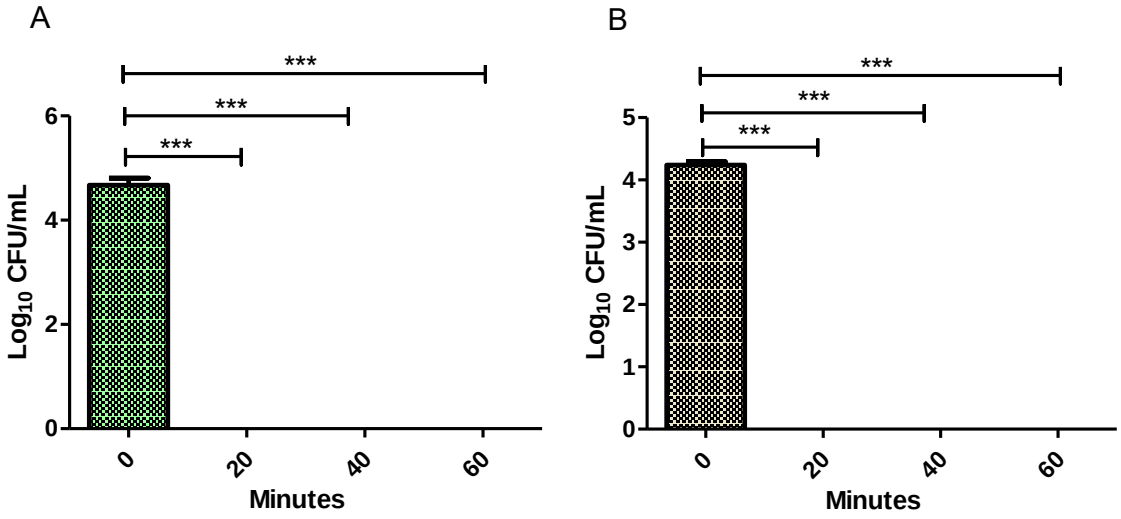


Figure 3.12: A: Number of heterotrophic bacteria in the Plankenburg River water before and after treatment with the second Swoxid prototype under controlled conditions over 60 min. **B**: Number of heterotrophic bacteria in the Plankenburg River water before and after treatment with the second Swoxid prototype under experimental conditions over 60 min.

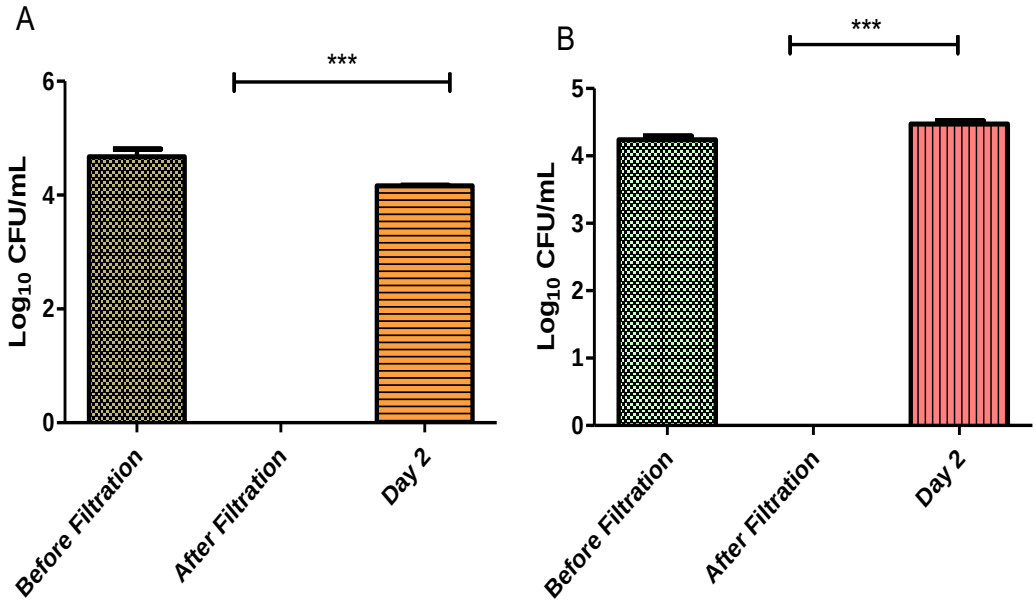


Figure 3.13: A: Number of heterotrophic bacteria in the Plankenburg River water after treatment with the second Swoxid prototype under controlled conditions over 2 days. **B**: Number of heterotrophic bacteria in the Plankenburg river water after treatment with the second Swoxid prototype under experimental conditions over 2 days.

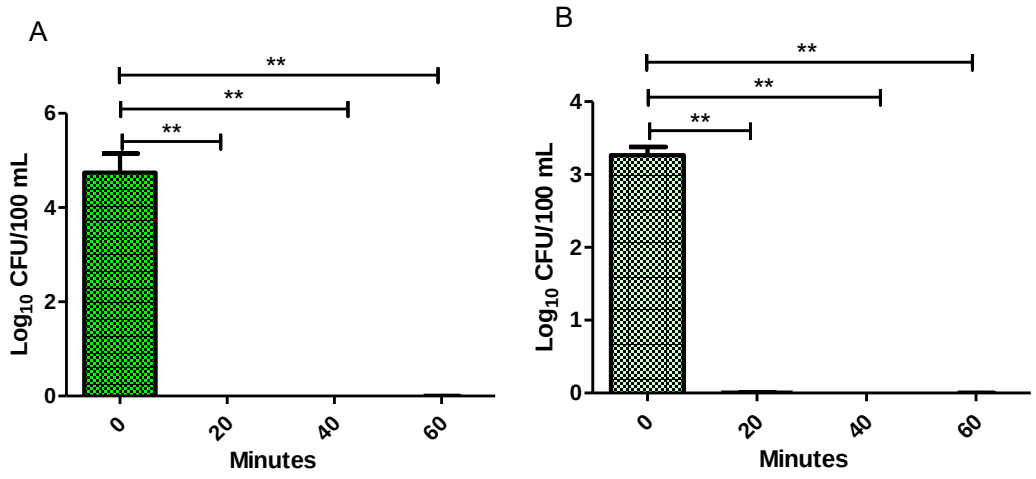
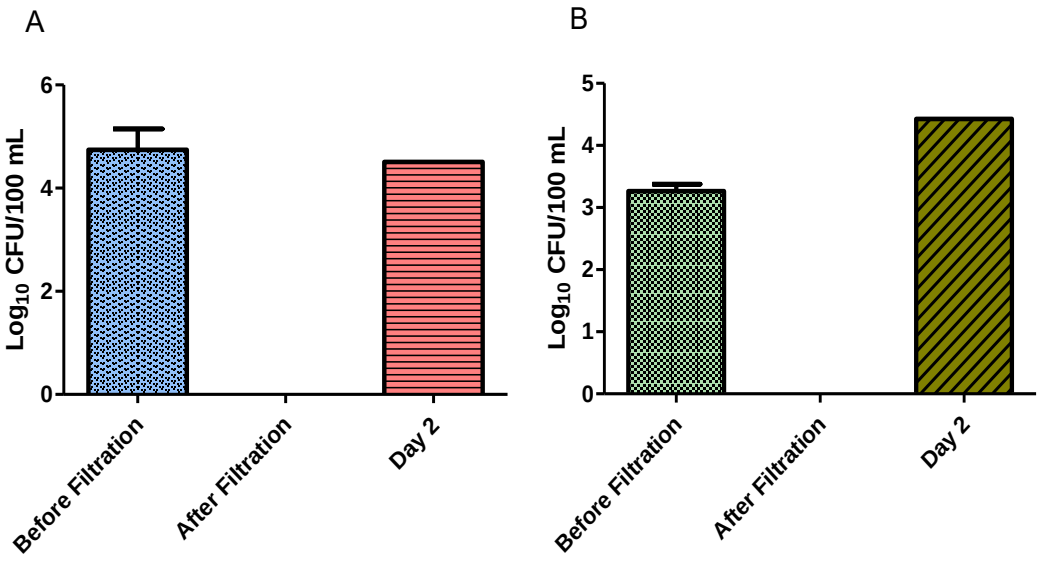


Figure 3.14: A: Number of coliforms in the Plankenburg River water before and after treatment with the second Swoxid prototype under controlled conditions over 60 min. **B**: Number of coliforms in the Plankenburg River water before and after treatment with the second Swoxid prototype under experimental conditions over 60 min.



.**Figure 3.15: A**: Number of coliforms in the Plankenburg river water before and after treatment with the second Swoxid prototype under controlled conditions over 2 days. **B**: Number of coliforms in the Plankenburg river water before and after treatment with the second Swoxid prototype under experimental conditions over 2 days.

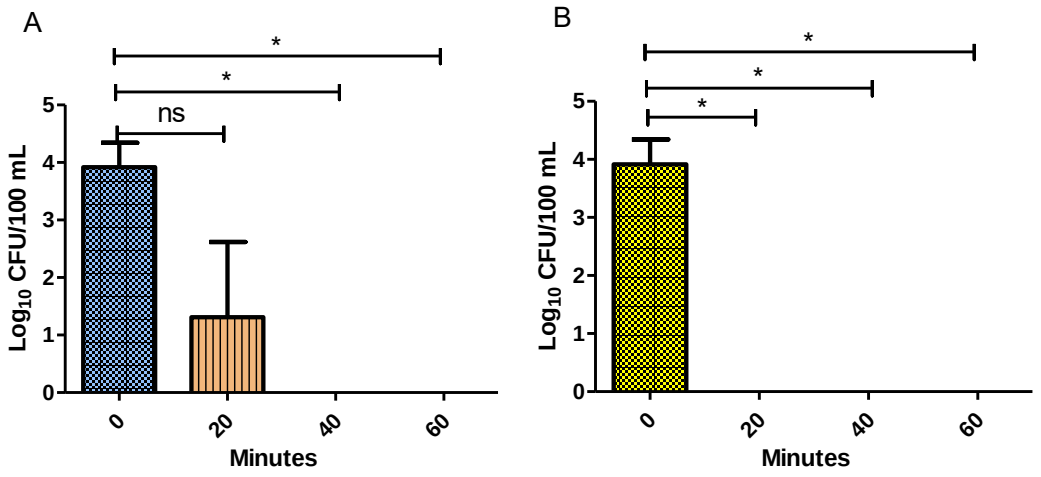


Figure 3.16: A: Number of the faecal coliforms in the Plankenburg River water before and after filtration with the second Swoxid prototype under controlled conditions over 60 min. **B**: Number of the faecal coliforms in the Plankenburg River water before and after filtration with the second Swoxid prototype under controlled conditions over 60 min.

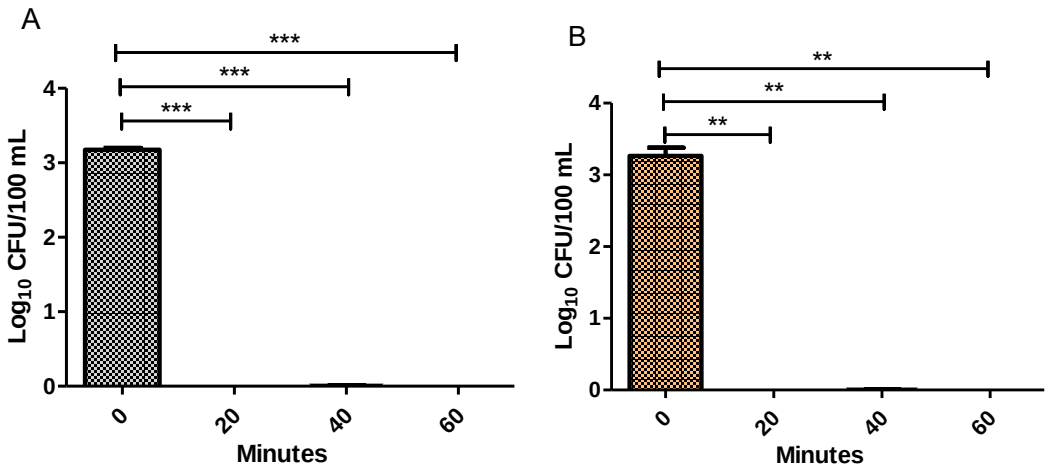


Figure 3.17 A: Number of *Enterococcus* spp. in the Plankenburg river water before and after filtration with the second Swoxid prototype under controlled conditions over 60 min. **B**: Number of *Enterococcus* spp. in the Plankenburg river water before and after filtration with the second Swoxid prototype under experimental conditions over 60 min.

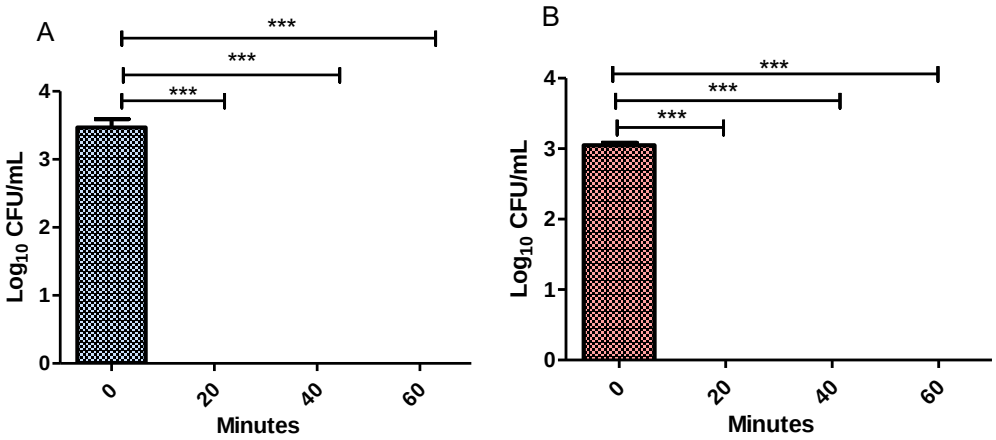


Figure 3.18 A: Number of *Salmonella* and *Shigella* spp. in the Plankenburg river water before and after filtration with the second Swoxid prototype under controlled conditions over 60 min. **B**: Number of *Salmonella* and *Shigella* spp. in the Plankenburg river water before and after filtration with the second Swoxid prototype experimental controlled conditions over 60 min.

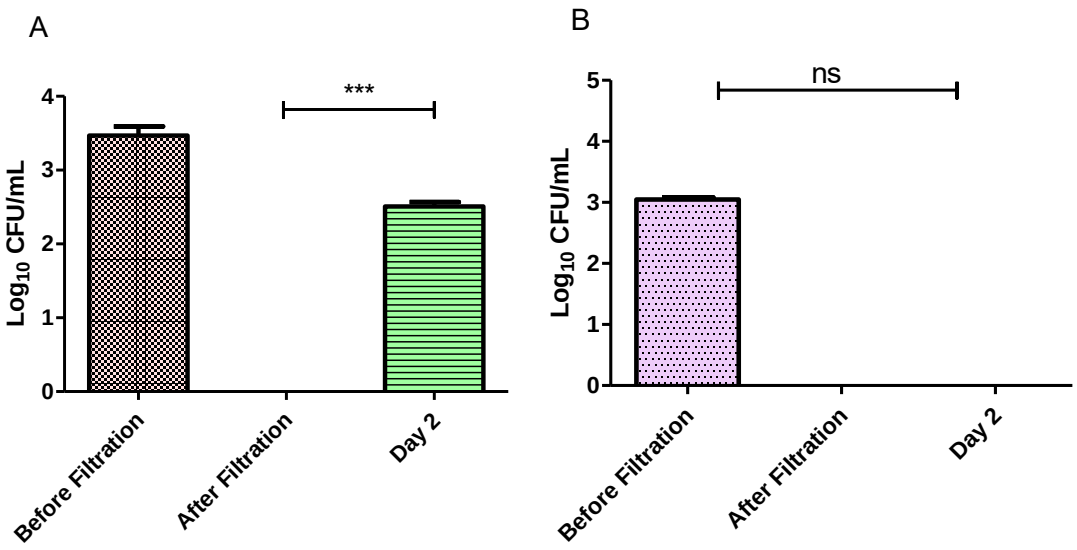


Figure 3.19 A: Number of *Salmonella* and *Shigella* spp. in the Plankenburg river water before and after treatment with the second Swoxid prototype under controlled conditions over 2 days. **B**: Number of *Salmonella* and *Shigella* spp. in the Plankenburg river water before and after treatment with the second Swoxid prototype under experimental conditions over 2 days.

3.3.2. Effect of Exposure to the Eheim UV Purifier and Swoxid Prototype on Bacteria

The effect of radical formation (upon exposure of the Swoxid membrane on the glass stub to light) on the bacterial cell surface was determined using SEM. Three controls were included and they assessed conditions under no exposure to UV but to the membrane (Figure 3.20), no exposure to the UV and the membrane (Figure 3.21) and exposure to UV but not to the membrane (Figure 3.23). The experimental condition (exposure to both UV and the membrane) is depicted in Figure 3.22. The images demonstrate that there is not much difference in the appearance of the *S. aureus* Xen 36 cells under any of the control conditions. For all three control conditions, the cells appear intact. This indicates that UV exposure did not damage the membrane of the cells (Figure 3.23). However, the cells exposed to the Swoxid membrane under UV appeared cracked. This indicates that the radicals generated by TiO_2 – UV light reaction may have damaged the membranes of the *S. aureus* Xen 36 cells. Although there were not enough radicals generated to inactivate bacteria completely, the radicals that were generated were sufficient to start oxidising the lipid bilayer of the bacterial cells.

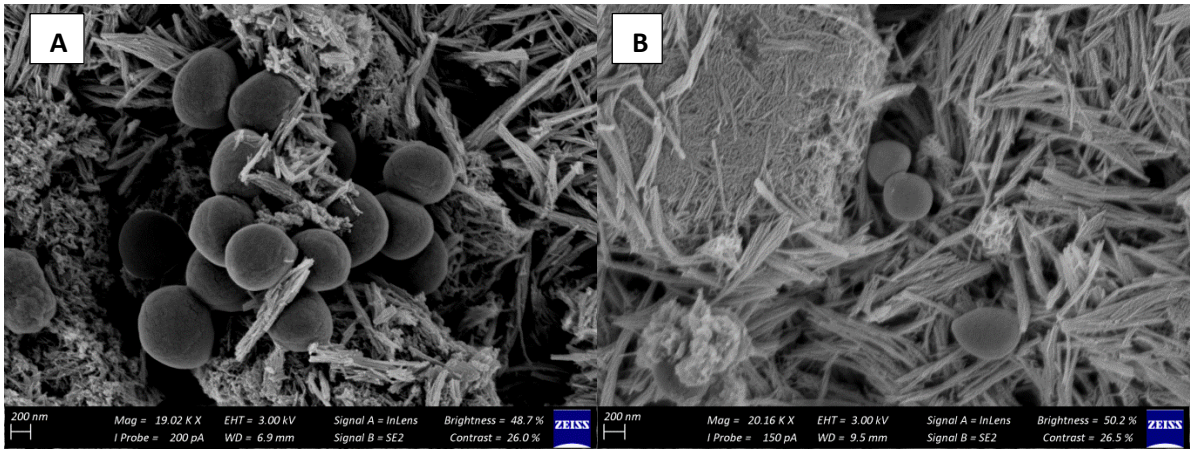


Figure 3.20 A and B: SEM image of *S. aureus* Xen 36 cells on the Swoxid membrane without exposure to UV.

