

**Evaluation of Membrane Filtration and UV Irradiation for the Control of
Flavobacterium psychrophilum in Recirculation Aquaculture Systems**

by

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ABSTRACT

EVALUATION OF MEMBRANE FILTRATION AND UV IRRADIATION FOR THE CONTROL OF *FLAVOBACTERIUM PSYCHROPHILUM* IN RECIRCULATION AQUACULTURE SYSTEMS

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Flavobacterium psychrophilum, the causative agent of bacterial cold water disease (BCWD), is tolerant of recommended ultraviolet (UV) doses used in recirculation aquaculture systems (RAS). Membrane filtration (MF) is used to remove pathogens from wastewater in many industries, but has not been thoroughly tested in RAS. In this study, bacterial removal efficiencies were assessed between MF and UV treatments over 30 days in an RAS. Bacterial removal efficiencies were not significantly different ($p > 0.05$) between MF and UV treatments, which removed 98.5 ± 0.4 % and 99.6 ± 0.1 % of total bacteria and 93.2 ± 5.2 % and 92.5 ± 4.1 % of heterotrophic bacteria, respectively. Under laboratory conditions, a MF system was challenged with concentrated doses of *F. psychrophilum* and achieved 5.8 ± 0.2 log reductions of the pathogen. Therefore, MF represents a potential alternative to UV irradiation and could be used to reduce the prevalence of *F. psychrophilum* in RAS, potentially reducing the incidence of BCWD and its impact on the aquaculture industry.

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List of Abbreviations

A	Area (m ²)
BCWD	Bacterial Coldwater Disease
cfu	Colony Forming Units
cm	Centimetre
DO	Dissolved Oxygen (mg/L)
F	Fouling Rate (/m/day)
g	Gravitational Acceleration (m/s ²)
h	Height (m) between the effluent pump and point of discharge
hr	Hour
J	Permeate Flux (L/m ² /hr)
kDa	Kilodalton
kg	Kilogram
kPa	Kilopascal
kV	Kilovolt
L	Litres
M	Molarity (mol/m ³)
m	Metre
m ²	Square Metre
m ³	Cubic Metre
MF	Membrane Filtration
mg	Milligram
min	Minute
mJ	Millijoule
mL	Millilitre
mm	Millimetre
MPN	Most Probable Number
mS	Millisiemen
mW	Milliwatt
MWCO	Molecular Weight Cut Off

nm	Nanometer
NTU	Nephelometric Turbidity Units
p_0	Initial Pressure (kPa)
Q_e	Effluent Flow Rate (L/min)
Q_p	Permeate Flow Rate (L/min)
RAS	Recirculation Aquaculture Systems
PCR	Polymerase Chain Reaction
PVDF	Polyvinylidene Fluoride
R_f	Fouling Resistance (/m)
R_m	Membrane Resistance (/m)
s	Second
SD	Standard Deviation
SE	Standard Error
SEM	Scanning Electron Microscopy
t	Time
TDS	Total Dissolved Solids (mg/L NaCl)
TMP	Transmembrane Pressure (kPa)
TOC	Total Organic Carbon (mg/L)
TSS	Total Suspended Solids (mg/L)
UV	Ultraviolet
UVT	UV Transmittance (% at 254 nm)
V	Volume
°C	Degrees Celsius
η	Viscosity (kPa·min)
μm	Micrometre
ρ	Fluid Density (kg/m ³)
τ	Residence Time

Declaration of Work Performed

All work reported in this thesis was performed by me with the exception of the following:

Water samples in Chapter 2 were analysed for total organic carbon, total Kjeldahl nitrogen and heterotrophic bacteria by the staff at the Agriculture and Food Laboratory, Laboratory Services, University of Guelph.

Isolation, amplification and sequencing of *Flavobacterium psychrophilum* was performed by Iwona Skulska at the Fish Health Laboratory, University of Guelph and the staff at the Genomics Facility, Advanced Analysis Centre, University of Guelph.

Chapter 1: Literature Review

1.1 Introduction

By 2050, global livestock production is estimated to double in order to meet the food demand of the growing human population (FAO, 2006). This demand cannot be easily met through the expansion of existing livestock operations because the availability of freshwater and farmable land is limited (FAO, 2006; Thornton, 2010; Wallace, 2000). For that reason, livestock operations are becoming more intensive in order to produce more food with fewer resources (FAO, 2006; Piedrahita, 2003; Sharrer et al., 2007; Thornton, 2010). However, disease control may be compromised in the process, resulting in clinical disease outbreaks and production losses (Ilea, 2009; Piedrahita, 2003; Thornton, 2010). Increasingly, pathogen control technologies are relied upon to limit pathogen loads in animal rearing systems and potentially reduce the prevalence of infection and disease (Thornton, 2010). Therefore, livestock operations need to adopt more efficient pathogen control technologies to preserve animal health while intensifying livestock production.

Increased production from recirculation aquaculture systems (RAS) may be an efficient way to produce food while reducing pressures on feed, water and land resources. RAS can produce fish at high stocking intensities, while reducing water consumption and land use (Chen et al., 2002; Piedrahita, 2003). In addition, fish can achieve substantially higher feed efficiencies compared to terrestrial livestock (FAO, 2006). However, inefficient removal of pathogens from wastewater in RAS can recirculate pathogens to cultured fish, which can potentially increase the incidence of

disease and result in substantial production losses (Piedrahita, 2003; Sharrer and Summerfelt, 2007). Therefore, an opportunity exists to improve fish health and production in RAS by implementing more efficient pathogen control technologies.