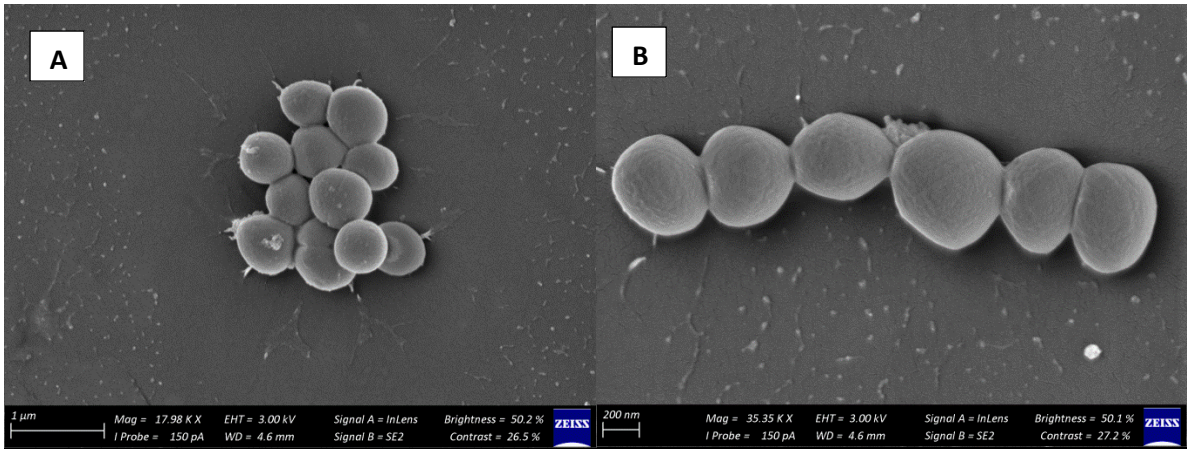


Figure 3.21 A and B: SEM image of *S. aureus* Xen 36 cells on the glass stub without exposure to UV.

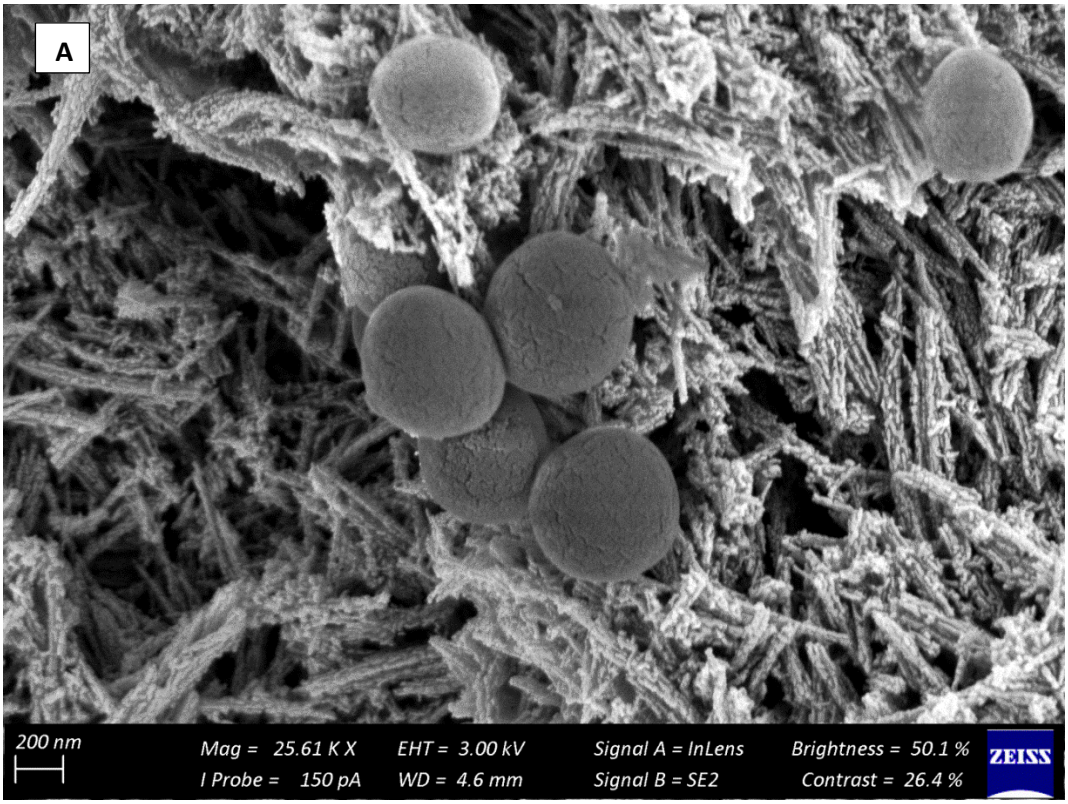


Figure 3.22 A: SEM image of *S. aureus* Xen 36 cells on the Swoxid membrane with exposure to UV.

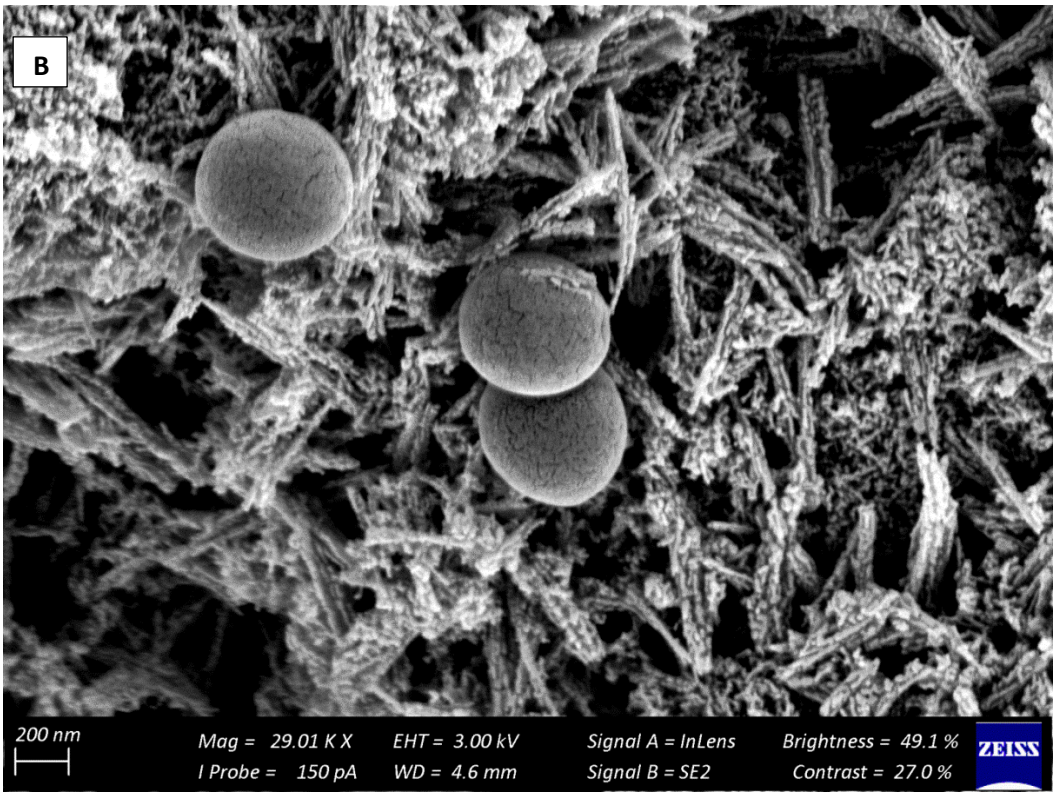


Figure 3.22 B: SEM image of *S. aureus* Xen 36 cells on the Swoxid membrane with exposure to UV.

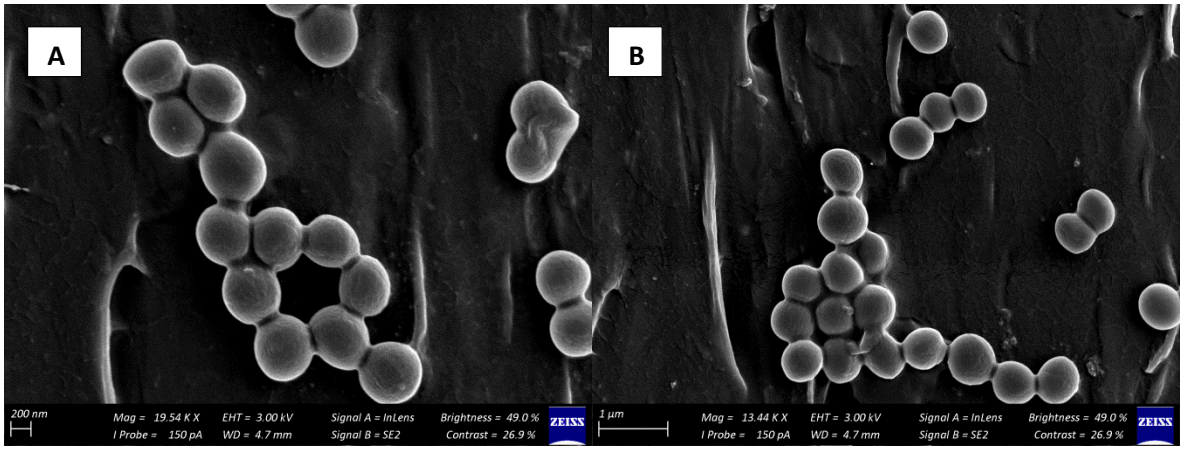


Figure 3.23: SEM image of *S. aureus* Xen 36 cells on the glass stub with exposure to UV

3.3.3. Determining the Efficiency of the Eheim UV Purifier and the second Swoxid Prototype in Removing Micropollutants

The results show that the Swoxid prototype is effective at removing several micropollutants. For example, for SMX (Figure 3.24 A) a 96 % removal was observed. However, it was lower than removal observed by the control, which yielded a 100 % although the difference was not statistically different. This suggests that in these tests, the removal of the compound was mainly due to UV bombardment and not due to exposure to radicals. This was unexpected, as UV treatment usually relies on co-treatment with ROS such as H_2O_2 to be effective in degrading chemical compounds (Ao & Liu, 2017).

Contrary to SMX, CBZ was harder to degrade and only yielded an 18 % removal effiency for the Swoxid prototype (Figure 3.24 B). Interestingly, although CBZ is subject to photolysis, treatment with the Eheim UV Purifier only yielded an 11 % removal (difference not statistically significant). Removal of CBZ was ineffective for both technologies, demonstrating the recalcitrance of CBZ, as also showed in the case wastewater treatment (Golan-Rozen et al., 2011).

Figure 3.24 depicts an array of the results of the experiment with micropollutants in the Plankenburg river water. CBZ is not shown as the levels were below the accurate detection limit. The results show that a negative removal of -37 % is observed for the Eheim UV Purifier in the removal of acetaminophen (ACTM) and a low removal of 18 % is observed for ACTM for the Swoxid (Figure 3.25 A). The negative removal of ACTM can potentially be explained by the fact that some of the ACTM that occurs in river is in the conjugated forms of

the compound (ACTM glucuronide, ACTM sulphate and N-acetyl-p-benzoquinone imine) as was metabolised and excreted by individuals that consumed the drug (Mazaleuskaya et al., 2015). Therefore, deconjugation of the conjugated form ACTM may have occurred after exposure to UV resulting in higher levels of ACTM in the effluent than in the influent (Al Qarni et al., 2016). Alternatively, the negative removal could be caused by matrix interference, which led to suppression in the detection of ACTM in the influent samples (Yadav et al., 2019).

Interestingly, despite the low removal efficiency obtained for the Swoxid prototype, higher removal efficiencies of ACTM have been obtained from treatment at WWTPs. Micropollutants are removed from WWTPs by coagulation-flocculation where coagulants are used to destabilize colloidal matter and emulsions. The alternative mechanism of removal of micropollutants is biodegradation and biotransformation where microorganisms are used to transform and degrade micropollutants into less potent derivatives (Das et al., 2017). A 76-98 % removal was obtained by two WWTPs in the North West province in a study by Kanama et al., (2018). Moreover, oxidation by the Fenton method and the zero valent aluminium-acid system also led to higher removal efficiencies, namely 98 % and above 99 %, respectively (Briones et al., 2012; Zhang et al., 2012). Furthermore, an alternative oxidative system consisting of a TiO_2 solution irradiated with a 250 W metal halide lamp led to 95 % removal of ACTM (Zhang et al., 2008). All the treatment processes mentioned above are more effective in removing ACTM than the Swoxid prototype.

A 6 % removal was observed for the Eheim UV Purifier for benzotriazole (BZT) and an 86 % removal was observed for the Swoxid prototype (Figure 3.25 B). The results were statistically significant with a P value of 0.0338. The low removal for the Eheim UV Purifier and the high removal for the Swoxid prototype demonstrates that in this case, it was not UV bombardment that degraded BZT but the radicals generated by the reaction of TiO_2 with UV. The removal efficiency of BZT by the Swoxid is slightly above the removal obtained in a separate study by Jorfi et al., (2017) where a system using a combination of the Fenton reaction and TiO_2 and UV to generate radicals was used to degrade BZT. The system had optimized conditions (varying pH, reaction times, and catalyst and BZT dosages) and the removal obtained was 72 % (Jorfi et al., 2017). The removal of BZT by the Swoxid prototype also exceeds the removal by some WWTPs, which have been shown to have a removal efficiency below 68 % (Asimakopoulos et al., 2012; Herzog et al., 2014). However, a study by Chen et al., (2018) showed an alternative TiO_2 – UV system where the TiO_2 was irradiated for 30 min yielded a higher removal efficiency of 97 % (Chen et al., 2018).

Very low removal efficiencies were observed for both the Eheim UV Purifier and the Swoxid prototype for caffeine (Figure 3.25 C) and cocaine (Figure 3.25 D). Interestingly, despite the low removal efficiency obtained for the Swoxid prototype, a high removal efficiency (between 95 -98 %) for caffeine has been obtained and reported with treatment by WWTPs in Egypt and Saudi Arabia (Younes et al., 2018; Qarni et al., 2016). Furthermore, an alternative oxidation process, namely electrochemical oxidation used in the study by Al-Qaim et al., (2015) yielded removal efficiencies between 82 % and 99 % removal of caffeine, which is significantly higher than the removal efficiency of the Swoxid prototype (Al-Qaim et al., 2015). This suggests that caffeine is not that persistent a micropollutant; however, it is not efficiently removed by the Swoxid. Furthermore, an alternative TiO_2-UV system in a study by Sacco et al., (2019) led to a 96 % removal of caffeine when the TiO_2 was irradiated for 6 h (Sacco et al., 2019). Although this system is more effective than the Swoxid prototype, it is impractical in that it takes very long to achieve its efficacy.

Cocaine has been reported to have a removal efficiency between 79 % and 99 % by WWTPs, which is also considerably high and exceeds the removal efficiency of the Swoxid prototype (Bones et al., 2007; Subedi & Kannan., 2014). The high removal efficiencies of cocaine by WWTPs suggest that it may not be persistent even though the Swoxid prototype does not remove the compound efficiently.

In both cases, when compared, the results for the two technologies were statistically non significant. The removal of codeine by the Swoxid prototype (73 %) was slightly higher than the removal by the Eheim UV Purifier (62 %); however because the two removal efficiencies are comparable, it indicates that removal is mainly due to UV bombardments (Figure 3.25 E). The removal efficiency of the Swoxid prototype for codeine slightly exceeds that of some WWTPs, for example the one in Verona which has been reported to have a removal efficiency of 60 % (Grande et al., 2013). In a study by Lai et al., (2015) an alternative oxidative process, namely MnO_2 oxidation led to the removal of codeine of 96-99 % at a pH of 5 and 6 (Lai et al., 2015). The removal obtained by MnO_2 oxidation exceeds that of the Swoxid. This indicates that the Swoxid is slightly more effective than some WWTPs at removing codeine yet less effective than MnO_2 oxidation.

The same applies to the removal of diclofenac (Figure 3.25 F) and efavirenz (Figure 3.25 G). A 91 % and 93 % removal was observed for the Eheim UV purifier and Swoxid prototype in the removal of diclofenac. Moreover, the difference between the results was statistically non significant. In the case of efavirenz, a 98 % removal was obtained by the Eheim UV purifier and a 99 % removal was obtained by the Swoxid prototype. The difference between the results for both technologies was statistically non-significant. The high removal of diclofenac

by both technologies corresponds to the removal of other technologies. For example, the removal efficiency of diclofenac by WWTPs is 69 %, which is considerable. (Schwaiger et al., 2004; Younes et al., 2018). Moreover, ozonation can lead to a removal of diclofenac of above 90 % when 5 – 7 mg/mL ozone is used (Altmann et al., 2014).

The Swoxid prototype performed better than WWTPs (in southern Gauteng, South Africa) in the removal of efavirenz which ranges between 27-95 % (Schoeman, Dlamini & Okonkwo, 2017).

The removal of methamphetamine (METH) (Figure 3.25 H), methaqualone (Figure 3.25 I) and 3,4-methylenedioxymethamphetamine (MDMA) (Figure 3.25 J) were all low for both the Eheim UV Purifier and the Swoxid prototype and the results between the two were statistically not significant. The removal of METH by WWTPs is between 44-99 % which is considerably higher than the removal obtained by the Swoxid prototype, which was 18 % (Boles & Wells, 2010). Advanced oxidative process (AOP) is also more effective at removing METH than the Swoxid prototype as demonstrated in a study by Gu et al., (2019) In the study, generation of sulphate and $^{\cdot}OH$ radicals led to complete removal of 100 µg/L METH in 30 minutes (Gu et al., 2019). Moreover, in this instance, the removal efficiency of the Swoxid prototype (24 %) is compared to another TiO_2-UV system in a hollow cylinder, which only led to 45 % (Lin et al., 2013). This suggests that in both instances, the irradiation of TiO_2 by UV was not effective in removing METH which can be explained by the possibility that in both systems, not enough radicals were generated.

The low removal of MDMA with the both the Swoxid prototype and Eheim UV Purifier corresponds to the low removal efficiency obtained by WWTPs of 13-57 % (Paciuszkiewicz et al., 2019; Andrés-Costa et al., 2014). This suggests that the micropollutant is recalcitrant.

For SMX, a high removal efficiency is observed for the UV and the Swoxid systems. Additionally, the removal efficiency of the Eheim UV purifier was higher than that of the Swoxid prototype and the difference was statistically significant (P value is 0.014). This further supports the deduction that UV bombardment is sufficient in removing certain micropollutants and that the radicals generated by the Swoxid prototype are not sufficient to degrade chemical pollutants. Interestingly, the removal efficiency obtained for the Swoxid prototype, which is 81 % exceeds that obtained for MBR treatment and WWTPs which is between 66-67 % and 34-56 %, respectively (Hai et al., 2011; Hendricks & Pool, 2012). Moreover, the removal efficiency obtained for the Swoxid prototype of 81 % is comparable to an alternative TiO_2-UV system where the TiO_2 is coupled to platinum and palladium and led to a removal of SMX of 90 % (Borowska et al., 2019).

With most of the micropollutants, the Swoxid prototype was less effective than other technologies and in some cases even other TiO_2-UV systems. This can be explained by the possibility that too little radicals were generated during experimentation. Moreover, it is also possible that the radicals that were generated were quenched by the organic matter in the river water, whereas the other technologies tested removal of the micropollutants with pristine water, except WWTPs. Additionally, the radicals only act over a short distance which may also hinder the efficacy of the prototype.

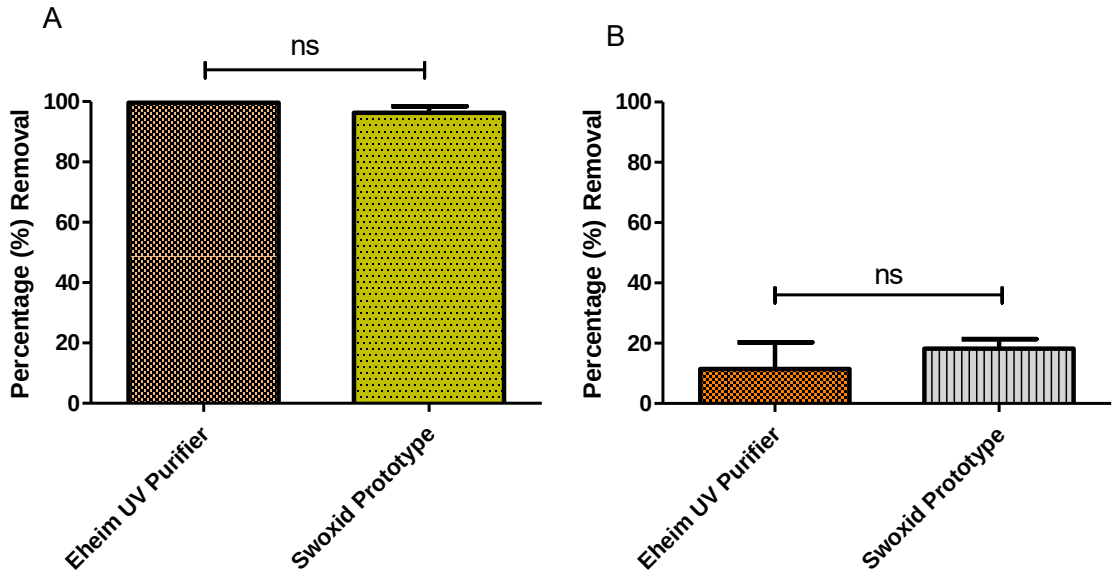
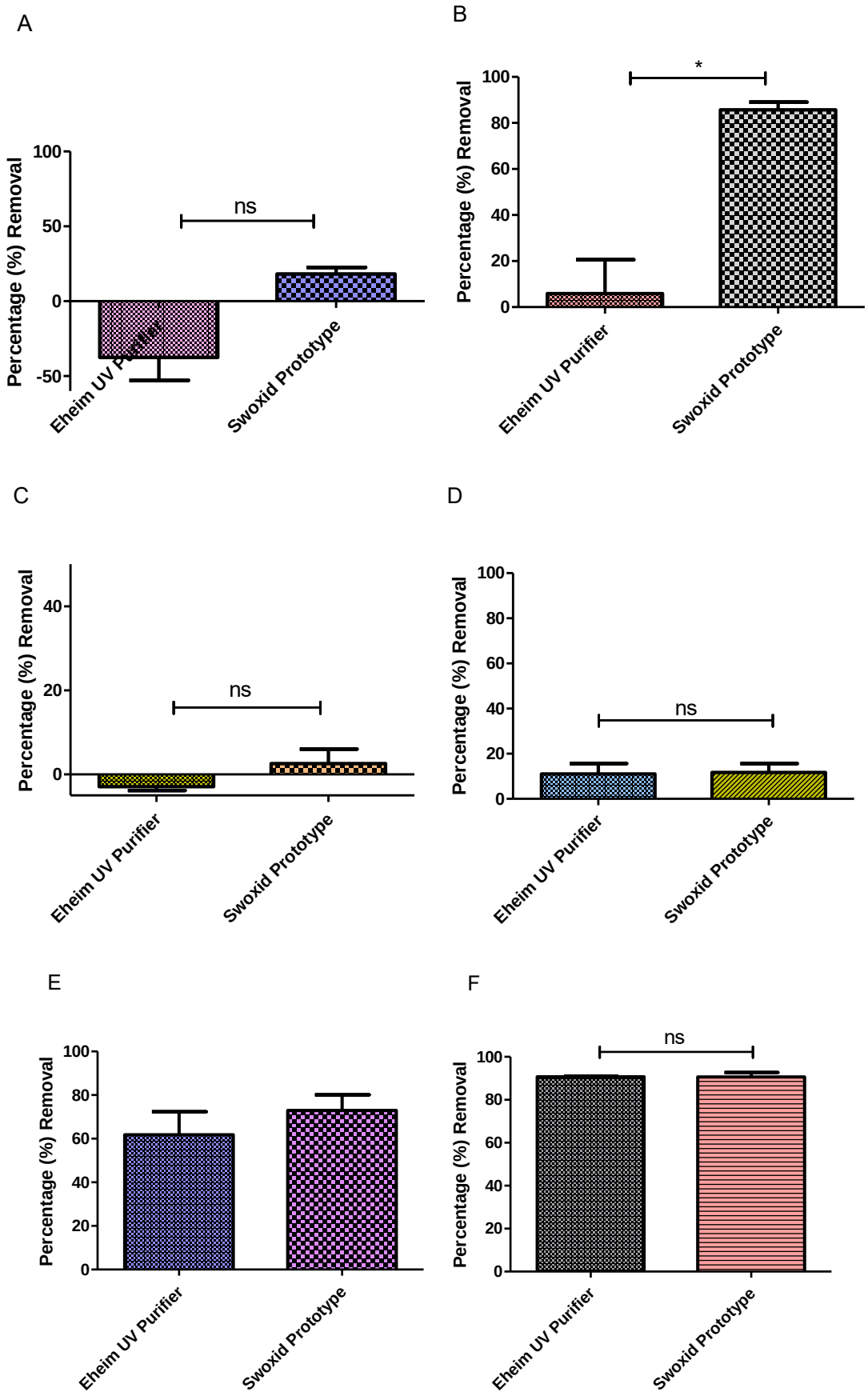


Figure 3.24. A. Efficiency of the Swoxid prototype in comparison to the Eheim UV purifier in the removal of SMX. **B**. Removal efficiency of the Swoxid prototype in comparison to the Eheim UV purifier in the removal of CBZ.



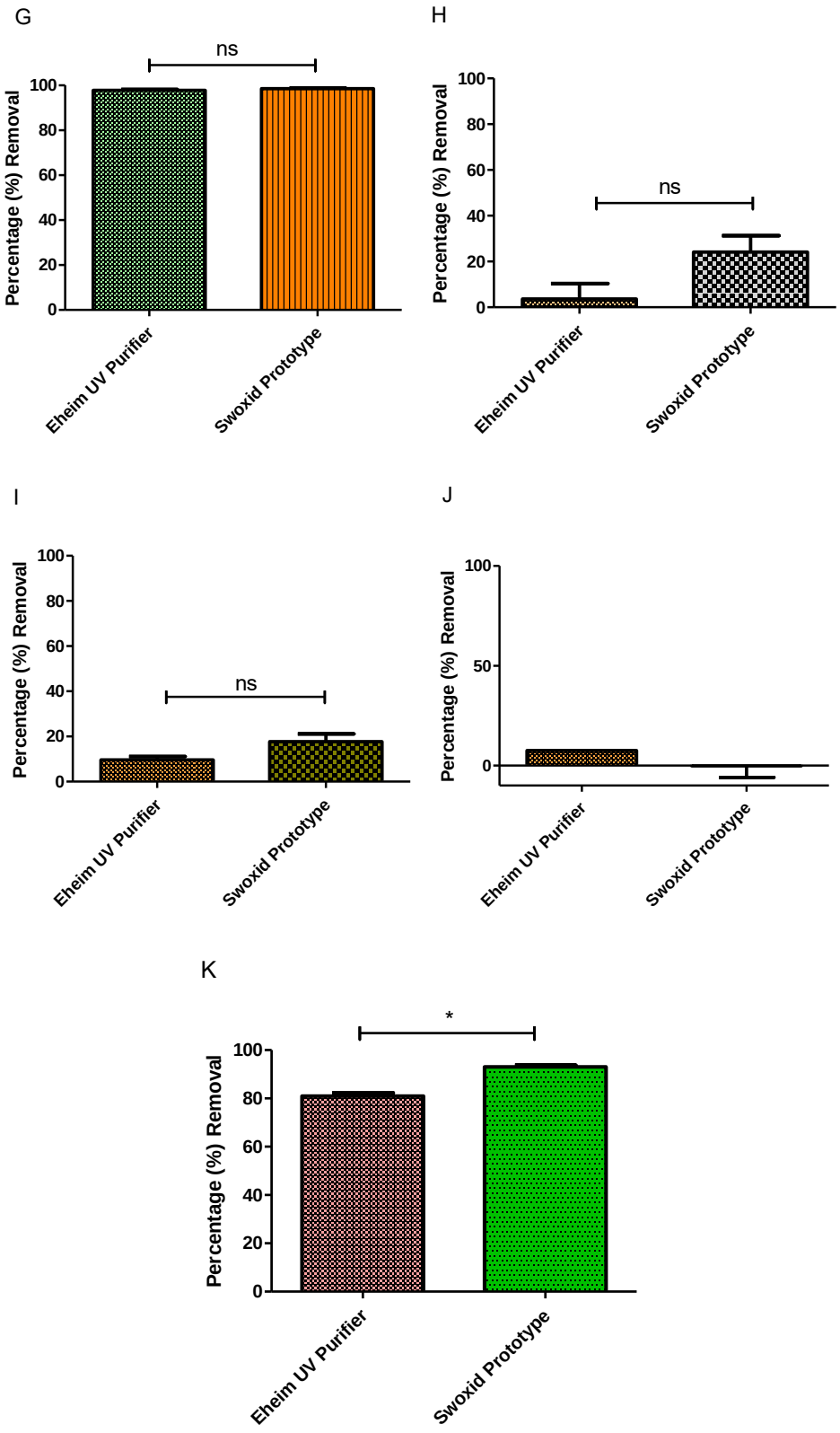


Figure 3.25. Efficiency of the Swoxid prototype and the Eheim UV purifier in the removal of **A**: Acetaminophen; **B**: Benzotriazole; **C**: Caffeine; **D**: Cocaine; **E**: Codeine; **F**: Diclofenac; **G**: Efavirenz; **H**: Methamphetamine; **I**: Methaqualone; **J**: MDMA and **K**: SMX in the Plankenburg River water.

Although the Swoxid prototype was efficient in removing bacteria and micropollutants, it may not necessarily be practical to use. For example, consumers may not understand how to use the technology and may become lost in translation due to language barriers. Moreover, the first prototype can be easily broken as it made of glass. Furthermore, although the second prototype is a modification of the first one, it is not the final model as the main objective is to use gravitational flow as the mechanism with which water transverses the filter.

Antimicrobial Nanofiber Membrane

3.3.4. Leaching of Cu- and Zn-ions from the nanofiber membranes

The concentrations of the Cu(II) and Zn(II) ions that leached from the antimicrobial nanofiber membranes are indicated in Figure 3.26. The recommended limits for the Cu(II) and Zn(II) ions according to the South African National Standards for drinking water are 2000 µg/L and 5000 µg/L, respectively. The limits set by the United States Environmental Protection Agency (USEPA) for Cu(II) and Zn(II) ions are 1000 µg/L and 5000 µg/L, respectively. Yet, the maximum quantity of Cu(II) that leached was below 40 µg/L and the maximum quantity of Zn(II) that leached was approximately 100 µg/L. Therefore, the quantities of Cu(II) and Zn(II) that leached from the membranes are below the recommended limit for each of the ions, thus making the membranes safe to use for filtration. However, a higher concentration of Cu(II) and Zn(II) leached from the membranes containing a higher density than from their low density counterparts (Figure 3.26). This may be due to the higher density membranes having a larger surface area enabling them to retain more of the biocide.

Additionally, although the BioClear biocide contains a higher concentration of Cu(II) than Zn(II), the concentration of Zn(II) that leached was more than Cu(II) (Figure 3.26). For example 0.43 % of the Zn(II) leached in comparison to 0.14 % of the Cu(II). The fact that the Zn(II) leaches more readily than the Cu(II) suggests that the Cu(II) may be more tightly bound to the membrane than the Zn(II).

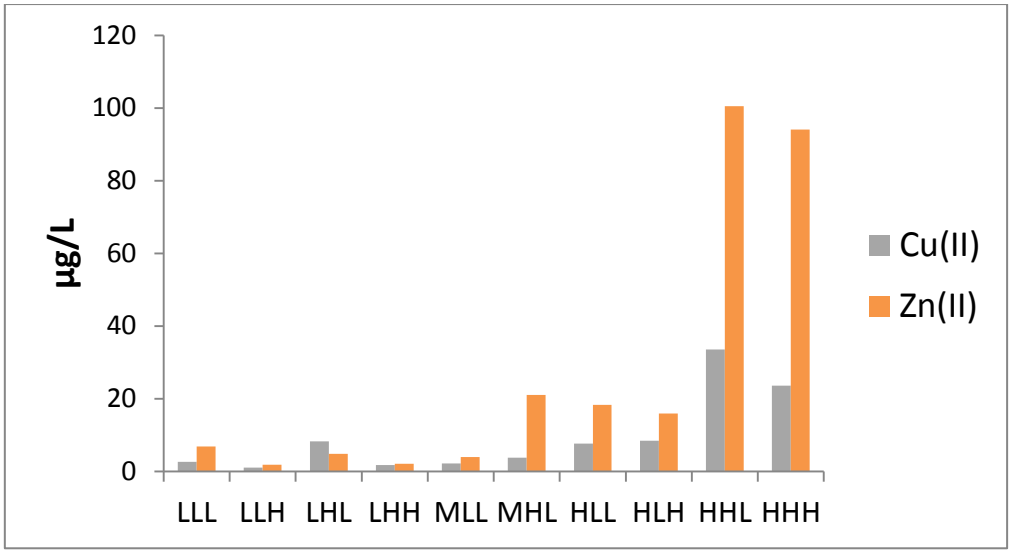


Figure 3.26: Concentrations of Cu(II) and Zn(II) from the antimicrobial nanofiber membranes with different concentrations of the biocide.

Similar results were observed with the biocide-containing high planar density (BHPD) membrane as with the initial thinner variations of the antimicrobial membrane. As shown in Figure 3.27, leaching of Zn(II) peaked almost instantaneously, compared to Cu(II), which showed a time-dependent increase over the course of an hour. Additionally, as with the thinner variations, the concentration of Zn(II) and Cu(II) that leached was below 100 µg/L.

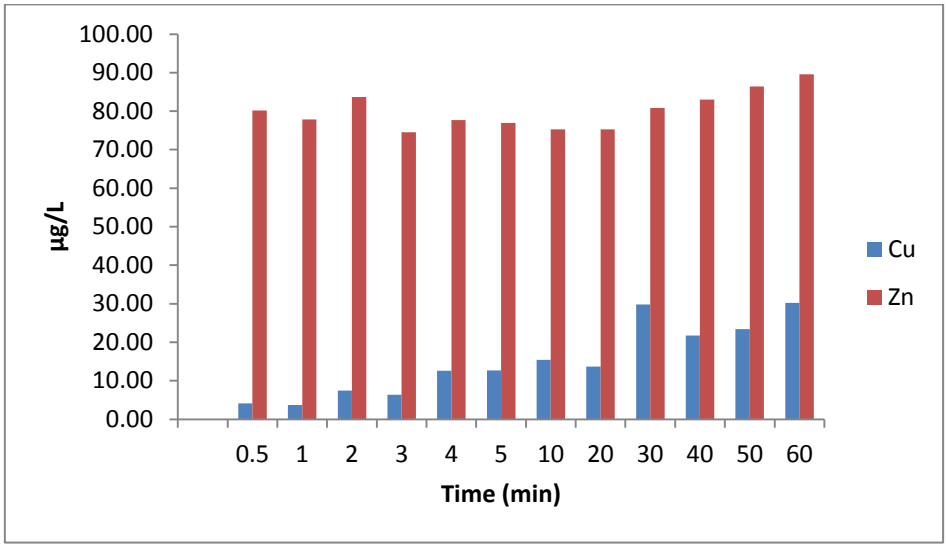


Figure 3.27: Concentrations of Cu(II) and Zn(II) that leached from the BHPD membrane over 60 min.

The experiments were performed only once, due to the high cost and consequently limited number of membranes available for tests.

3.3.5. Determining the shortest contact time needed for the inactivation of the metabolism of *E.coli* Xen 14 and *S.aureus* Xen 36

One of the ways to determine the efficiency of the filters against bacteria is to determine the effect that the membranes have on the metabolism of bacteria. Therefore, *E. coli* Xen 14 and *S. aureus* Xen 36 were exposed to the ten antimicrobial nanofibers and the decrease in bioluminescence was observed over a period of 60 min or until complete inactivation of bioluminescence occurred. The bacterial strains used contain a stable copy of the lux operon from the bacterium *Photoharbdus luminescens* on their chromosomes (http://www.perkinelmer.com/product/xen14-escherichia-coli-119223). The lux operon consists of the genes *luxABCDE*. The gene *luxCDE* encodes a fatty acid reductase complex which is involved in the biosynthesis of the long chain fatty aldehyde that acts a substrate in the luminescence reaction. The gene *luxAB* is more directly involved in the luminescence reaction by oxidising the long chain fatty aldehyde and reduced riboflavin phosphate using oxygen and hydrolysing ATP. When the bacteria are metabolically active, the reactions occur and blue-green light is emitted (Meighen & MacKenzie, 1973). These reactions occur within Xen bacteria and the blue green light emitted is detected by the light detectors of the IVIS Imaging instrument. Figure 3.28 depicts the bioluminescence colour scale where the blue-purple spectrum indicates a low photon/sec count and the red-orange spectrum indicates high bioluminescence activity.

The nanofiber membranes displayed antimicrobial activity of varying degrees. Although some of the membranes containing a low biocide loading led to a considerable decrease in cell metabolism, none of them led to complete inactivation of the metabolism of the bacteria. The LLL membrane displayed very little activity against *E.coli* Xen 14 (Figure 3.29 and Figure 3.31 A) after 60 min as no significant decrease in bioluminescence was observed. However, exposure to the LLH, LHL and the LHH membranes reduced bioluminescence of the *E. coli* Xen 14 cells to some extent as a decrease of 3.272×10^6 photons/sec, 1.935×10^6 photons/sec and 2.607×10^6 photons/sec were observed, respectively over the course of 60 min. The LHL led to a more rapid decrease in bioluminescence than the LLH and the LHH by causing a decrease of bioluminescence by 1.836×10^6 photons/sec within 10 min (Figure 3.29 and Figure 3.31 A).

Similar results were observed for *S. aureus* Xen 36 as with the *E. coli* Xen 14. Exposure to the LHL and LHH membranes reduced the metabolism of the cells. Both led to a decrease in bioluminescence after 10 min and at the end of 60 min, the reduction in bioluminescence was 2.366×10^6 photons/sec for the LHL membrane and 2.683×10^6 photons/sec for the LHH membrane (Figure 30 and Figure 3.31 B). However, unlike with the *E. coli* Xen 14, the

LLL slightly reduced the metabolism of *S. aureus* Xen 36 as the bioluminescence was reduced by 1.009×10^6 photons/sec after 60 min (Figure 30 and Figure 3.31 B). Additionally, no decrease in bioluminescence was observed after exposing the *S. aureus* Xen 36 to the LLH for 60 min.

Complete inactivation of the metabolism of *E. coli* Xen 14 was observed after 20 min with the MLL and MHL membranes (Figure 3.32 and Figure 3.34 A). The MLL reduced the bioluminescence of *S. aureus* Xen 36 by 4.99×10^5 photons/sec whereas the MHL membrane completely inactivated the metabolism of the bacteria after 10 min (Figure 3.33 and Figure 3.34 B).

Complete inactivation of the metabolism of *E.coli* Xen 14 was observed after 10 min with the HLL and HLH just as with the medium biocide loading membranes (Figure 3.35 and Figure 3.37 A). The same was observed for *S. aureus* Xen 36 (Figure 3.36 and Figure 3.37 B). However, the HHL reduced the metabolism of *E. coli* Xen 14 completely after 5 min and the HHH after 1 min (Figure 3.37 A). The same was also observed for *S. aureus* Xen 36 (Figure 3.36 and Figure 3.37 B). Based on the results, it appears as if the high density containing, high fibre diameter membranes are more effective in inactivating the metabolism of the test bacteria than their counterparts. Additionally, based on the difference in the intensity of bioluminescence emitted by *E. coli* Xen 14 and *S. aureus* Xen 36 after exposure to high biocide loading membranes, it appeared as if the membranes were more active against inactivating the metabolism of *S. aureus* Xen 36 than *E. coli* Xen 14. This is also supported by the results obtained with the MHL membranes where it took 20 min for the inactivation of *E. coli* Xen 14 and only 10 min for the inactivation of *S. aureus* Xen 36 (Figure 3.32 and Figure 3.33).

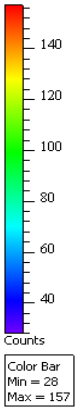


Figure 3.28: Bioluminescence colour scale. Blue-purple end of the spectrum indicates low photons/sec count whereas red-orange spectrum indicates high photons/sec count.

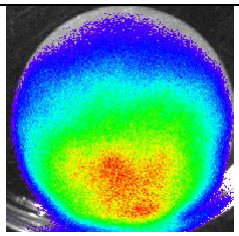
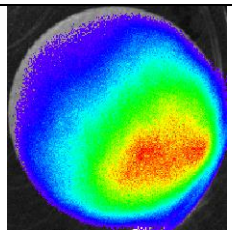
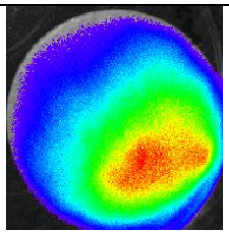
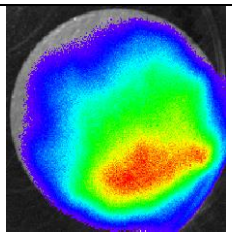
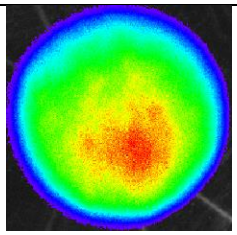
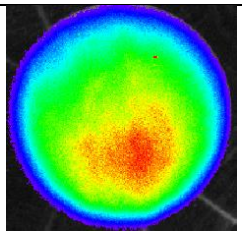
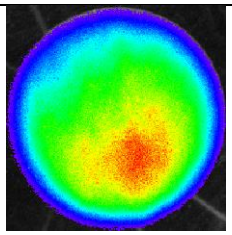
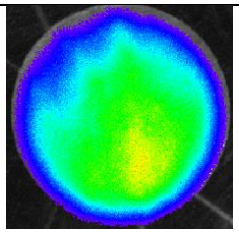
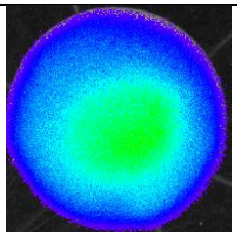
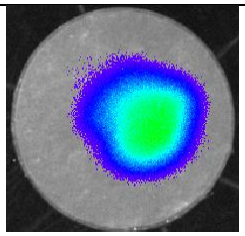
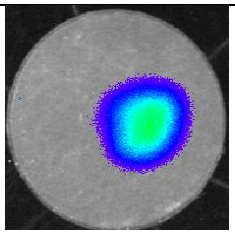
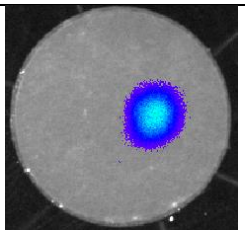
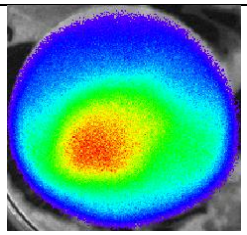
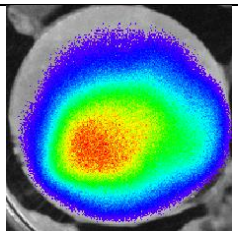
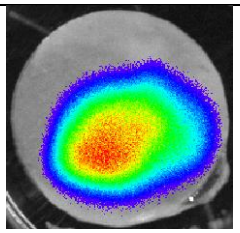
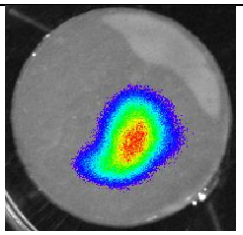
Nanofiber filter	1 min	10 min	20 min	60 min
Low biocide loading, low density, low fibre diameter **(LLL)**				
Low biocide loading, low density, high fibre diameter **(LLH)**				
Low biocide loading, high density, low fibre diameter **(LHL)**				
Low biocide loading, high density, high fibre diameter **(LHH)**				

Figure 3.29: IVIS images of nanofiber filters with low biocide loading membranes, exposed to 10^8 CFU/mL *E. coli* Xen 14 for 60 min

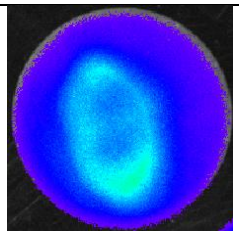
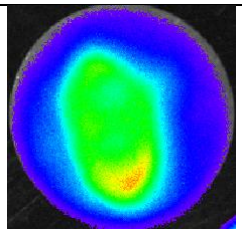
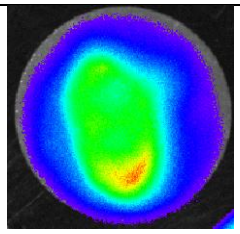
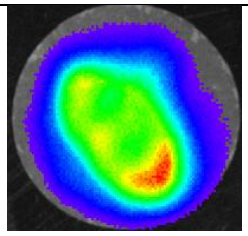
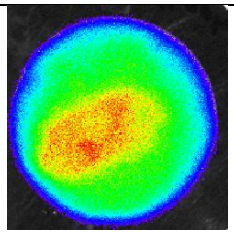
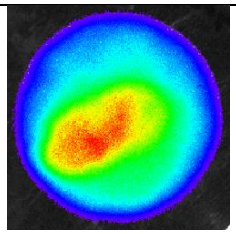
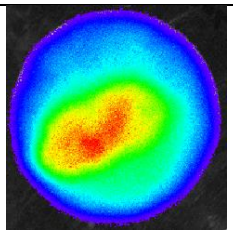
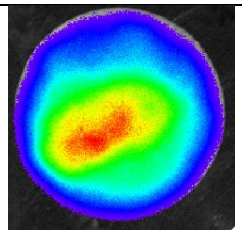
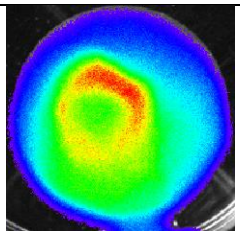
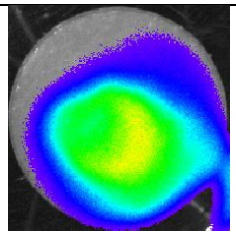
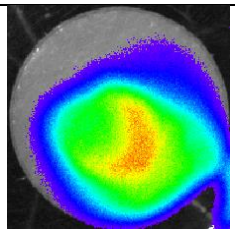
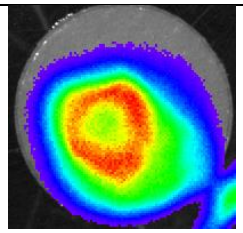
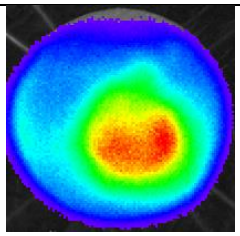
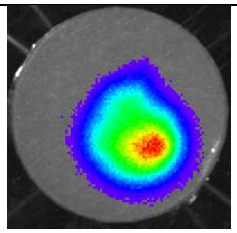
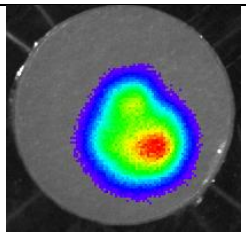
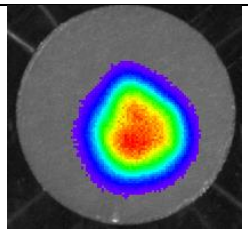
Nanofiber filter	1 min	10 min	20 min	60 min
Low biocide loading, low density, low fibre diameter **(LLL)**				
Low biocide loading, low density, high fibre diameter **(LLH)**				
Low biocide loading, high density, low fibre diameter **(LHL)**				
Low biocide loading, high density, high fibre diameter **(LHH)**				

Figure 3.30: IVIS images of nanofiber filters with low biocide loading, exposed to 10^8 CFU/mL *S. aureus* Xen 36 for 60 min

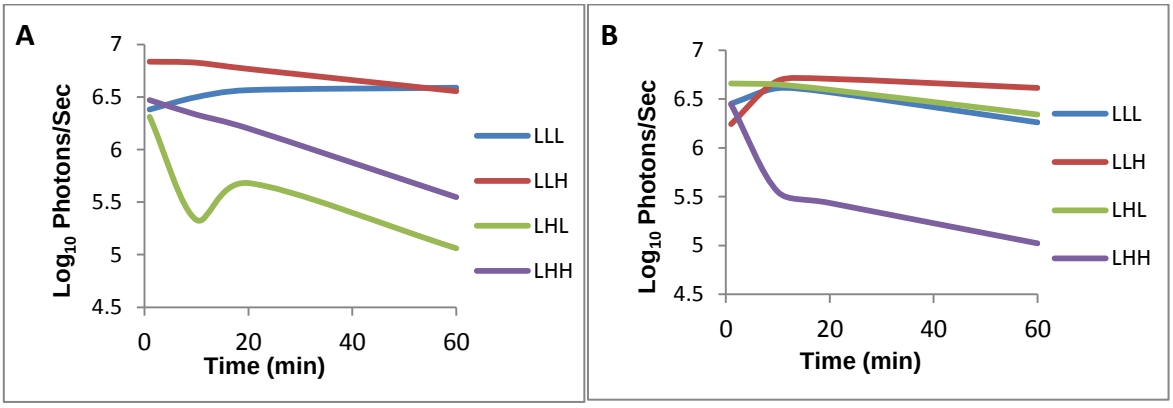


Figure 3.31. A. Change in bioluminescence of *E. coli* Xen 14 over time after exposure to low biocide containing membranes. **B.** Change in bioluminescence of *S. aureus* Xen 36 over time after exposure to low biocide containing membranes.

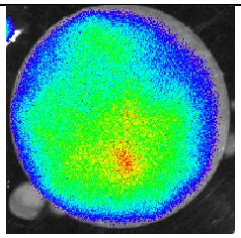
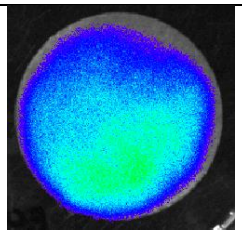
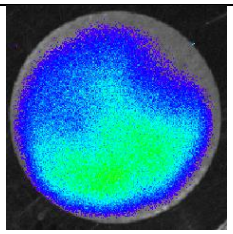
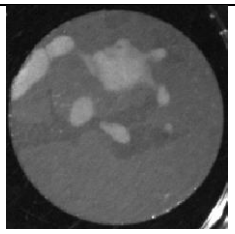
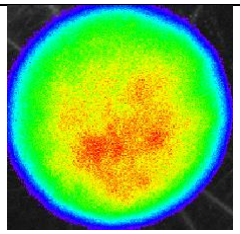
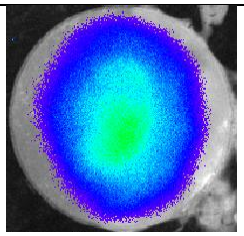
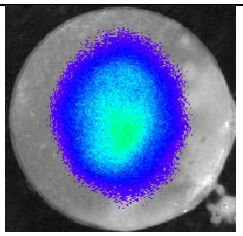
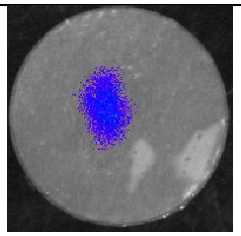
Nanofiber filter	1 min	10 min	20 min	60 min
Medium biocide loading, low density, low fibre diameter **(MLL)**				
Medium biocide loading, high density, low fibre diameter **(MHL)**				

Figure 3.32: IVIS images of nanofiber filters with medium biocide loading, exposed to 10^8 CFU/mL *E. coli* Xen 14 for 60 min

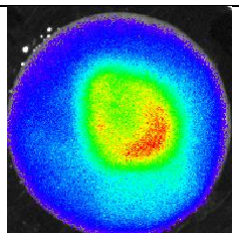
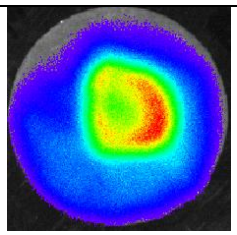
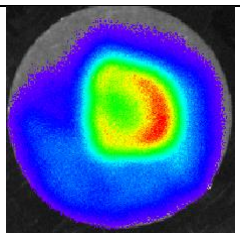
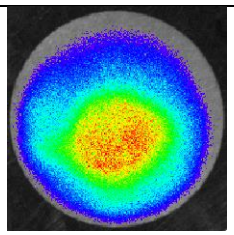
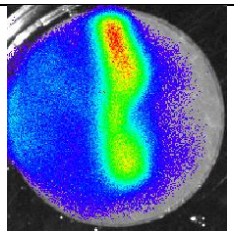
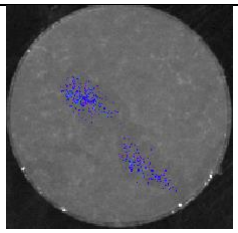
Nanofiber filter	1 min	10 min	20 min	60 min
Medium biocide loading, low density, low fibre diameter **(MLL)**				
Medium biocide loading, high density, low fibre diameter **(MHL)**				

Figure 3.33: IVIS images of nanofiber filters with medium biocide loading, exposed to 10^8 CFU/mL *S. aureus* Xen 36 for 60 min

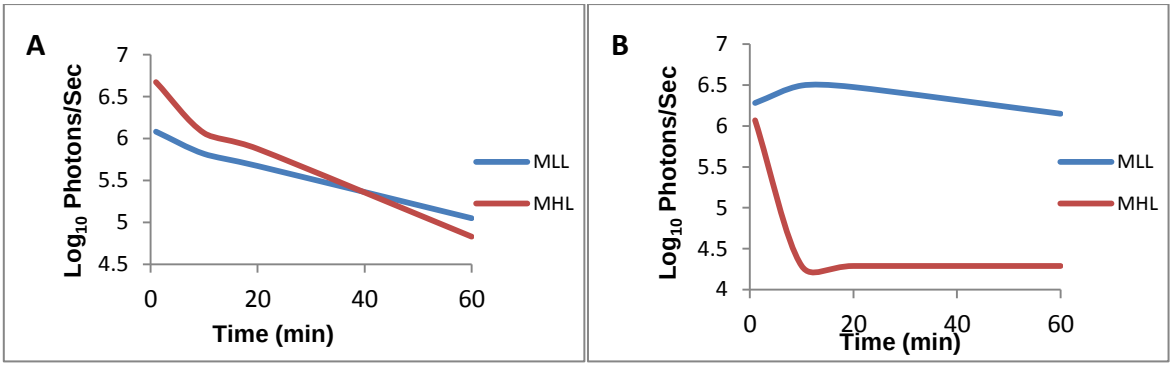


Figure 3.34 A. Change in bioluminescence of *E. coli* Xen 14 over time after exposure to medium biocide containing membranes. **B.** Change in bioluminescence of *S. aureus* Xen 36 over time after exposure to medium biocide containing membranes.

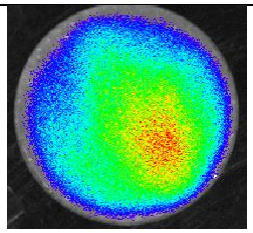
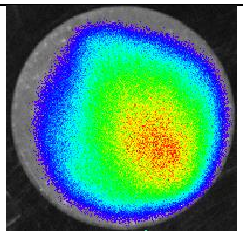
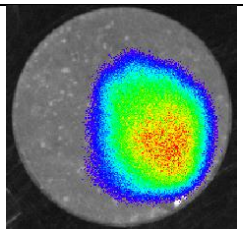
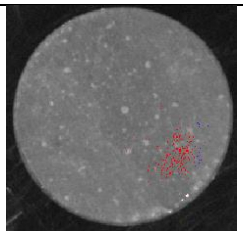
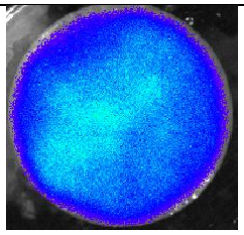
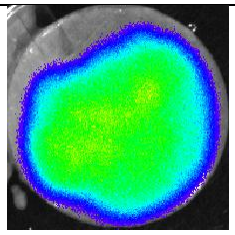
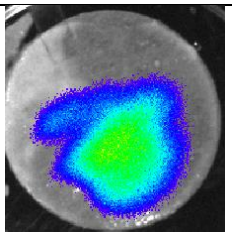
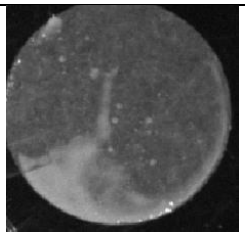
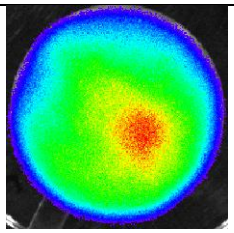
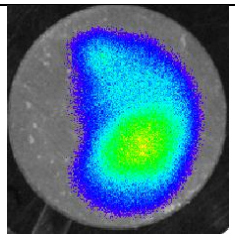
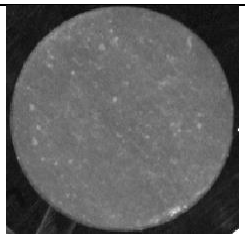
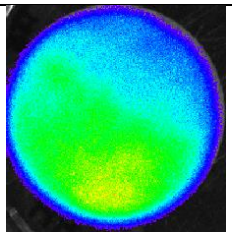
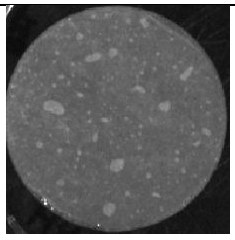
Nanofiber filter	1 min	5 min	10 min	20 min
High biocide loading, low density, low fibre diameter **(HLL)**				
High biocide loading, low density, high fibre diameter **(HLH)**				
High biocide loading, high density, low fibre diameter **(HHL)**				
High biocide loading, high density, high fibre diameter **(HHH)**				

Figure 3.35: IVIS images of nanofiber filters with high biocide loading, exposed to 10^8 CFU/mL *E. coli* Xen 14 for 20 min

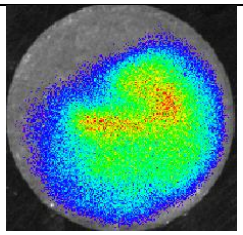
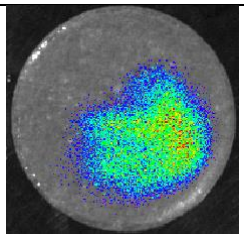
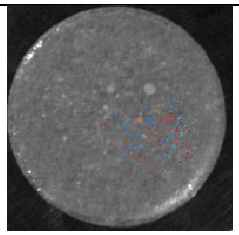
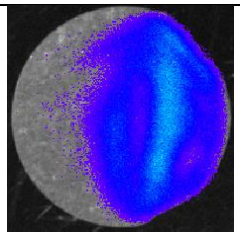
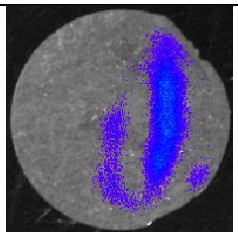
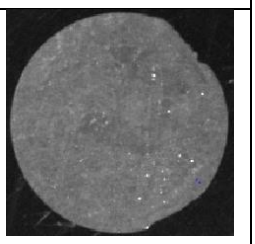
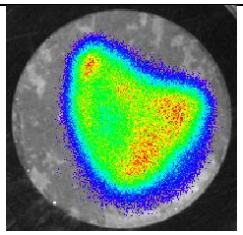
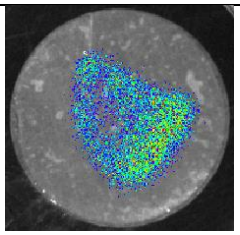
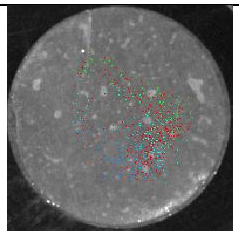
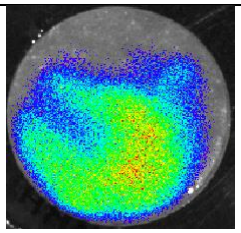
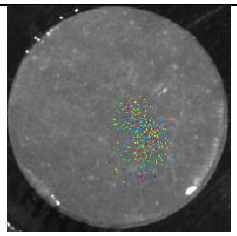
Nanofiber filter	1 min	5 min	10 min
High biocide loading, low density, low fibre diameter **(HLL)**			
High biocide loading, low density, high fibre diameter **(HLH)**			
High biocide loading, high density, low fibre diameter **(HHL)**			
High biocide loading, high density, high fibre diameter **(HHH)**			

Figure 3.36: IVIS images of nanofiber filters with high biocide loading, exposed to 10^8 CFU/mL *S. aureus* Xen 36 up to 10 min

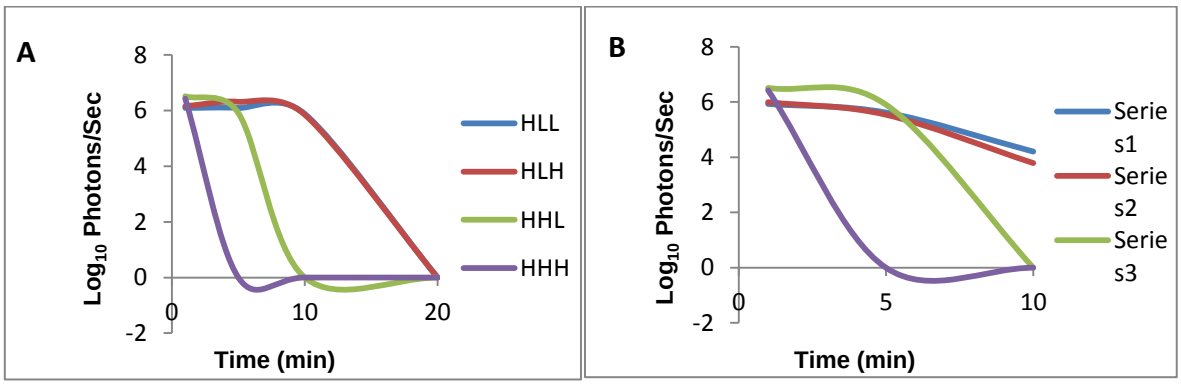


Figure 3.37 A. Change in bioluminescence of *E. coli* Xen 14 over time after exposure to high biocide containing membranes. **B.** Change in bioluminescence of *S. aureus* Xen 36 over time after exposure to high biocide containing membranes.

3.3.6. Determining the antimicrobial activity of the high planar density, biocide-containing membranes

Based on the bioluminescence experiments, it would appear that the high biocide containing membranes performed better than the low and medium biocide-containing membranes. Therefore, growth inhibition experiments were performed with these membranes to determine whether or not the high biocide containing membranes inhibited the growth of the bioluminescent bacteria in addition to halting metabolism.

According to the results of the antimicrobial activity experiments, it is clear that the membranes did not significantly decrease the growth of the test bacteria *E. coli* Xen 14 and *S. aureus* Xen 36 (Figures 3.38 and 3.39) although they significantly hindered the metabolism of the test bacteria. Interestingly, the HLL showed the greatest decrease in the growth of *E. coli* Xen 14, with a log reduction of 1.5 after 20 min as opposed to the HHH membrane which was expected to be more effective based on the results of the bioluminescence experiments.

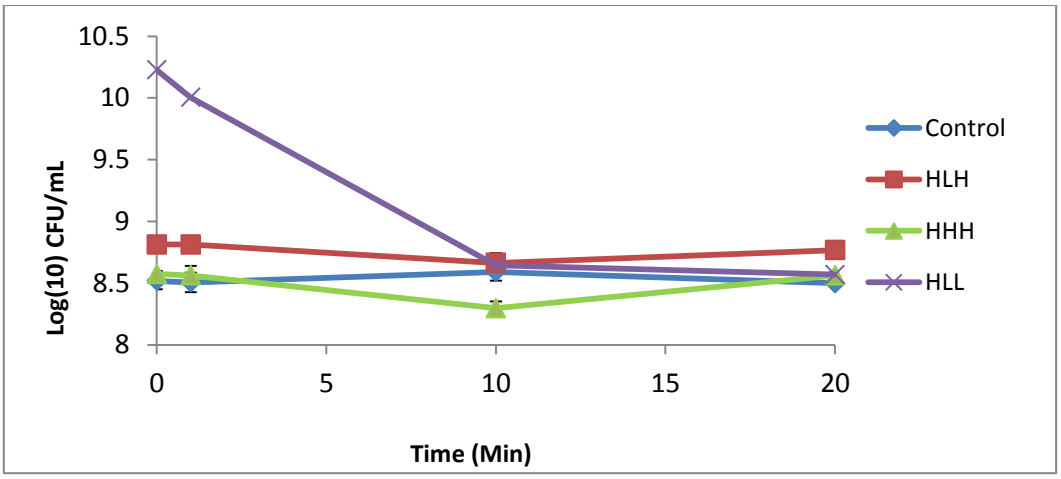


Figure 3.38. Growth of *E. coli* Xen 14 after exposure to the high biocide containing membranes over the course of 20 min

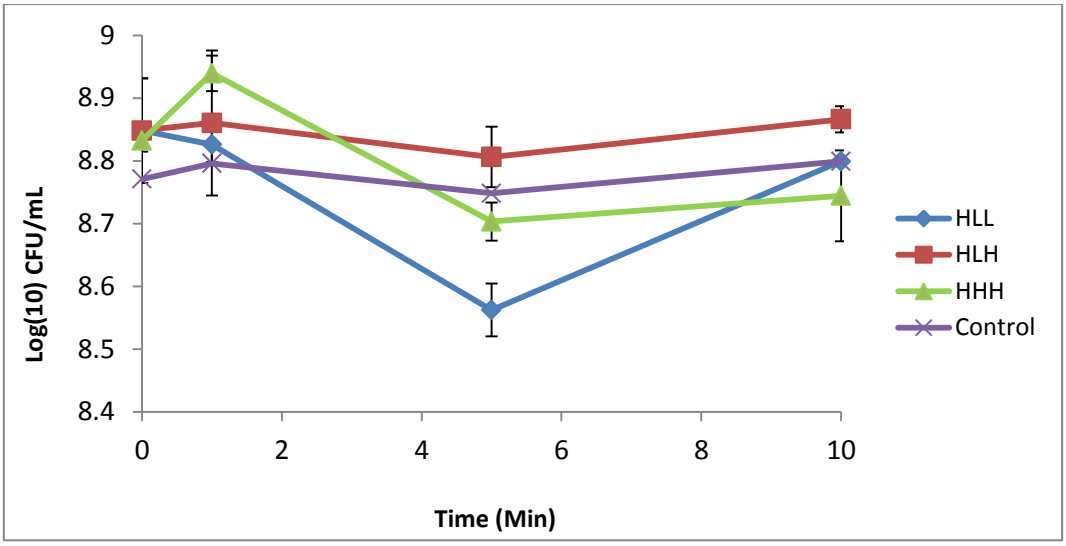


Figure 3.39. Growth of *S. aureus* Xen 36 after the exposure to the high biocide containing membranes over the course of 10 min

3.3.7. Determining the efficiency of the antimicrobial nanofiber membranes filter to remove bacteria from water

In the first trial, 1 litre of RO water spiked with *E. coli* Xen 14 and *S. aureus* Xen 36 was filtered through the HHL and HHH membranes. Although the HHH and HHL membranes showed antimicrobial activity in the bioluminescence experiments, they were ineffective in filtering out and inactivating the test bacteria as there was no significant reduction in bacterial growth before and after filtration (Figures 3.40 – 43). Furthermore, the results of filtration with the biocide containing membranes did not differ significantly to those observed with the negative control. The poor performance could be due to short contact periods of the bacteria with the membrane and the low quantity of Cu(II) and Zn(II) that leach from the membranes. At low concentrations, Cu(II) and Zn(II) cannot effectively inactivate bacteria and this is supported in Figure 5.1. in the appendix. At high biocide concentrations, the growth of bacteria was inhibited whereas doses near the recommended drinking limit for Cu(II), namely the 10 000X dilution at 1363.219 µg/L Cu(II) had little effect on the growth and viability of bacteria. Therefore, exposure of bacteria to the estimated 100 µg/L Cu(II) that leached into the filtrate would have been inadequate to have any biocidal effect. Furthermore, it is also possible that the lack of growth reduction can also be attributed to the short contact time of the bacteria with the fibres. For example, the RO water was filtered at a rate of 0.079 L/min. This indicates that the pore sizes of the membrane may be too large to retain bacteria onto the membrane and allowed for adequate removal of the bacteria and increased contact time with the membrane.

Overall these results are considerably lower than the reduction of bacterial growth reported in literature. For example, similar experiments have been conducted with the antimicrobial nanofiber membranes using a biocide (AquaQure) containing Cu and Zn ions. These poly vinyl acetate (PVA) nanofibers, with the biocide incorporated, led to a 5 log reduction of water spiked with *E. coli* Xen 14, *S. aureus* Xen 36, *S. typhimurium* Xen 26, *P. aeruginosa* Xen 5 and *K. pneumoniae* Xen 39 (Gule, de Kwaadsteniet, Cloete & Klumperman B, 2012). Interestingly, the biocide used had significantly lower content of Cu(II) and Zn(II) ions than BioClear. Furthermore, a higher quantity of the ions (approximately 531-1670 µg/L more Cu(II) and 92-476 µg/L more Zn(II) leached from these membranes and can be explained by the cations being more tightly bound to the PLA nanofibers than to the PVA (Gule, de Kwaadsteniet, Cloete & Klumperman B, 2012).

The filtration experiments were repeated by stacking the high biocide containing membranes, HHL and HHH on top of one another. No bacteria were removed through filtration (particularly the HHL and the HHH). As depicted in Figures 3.44 and 3.45, stacking the membranes on top of one another did not improve filtration efficiency as the colony counts before filtration and after filtration did not differ significantly. Therefore, the membranes, whether singular or stacked on top of each other, were ineffective to filter out or inactivate bacteria.

The use of the BHPD membrane, with a thicker density, removed less than 1 log of *E. coli* Xen 14 but was considered statistically significant (P value of 0.0056) (Figure 3.46 A). The BHPD membrane did not have a significant removal of *S. aureus* Xen 36 (Figure 3.46 B). This suggests that one thick BHPD membrane is almost as inefficient as the less dense membranes. These results were in contrast with the bioluminescence results where a higher number of *S. aureus* Xen 36 cells were killed than *E. coli* Xen 14.

Two BHPD membranes combined showed approximately 2 log reduction in *S. aureus* Xen 36 after filtration (Figure 3.47). Similar results were observed with the combination of a non biocide-containing high planar density (NBHPD) and a BHPD membrane. Complete reduction in the bacterial count was observed when one BHPD and two NBHPD membranes were combined (Figure 3.48). Therefore, it appears that the ideal membrane has three high planar density membranes combined together in one. The results obtained could be due to the enhanced filtration capacity when the three membranes are stacked on top of each other, therefore, reducing pore size, and not because the bacteria made contact with the biocide in the membrane. For example, the pore sizes of one BHPD membrane range within 0.5 – 1.8 µm, however, the stacking of BHPD membranes appear to improve efficiency potentially be reducing the pore sizes through which the bacteria transverse. Alternatively,

the results observed could be due to the fact that when the membranes were stacked on top of each other, the bacteria were forced to filter through the stack and in that manner had more contact time with the BHPD membrane in the middle. However, a conclusive deduction can only be made with a repeat of the experiment using solely NBHPD membranes.

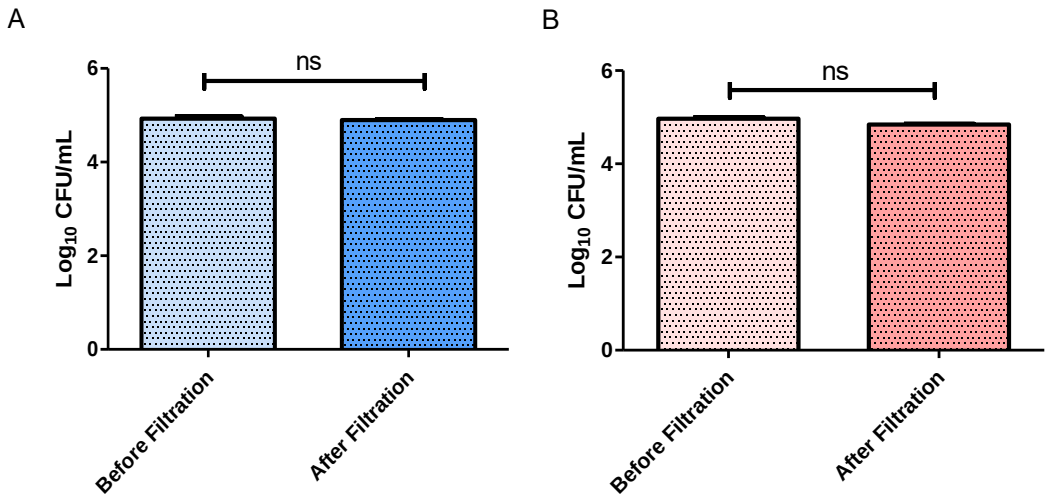


Figure 3.40. A. Number of *E.coli* Xen 14 after filtration with the negative control membrane. **B.** Number of *E.coli* Xen 14 after filtration with the HHH membranes (trial 1).

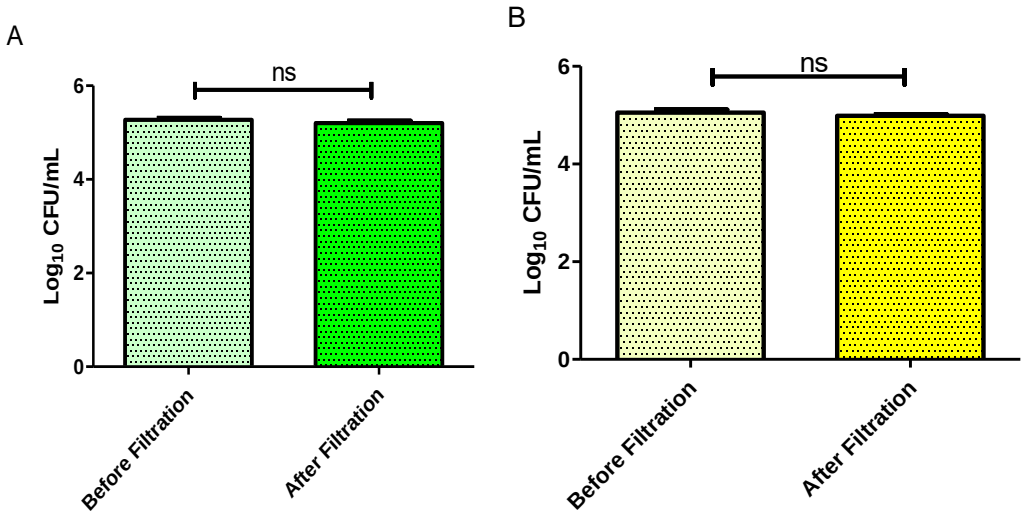


Figure 3.41. A. Number of *S.aureus* Xen 36 after filtration with the negative control membrane. **B.** Number of *S. aureus* Xen 36 after filtration with the HHH membranes (trial 1).

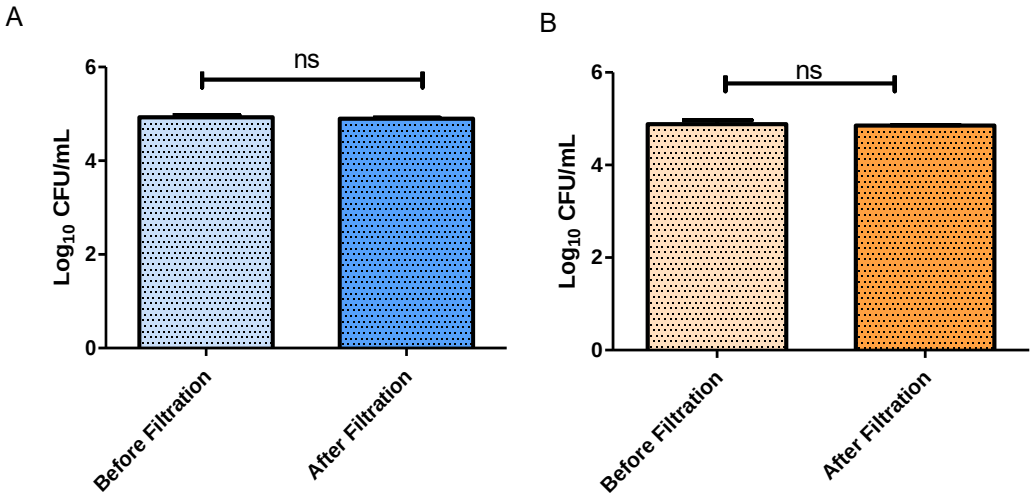


Figure 3.42. A. Number of *E.coli* Xen 14 after filtration with the negative control membrane. **B.** Number of *E.coli* Xen 14 after filtration with the HHL membranes (trial 1).

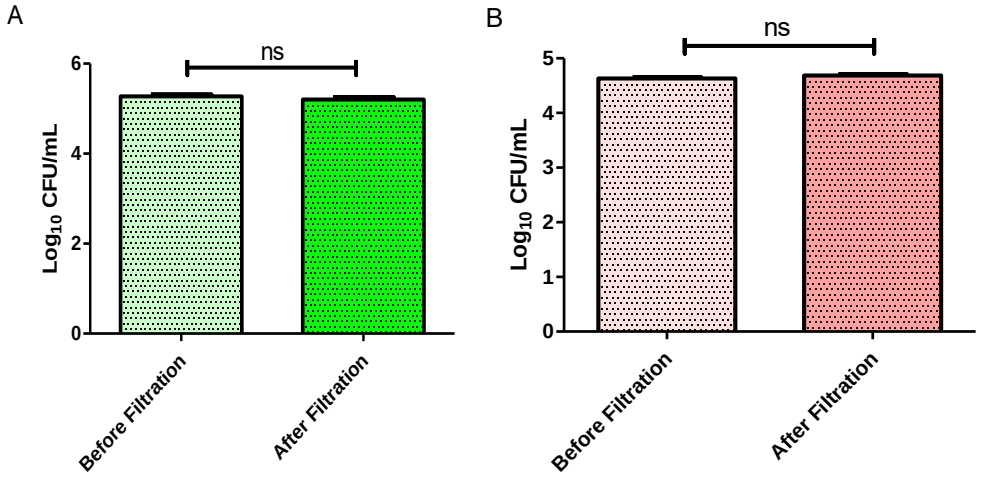


Figure 3.43. A. Number of *S. aureus* Xen 36 after filtration with the HHL membranes. **B.** Number of *S. aureus* Xen 36 after filtration with the HHL membranes (trial 1).

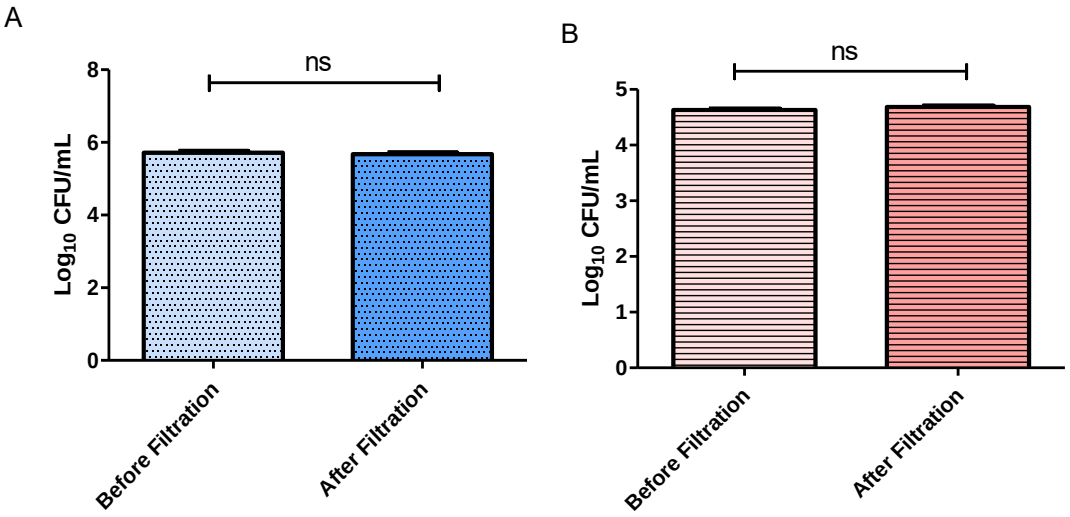


Figure 3.44. A. Number of *E.coli* Xen 14 after filtration with the two negative control membranes stacked on top of each other. **B.** Number of *E.coli* Xen 14 after filtration with the four HH membranes (2 HHH and 2 HHL membranes) stacked on top of each other (trial 2).

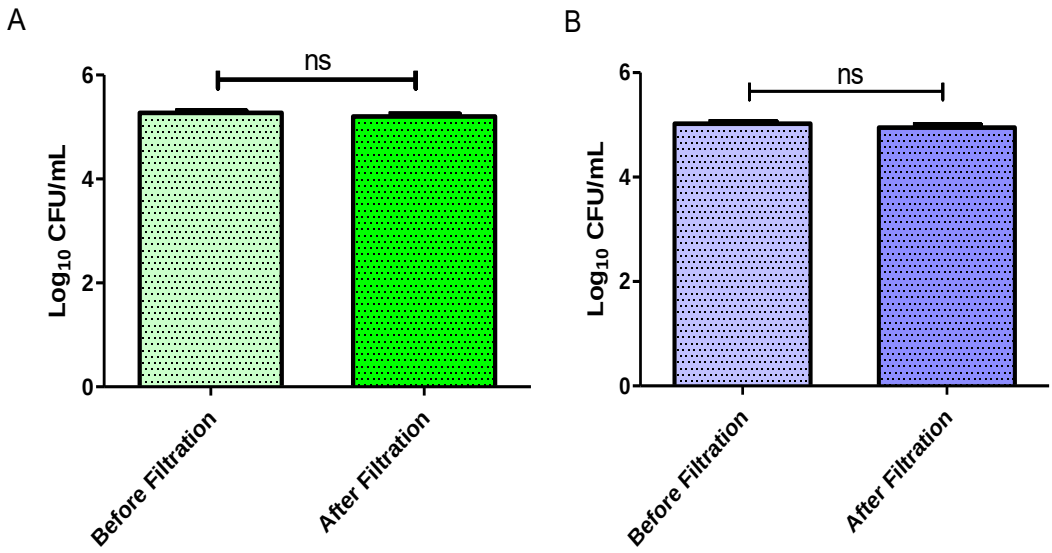


Figure 3.45. A. Number of *S. aureus* Xen 36 after filtration with the two negative control membranes stacked on top of each other. **B.** Number of *S. aureus* Xen 36 after filtration with the four HH membranes (2 HHH and 2 HHL membranes) stacked on top of each other (trial 2).

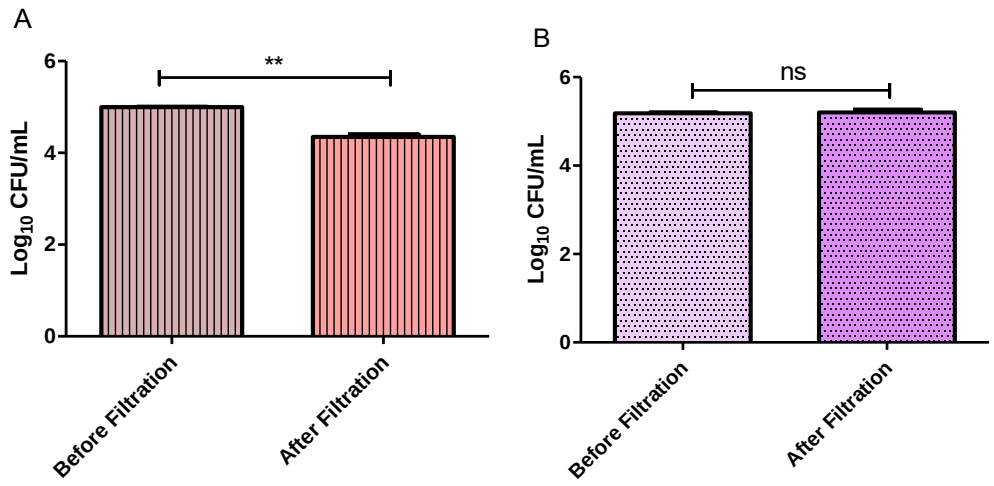


Figure 3.46. A. Number of *E.coli* Xen 14 after filtration with the BHPD membrane. **B**. Number of *S. aureus* Xen 36 after filtration with the BHPD membrane (trial 3).

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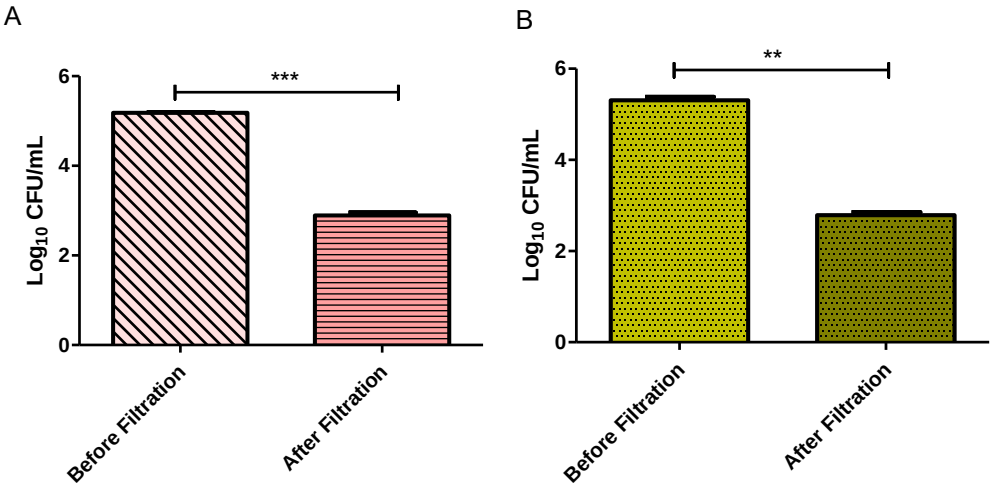

Figure 3.47 A. Number of *S. aureus* Xen 36 after filtration with two BHPD membranes (trial 4). **B**. Number of *S. aureus* Xen 36 after filtration with one NCHPD and one BHPD membrane together (trial 5).

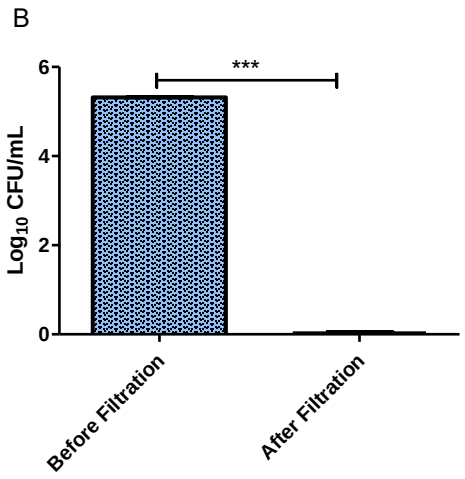

Figure 3.48. Number of *S. aureus* Xen 36 after filtration with the BHPD membrane and two NCHPD membranes combined together (trial 6).

3.3.8. Characterization of the High Planar Density Membranes Pre- and Post-Filtration

For these set of experiments, the effect of filtration on the structure of the membrane was determined and the pore sizes of the membranes were measured using SEM imaging and the software Image J. Additionally, the presence and the distribution of the Cu(II) and Zn(II) cations on the membrane was determined using EDS and the morphology and distribution of *S. aureus* Xen 36 on the membrane was investigated. Furthermore, there were deposits on the nanofibers that may have been the biocide (Figures 3.49 and 3.50).
Filtration did not affect the structure of the membrane as the fibrous network appeared intact and not damaged or disrupted during filtration. This suggests that the pressure applied was not too high.

As indicated in figure 3.5, all of the pores of one BHPD membrane are above 0.500 µm, with several exceeding 1.500 µm. Therefore, these pores are too big to retain the *S. aureus* cells on the membrane and to prevent the bacterial cells from entering the filtrate. The average diameter of a bacterium is within the range 0.5 – 1 µm and the average length is within the range 2.0 – 5.0 µm (Srivastava, 2013). The bacterium used in this case, *S. aureus* Xen 36, has a diameter in the range of 0.5 – 1.5 µm (Yousef & Carlstrom, 2003). Therefore, a single high planar density membrane on its own is not adequate enough to filter out bacteria.

In the case of 2 combined high planar density membranes, although the entire sections of the membranes were not imaged, more pores were observed when two membranes were stacked on top of each other than when three membranes were stacked on top of each

other. This is supported by the fact that 620 CFU/mL was observed after filtration with two membranes whereas no CFU/mL was observed after filtration with three membranes. The pores in Figure 3.51 A are all larger than 0.500 µm in width. For example, pore #2 is 1.426 µm, pore #3 is 0.744 µm, pore #4 is 0.848 µm, pore #5 is 0.643 µm, pore #6 is 1.057 µm, pore #7 is 0.608 µm and pore #8 is 0.848 µm in width. In Figure 3.51 B, pores #1 and #3 are 0.781 µm, pore #2 is 0.981 µm, pore #4 is 1.582 µm and pore #5 is 1.957 µm in width – the last two pores are wide enough to allow even the largest *S. aureus* cells through.

There were very few pores observed when three high planar density membranes were stacked on top of each other. Some of the pores observed are indicated in Figure 3.52 by numbers 1, 2 and 3. In Figure 3.52, the width of pores #1, #2 and #3 are 0.387 µm, 0.439 µm and 0.699 µm, respectively. In Figure 3.53, pores #1 is 1.212 µm. Given that the average diameter of an *S. aureus* cell (which is not the smallest bacterium), falls between the range of 0.5 – 1.5 µm, the ideal pore width would be lower than 0.5 µm, therefore, these pore sizes are still too wide.

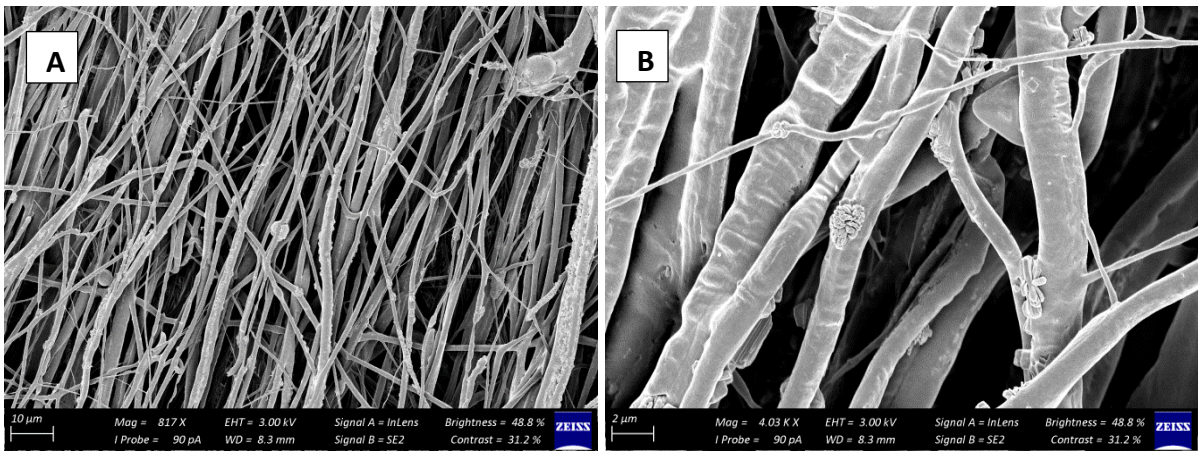

Figure 3.49: A and B. SEM image of the PLA layer of an unused BHPD membrane at different magnifications.

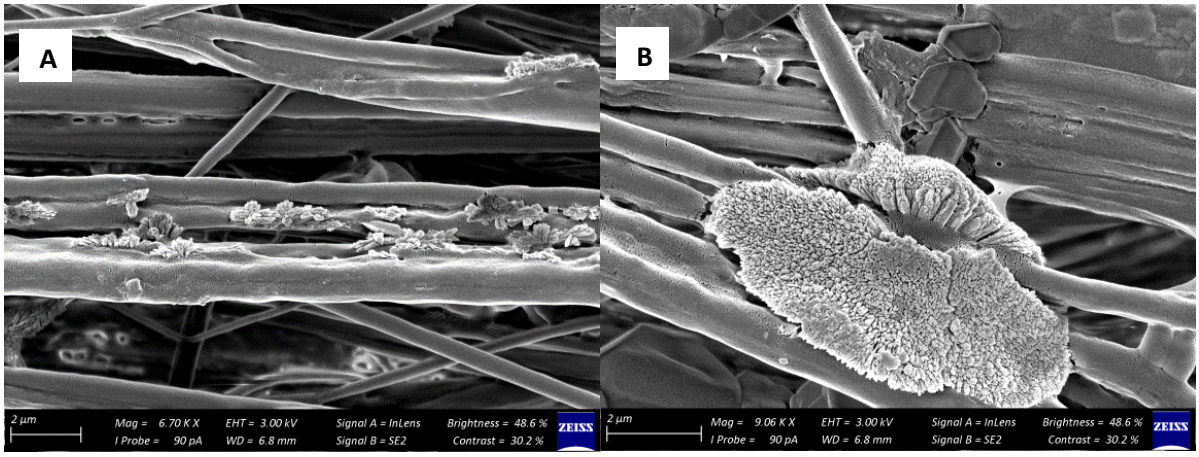

Figure 3.50: A and B: SEM images of the PLA layer of the used BHPD membrane at different magnifications.

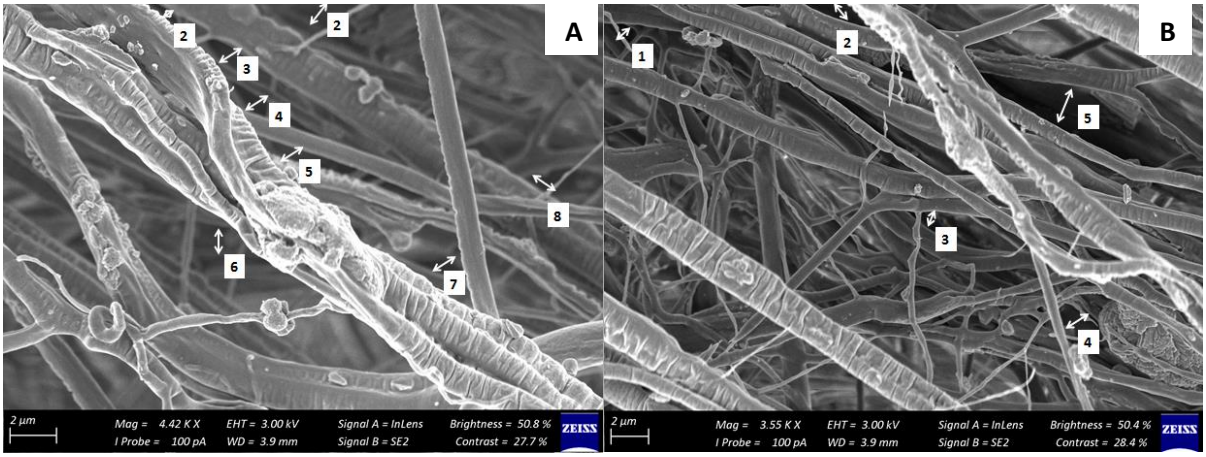

Figure 3.51: A and B. SEM image of 2 high planar density membranes stacked ontop of each other at different magnifications.

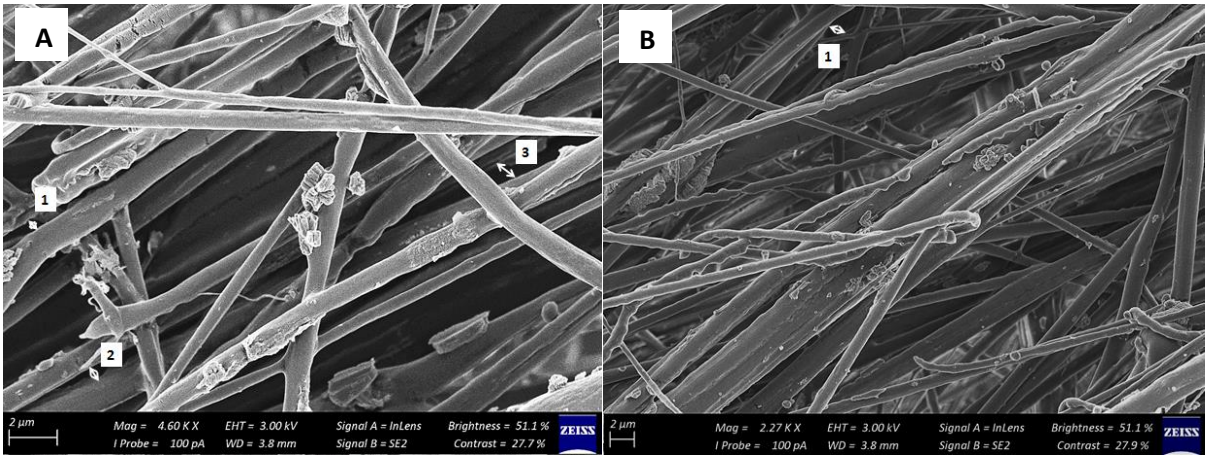

Figure 3.52: SEM image of 3 high planar density membranes stacked ontop of each other.

In addition to the analyses performed and described above, EDS was also performed on a used and unused BHPD membranes. As depicted in the map spectrum in Figures 3.53 and 3.54, traces of Cu and Zn ions are present on both membranes. However, the area maps generated by the AZTEC software indicate that even though a drastic reduction in Zn is observed after filtration, the intensity of Cu before and after filtration remains similar (Figure 3.55). These results indicate that the Cu is more tightly retained by the PLA even after filtration and the results correlate with the results of the leaching experiment depicted in Figure 3.26 and 3.27. The tight retention of the Cu(II) could be due to the fact that the metal adsorbs onto the PLA fibres which contain carboxyl function groups (Neghlani, Rafizadeh & Taromi, 2011). On the contrary, there was a significant decrease in the content of Zn in the used membrane than in the unused BHPD membrane. Additionally, it would appear as if the Cu(II) is not as evenly distributed across the membranes as the Zn ions (refer to Figure 3.55). The considerable decrease in Zn (II) also correlates to the leaching experiments where larger quantities of Zn (II) leached, more than the Cu(II).

For the BHPD variation, Cu(II) and Zn(II) are present on both the used and unused membrane.

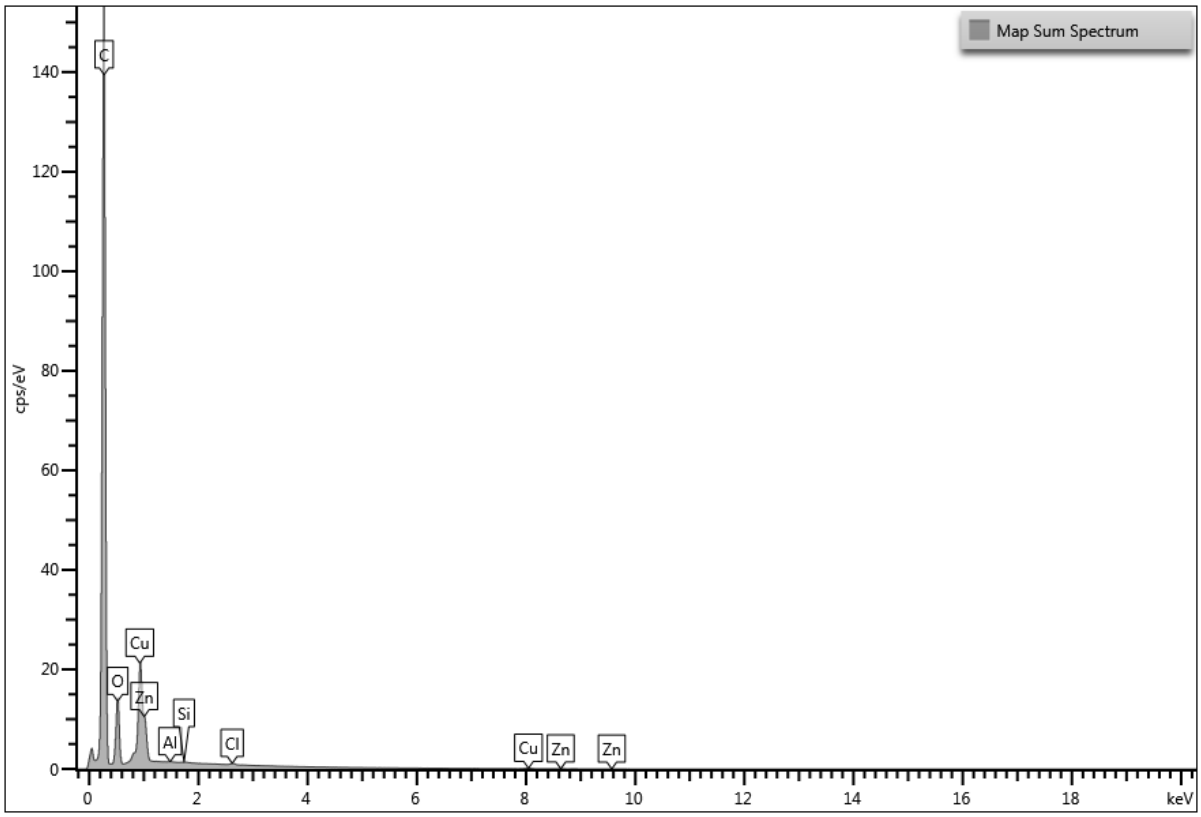

Figure 3.53. EDS map spectrum of the unused BHPD membrane.

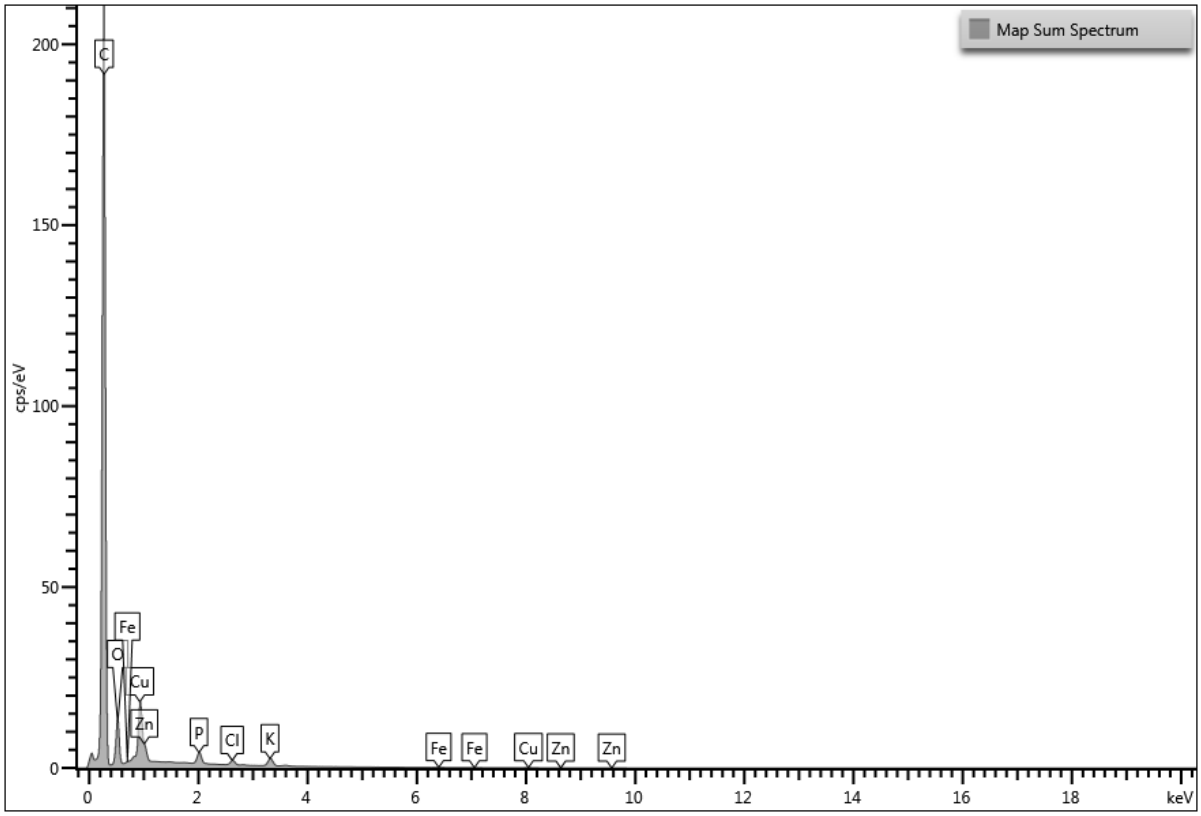

Figure 3.54. EDS map spectrum of the used BHPD membrane.

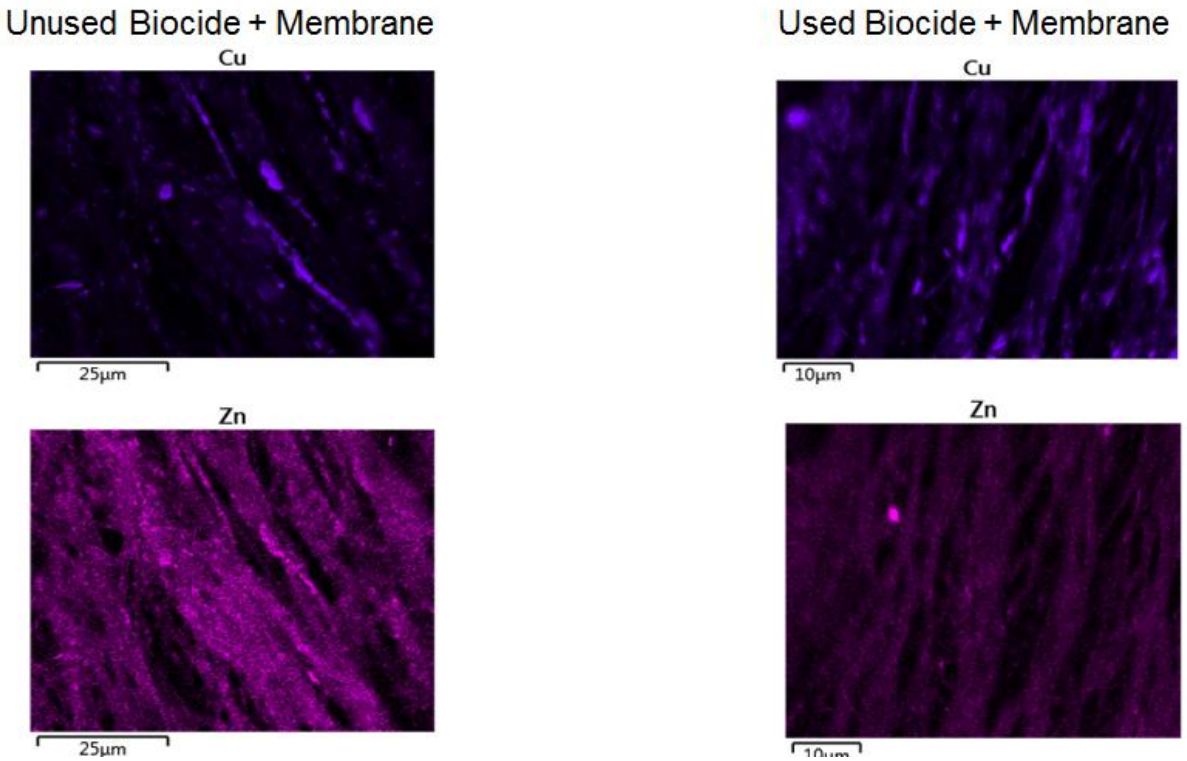

Figure 3.55. Distribution of Cu and Zn on the unused and used BHPD membranes.

Two deformed objects (1 and 2) in Figure 3.56 are likely to be *S. aureus* Xen 36 cells that have shrunk due to leakage of intracellular contents as a result of damage to the membrane after exposure to the biocide. As measured, the diameters of objects 1 and 2 are 0.875 and 0.882 µm, respectively, which fall within the range of the size of an *S. aureus* cell. However, the effect of the biocide can only be validated by comparing the morphology of cells trapped on an NHPD membrane.

Figure 3.57 reveals numbered circular structures embedded in the crystal-like agglomerate of the biocide, which are hypothesised to be *S. aureus* Xen 36 cells with damaged and flattened cell walls possibly due to contact with the biocide. Structure 1 has a diameter of 0.499 µm, structure 2 has a diameter of 0.615 µm, structure 3 has a diameter of 0.630 µm and structure 4 has a diameter of 0.772 µm. All these diameters also fall within the range of the size of an *S. aureus* cell. An additional example of cells with damaged structures embedded in an agglomerate of biocide is depicted in Figure 3.58. Structure 1 has a diameter of 0.545 µm, structure 2 has a diameter of 0.515 µm and structure 3 has a diameter of 0.495 µm. More examples of cells with damaged walls, which however, are not located near biocide agglomerates, are depicted in Figures 3.59 and 3.60.

However, there were several cells observed that appeared undamaged and intact (Figure 3.61). This indicates that the biocide may not be evenly distributed across the membrane; therefore, it is highly probable that not all cells that will make contact with the membrane will be exposed to the biocide. However, this may be compensated for by the fact that the filter has multiple layers.

Another observation was the presence of unidentifiable foreign matter embedded on the membrane and is depicted in Figures 3.62 A and B. The foreign matter could potentially be the lysate of bacterial cells that lysed upon exposure with the biocide. However, it cannot be confirmed whether this is the case. More of these images can be found in the appendix in Figures 5.2.1.

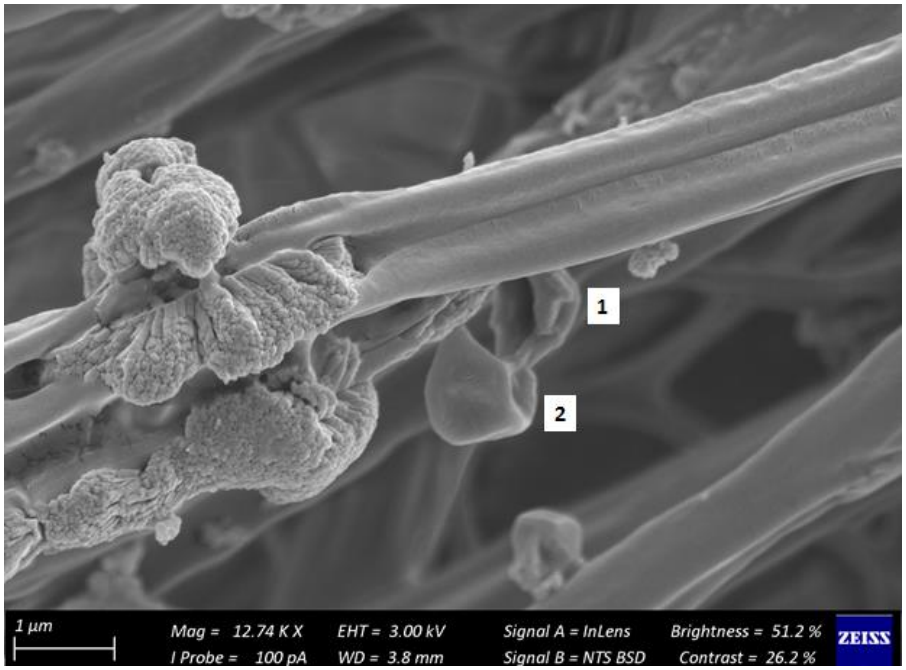

Figure 3.56: SEM image of damaged *S. aureus* Xen 36 cells in contact with the biocide.

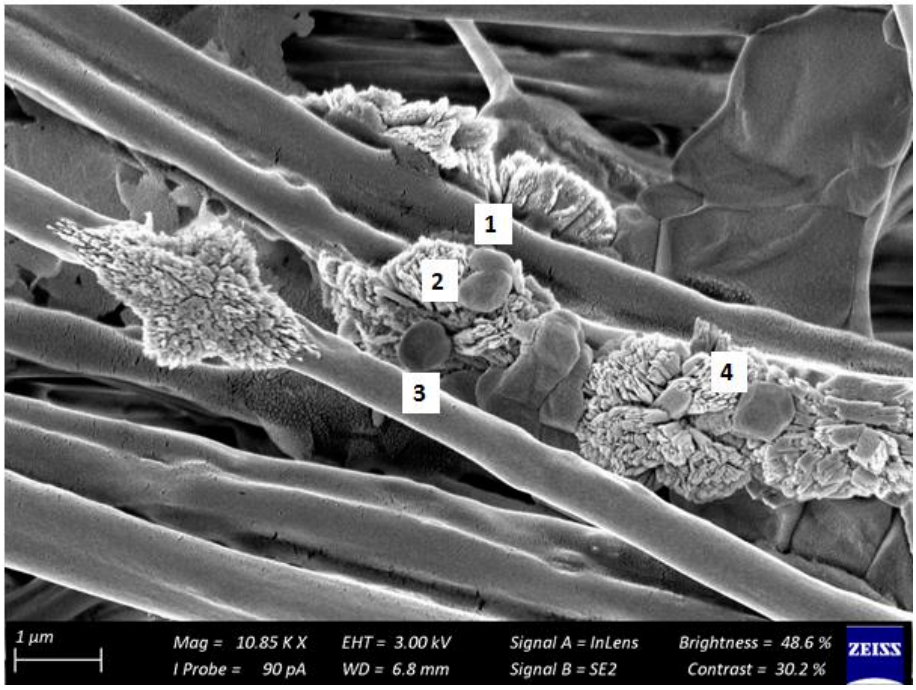

Figure 3.57: SEM image of damaged *S. aureus* Xen 36 cells in contact with the biocide.

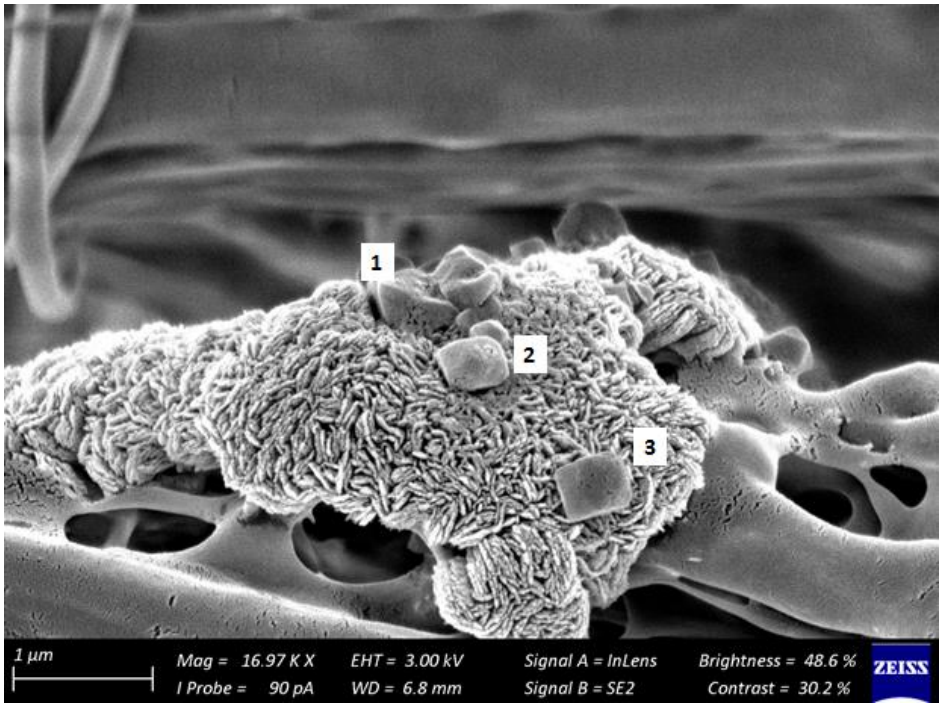

Figure 3.58: SEM image of damaged *S. aureus* Xen 36 cells in contact with the biocide.

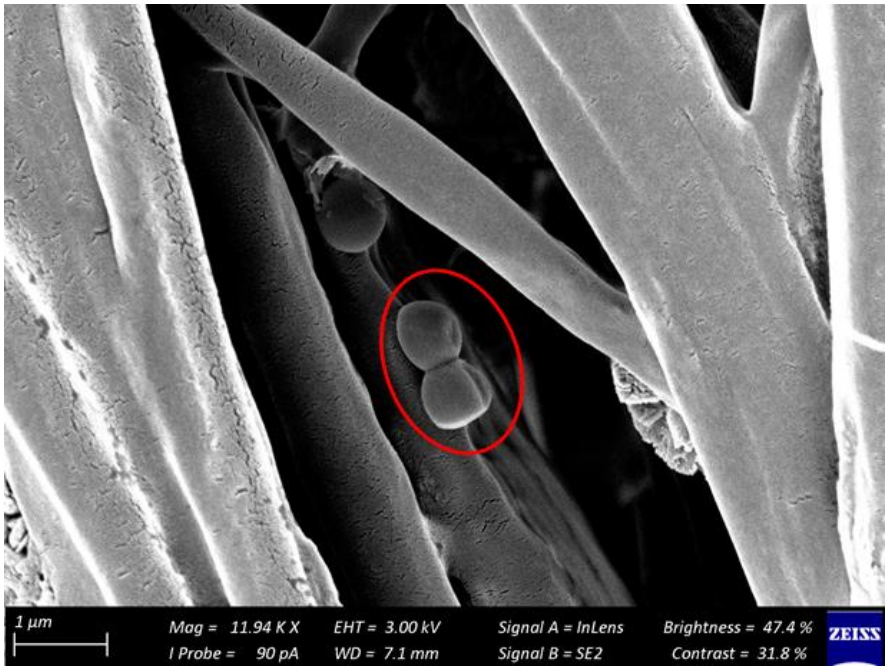

Figure 3.59: SEM image of damaged *S. aureus* Xen 36 cells retained on the BPHD membrane.

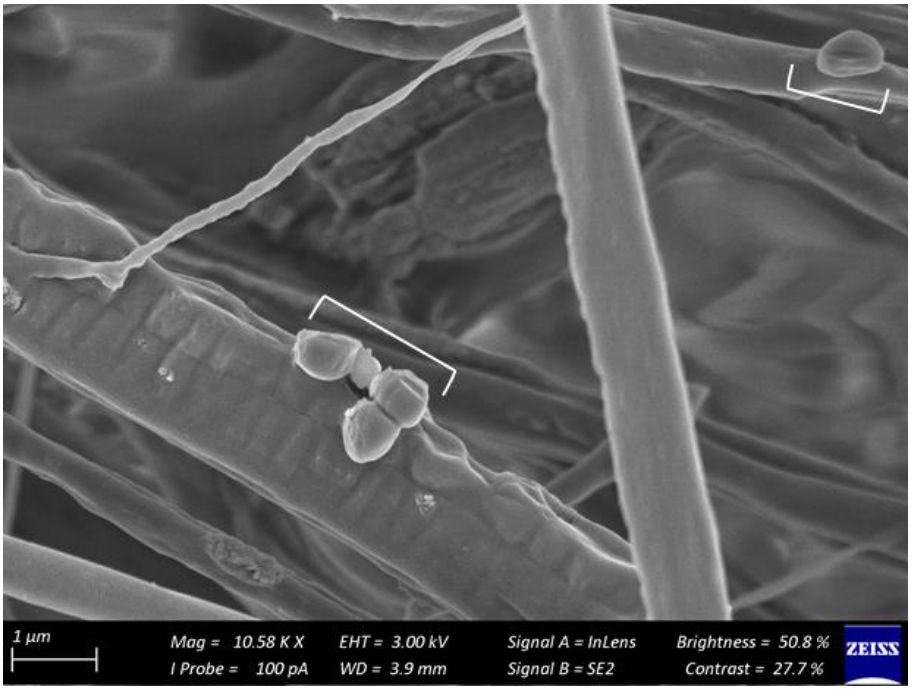

Figure 3.60: SEM image of damaged *S. aureus* Xen 36 cells retained on the BPHD membrane.

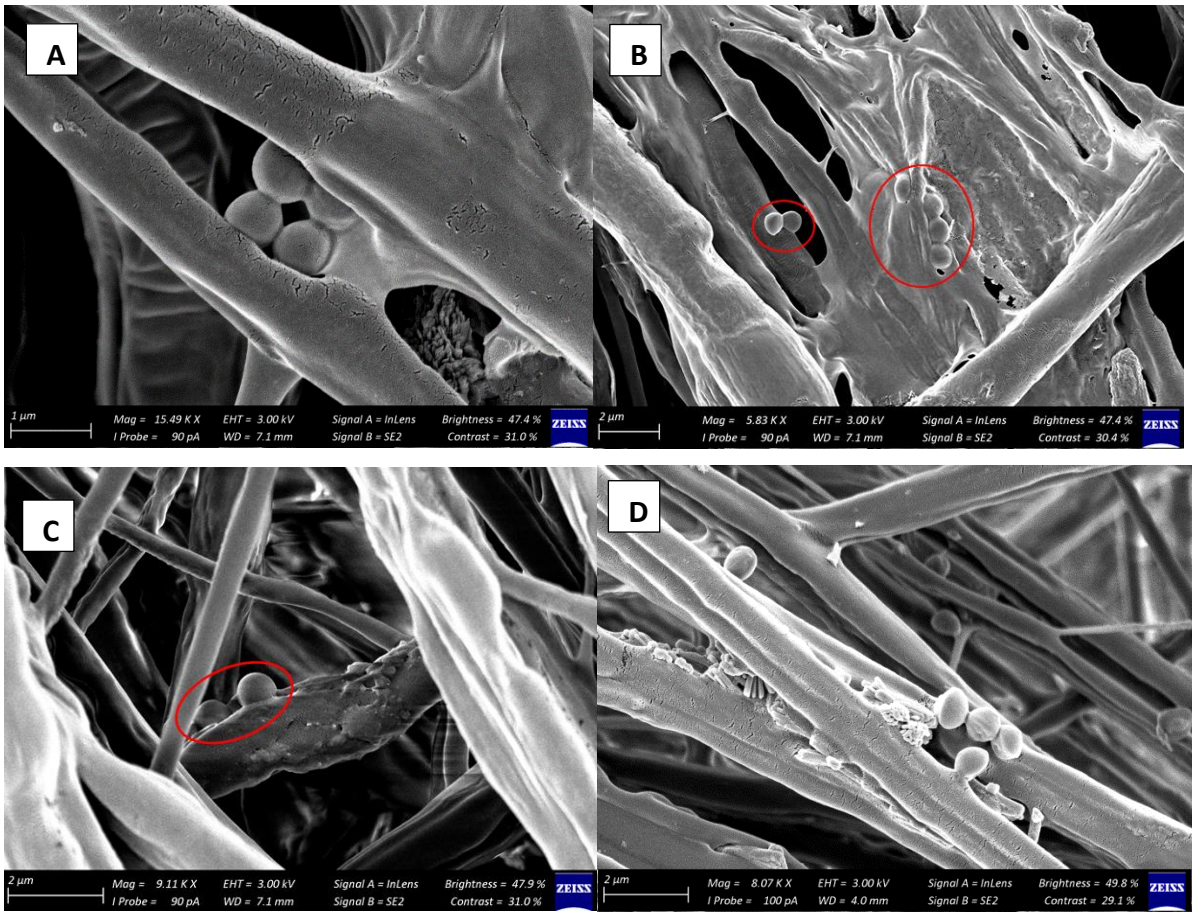

Figure 3.61: A. - D. SEM image of undamaged *S. aureus* Xen 36 cells retained on the BPHD membrane.

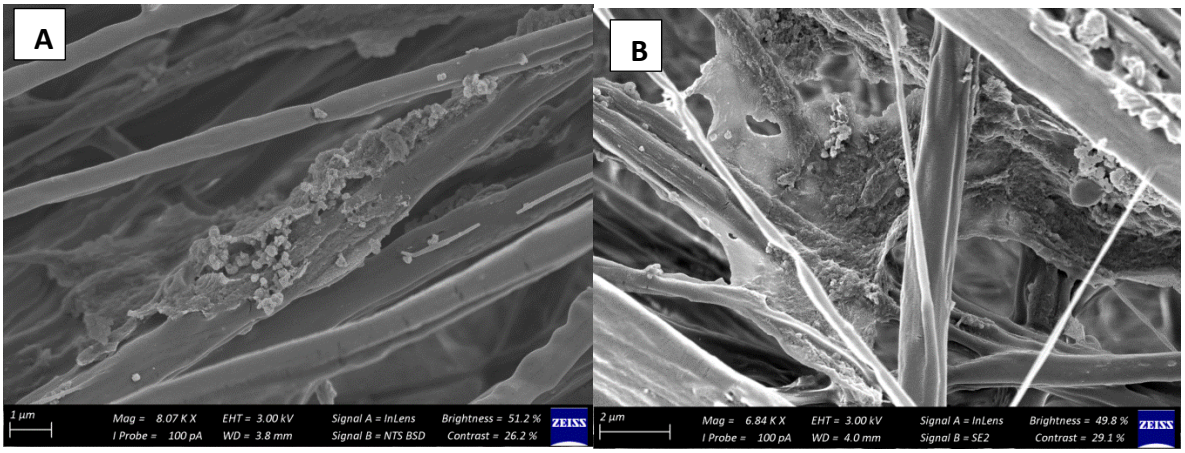

Figure 3.62. A and B: SEM images of unidentified foreign matter that could be the lysate of bacteria after exposure to biocide.

3.4. Conclusion and Recommendations

The first Swoxid prototype was effective against enteric bacteria / faecal coliforms, as well as *Enterococcus*, *Salmonella* and *Shigella* spp. and completely eradicated the bacteria without regrowth for six days after exposure. However, in the case of coliforms there was regrowth after 2 days, while a 1.5-log reduction could be achieved for the heterotrophic bacteria. It is possible that the bacterial counts in the inlet water were affected by sunlight under the experimental set-up used in this project, because of the slow filtration and the fact that the reservoir was not shielded from the sun.

The second Swoxid prototype also appeared effective in the inactivation of bacteria. The system killed off the bacteria tested within 20 min. However, similar to the first prototype, there was re-growth of heterotrophic and coliform bacteria; in this case after two days of incubation at 4ºC.This period is shorter than the four – six day period it takes for bacteria to appear in the filtrate of the first Swoxid prototype. Overall, there was little difference between the performances of the two Swoxid prototypes in the inactivation of bacteria.

The results with SEM suggested that the radicals generated by the TiO_2-UV reaction damaged the surface of the cell membrane due to the cracks that were observed on the surface of the *S. aureus* cells. This can be explained by the fact that the radicals are oxidants, which oxidise macromolecules such as the lipids which make up an important component of bacterial cell membranes.

The results further showed that the Swoxid system effectively reduces selected micropollutants, namely benzotriazole, codeine, diclofenac, efavirenz and SMX. However,

the fact that the UV device (control) compared well, and showed better results with some of the micropollutants, implies that UV light plays a larger role in the reduction of the micropollutants than the radicals generated. Compared to most of the alternative technologies mentioned in the thesis, the Swoxid prototype appeared less effective in removing micropollutants. As mentioned above, this may be explained by the fact that the respective treatments were tested with pristine water, whereas the Swoxid prototype treated river water, where the radicals could have been quenched by the organic matter in the river water. Additionally the radicals generated by the Swoxid acted only over a short distance, which may hinder the efficacy of the prototype.

Future research can focus on finding methods to improve it. For example, the quantity of TiO_2 in the membrane can be determined and tested at varying quantities that are higher than the quantity currently used in this study. Alternatively a different study could entail determining the leaching profile of TiO_2 after filtration on the ceramic membrane and comparing it to other profiles for other materials such as polymers, which may retain the TiO_2 more effectively. Moreover, the design of the filter can be altered in such a way that it will have a more robust structure and will allow for faster filtration.

Future research can also focus on determining the mechanism of action of the Swoxid prototype against bacteria. It is MOA of the Swoxid relies on the oxidative stress that the ROS it generates causes on major cellular components such as DNA, proteins and lipids. To view the effect of ROS on the membrane, the membrane dye FM1-43 can be used. One of the ways to validate the proposition that ROS inactivate by oxidative stress, mutagenic experiments with the Swoxid prototype can be conducted as mutagenesis is likely to result from oxidative damage (Macomber et al., 2007).

From the bioluminescent experiments with the antimicrobial nanofiber membranes, it is evident that a high biocide concentration rapidly inactivates the metabolism of bacteria. The effect on metabolism is enhanced when the membrane consists of nanofibers with a high density as opposed to a low density. However, the inactivation of metabolism does not always translate into cell death. The growth inhibition experiments demonstrated that although the high biocide containing membranes were able to rapidly inactivate the metabolism of the test bacteria *E. coli* Xen 14 and *S. aureus* Xen 36, they did not impact the viability of the cells.

These experiments also demonstrated that the metal cations leached off more readily from the high density membranes than the low density membranes and that although Zn(II) is present at a lower concentration in the biocide, it leaches out in larger concentrations than the Cu(II). This was confirmed by the EDS analysis.

The first series of filtration experiments showed that the membranes were ineffective in filtering out bacteria and that the water filters too rapidly through the membranes and therefore the bacteria do not have enough contact time with the biocide on the membrane to be biocidal. The results suggest that, for the filter thicknesses applied in this research, the Cu(II) and Zn(II) leached at too low concentrations to effectively render the water sterile. However, subsequent filtration experiments with combinations of high planar density membranes displayed significantly improved results and therefore it would seem that the matrix effect created by the stacking of one membrane on top of another is more effective in retaining bacteria on the membrane and also provides more contact time with the biocide on the membrane before entering the filtrate. However, these experiments need to be repeated with the negative control membranes on their own to determine if the improved efficiency is due to longer contact with the biocide on the membrane or enhanced filtration efficiency due to a decrease in pore size. Similar tests with *E. coli* Xen 14 will further demonstrate the potential utility of this approach.

The experiments also showed that contact between the bacteria and the biocide led to the deformation of bacteria. This may possibly be explained by the Cu and Zn ions disrupting the membrane of the cells causing the intracellular contents of the bacteria to leak. However, this requires validation by repeating the experiment with the controls.

Future research should focus on smaller pore sizes of the antimicrobial nanofiber membranes, and to optimize the pore size vs. filter thickness to achieve longer contact time for effective biocidal effect. Additionally, a method to ensure that the Cu(II) binds less tightly and that more of the metal cations leach during filtration should be sought and implemented. A study by Dankovich et al. (2014) demonstrated that when 206.9±34.1 ppb Cu leaches from a cellulose based filter, high log reductions of *E. coli* (8.8 and 4.6) were obtained after the filtration of water spiked with the bacterium. (Dankovich & Smith, 2014). This indicates that enhanced leaching of the metal might improve the efficiency of inactivation of bacteria. However, the concentration of metal that leaches still needs to be kept below the limit of the respective Drinking Standards. More in-depth investigations on the MOA of the antimicrobial nanofiber membrane against bacteria are needed. It is proposed that Cu(II) and Zn(II) disrupt the membrane potential and structure as part of their MOA. The fluorescent dye bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DIBAC4(3)) can be helpful in determining whether Cu(II) and Zn(II) have an influence on the membrane potential as its fluorescence is influenced by the potential of the membrane. To view the effect of Cu(II) and Zn(II) on the membrane, the membrane dye FM1-43 can be used.

In addition to the efficacy testing on POU filters performed in this study, it also provided information about the practicality and feasibility of POU filters. For example, the lack of knowledge on how to use the filters is an aspect in which POU filters may be inaccessible to the consumer. This may be caused by language barriers between the individuals distributing the filters and the recipients of the filters. Moreover, the design of POU filters may be impractical. For example, the first Swoxid prototype was made of two glass panes covering a ceramic membrane, yet glass can easily be broken.

Moreover, the average consumer may not know when they can no longer use the filters or how to discard of the filters. This may be caused by a lack of understanding or language differences between individuals distributing the filters and the recipients of the filters.

Furthermore, this study indicated is that not all POU filters are cost-effective to produce. For example, the thinner membranes were vastly inefficient and were R 3333.33 per membrane. A primary target market for the POU filters are people living in poorly-serviced areas that are typically burdened by poverty, who will not be able to afford expensive treatment devices. For this target group, alternative POU systems will have to be compared with inexpensive approaches such as the SODIS system. However, products manufactured during the experimental phase tend to be more expensive than when they are commercialized. Therefore, methods to reduce the cost of production of the membranes should be sought to make them more affordable.

The ideal POU filter should not be complicated to use but the use thereof can easily be explained. Furthermore, it should have a long life-span, be physically stable and robust. Moreover, it should be cost-effective.

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Appendix

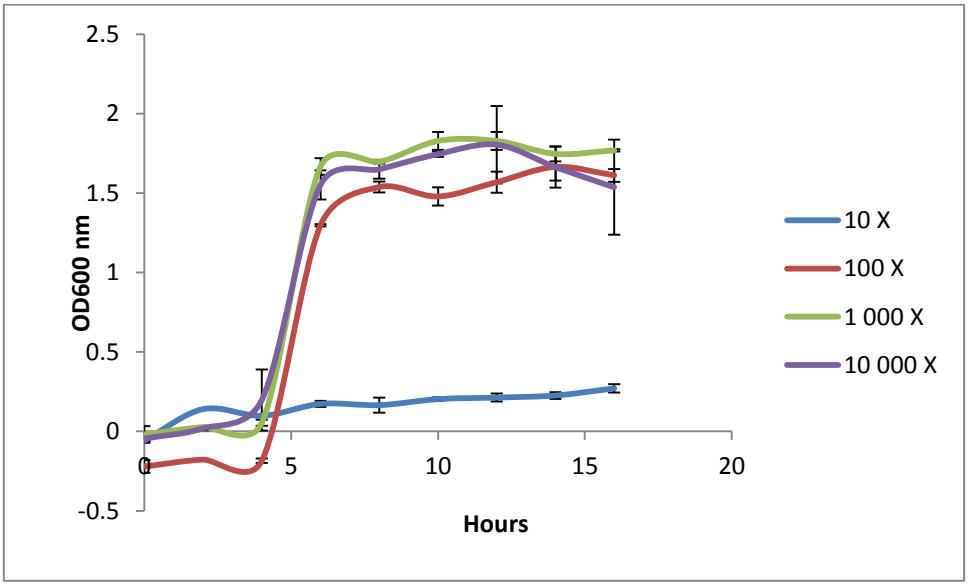

Figure 5.1: Growth curve of *S. aureus* Xen 36 under various dilutions of BioClear biocide

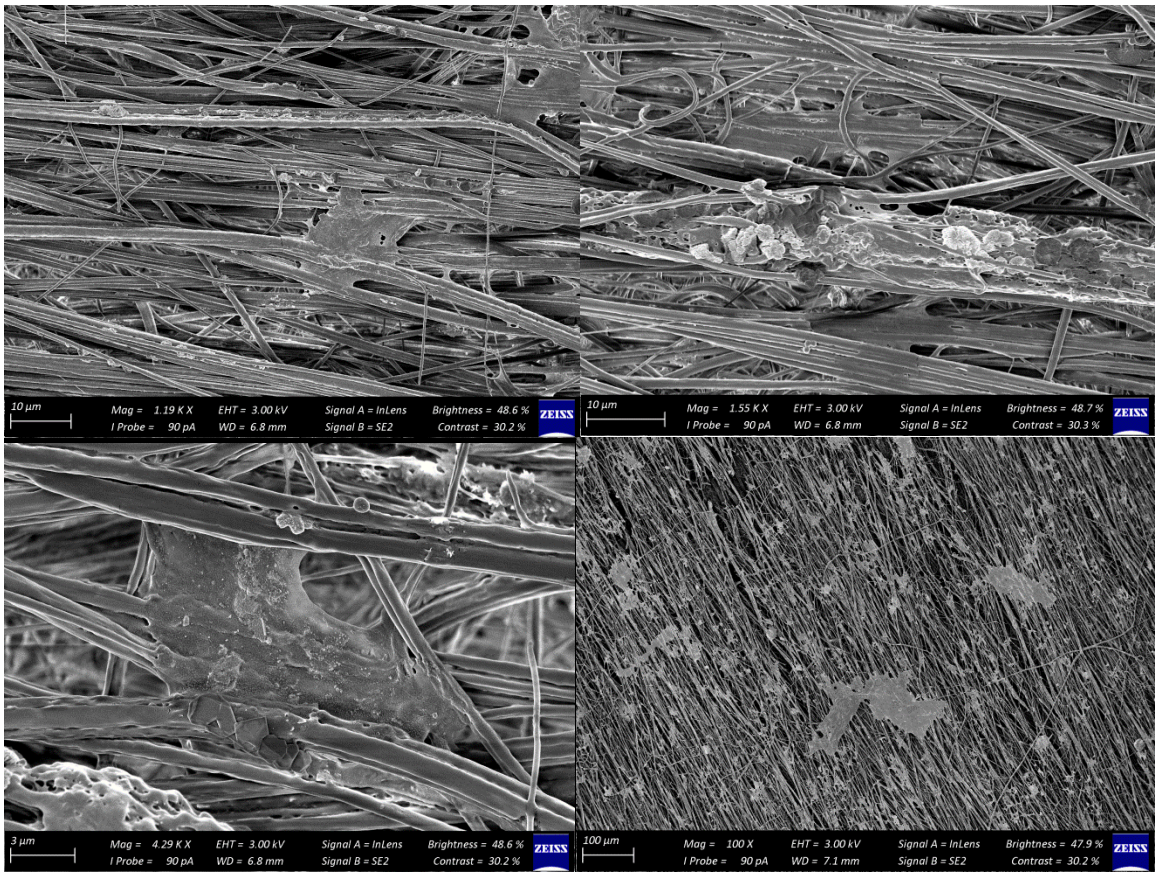

Figure 5.2. A-D: SEM images of BHPD membrane containing foreign matter