This chapter reviews the limitations of using UV irradiation and gives an insight into how the use of membrane filtration (MF) may improve the control of bacteria in RAS.

1.2 Recirculation Aquaculture Systems

Globally, open-water cage systems are commonly used to produce fish (Bostock et al., 2010; FAO, 2010; Tacon and Halwart, 2007), but potential exists for increased production from land-based systems. Aside from ponds, land-based aquaculture systems are more technologically advanced and require higher capital investment (FAO, 2010; Otchere et al., 2004), but continual developments of cost-effective technologies has increased interest in land-based production (Piedrahita, 2003). In addition, land-based systems are advantageous compared to open-water cage systems because they offer increased control over waste and pathogens (Otchere et al., 2004). Standards for waste and pathogen control may become more stringent over time as competition for land and water increases (Otchere et al., 2004; Piedrahita, 2003). Therefore, fish production from land-based systems may become more important in the future due to their ability to comply with increasing environmental regulations.

In land-based aquaculture, water can be supplied to fish in tanks and raceways via flow-through or recirculation strategies (Piedrahita, 2003). Recirculation systems are advantageous compared

to flow-through because they reduce water consumption and concentrate wastewater for easier treatment (Otchere et al., 2004; Piedrahita, 2003; Sharrer et al., 2007). For example, warm-water recirculation systems can produce 5,000 kg of fish/year/(L/min), compared to 16 kg of fish/year/(L/min) produced from flow-through systems (Chen et al., 2002). Water conservation is a major benefit of RAS, but wastes and pathogens from fish excretions, such as feces and urine, and uneaten feed must be removed to avoid recirculating these components to cultured fish (Piedrahita, 2003). Inefficient removal of wastes and pathogens allows them to accumulate in rearing systems, which can negatively impact fish health (Conte, 2004; Patterson et al., 1999). Therefore, combinations of physical, chemical and biological treatments are relied upon to remove these waste components and optimize rearing conditions in RAS (Piedrahita, 2003).

Removing pathogens from rearing systems is important because high pathogen loads can stress fish and increase the incidence of infection and disease (Conte, 1992). Clinical disease can reduce fish growth rates and increase fish mortality rates, thereby reducing production and increasing economical losses (Conte, 1992; Thornton, 2010; Wedemeyer, 1996). Management strategies, such as sourcing high quality water and equipment disinfection, can reduce the introduction of pathogens into rearing systems, but these strategies are ineffective once pathogens proliferate within the system (Conte, 2004; Sharrer and Summerfelt, 2007). Therefore, continuous pathogen removal technologies are relied upon to prevent accumulations of pathogens in RAS, consequently reducing the incidence of infection and disease (Sharrer and Summerfelt, 2007; Wedemeyer, 1996).

UV irradiation is the most commonly used water disinfection technology (Gomez et al., 2007) and is widely applied within aquaculture systems (Summerfelt et al., 2003). Its widespread use is largely due to factors such as high disinfection efficiency, minimal presence of disinfection by-products, ease of use and low cost (Gomez et al., 2007; Lazarova et al., 1999). UV irradiation inactivates microorganisms by damaging their DNA and RNA, which prevents them from replicating and causing infection (Summerfelt et al., 2003; Wedemeyer, 1996). The ability of UV to inactivate microorganisms is dependent on the applied UV dose, given as mJ/cm^2 , which is the product of UV light intensity, residence time and UV transmittance through water (Summerfelt et al., 2003; Wedemeyer, 1996).

1.3 Limitations of UV Irradiation

Several factors can reduce the disinfection efficiency of UV, which allows pathogens to accumulate in the rearing system and potentially infect cultured fish (Sharrer and Summerfelt, 2007). The main disadvantage of UV treatment is the ability of pathogens to avoid and tolerate UV irradiation (Wedemeyer, 1996). Other disadvantages of UV irradiation include rapid declines in lamp life efficiency (Summerfelt et al., 2003), bacterial regrowth after treatment (Lazarova et al., 1999) and the production of photo-products that cause cataracts (Bjornsson, 2004). Inefficient removal of pathogens by UV irradiation may increase pathogen loads in RAS, potentially increasing the incidence of infection and disease.

Particles suspended in influent wastewater or adhered to UV sleeves can shield pathogens from UV exposure and allow pathogens to bypass UV treatment (Wedemeyer, 1996). Increasing levels

of suspended particles has been reported to reduce UV inactivation (Lazarova et al., 1999; Liltved and Cripps, 1999; Wedemeyer, 1996), thus pre-treatments, such as micro-screen filtration, are used to remove suspended particles and increase the efficiency of UV disinfection (Gomez et al., 2007). However, Patterson et al. (1999) suggested that implementing micro-screens with pore sizes smaller than 50 μm is not economically practical in RAS. Additional treatments, such as granular media or foam fractionation filters, can be implemented to increase solids removal efficiency, but they are unable to achieve high water production and recovery rates (Chen et al., 1993; Chen et al., 2002). Thus, fine solids bypass micro-screen filtration and accumulate in the rearing system, consequently shielding pathogens from UV inactivation (Liltved and Cripps, 1999; Wedemeyer, 1996). Therefore, the dependence of UV irradiation on water quality may limit its reliability as an effective disinfection treatment in RAS.

Pathogens can also bypass UV treatment by tolerating UV damage due to DNA repair mechanisms, such as photo-reactivation and dark repair (Kudson, 1985). Wedemeyer (1996) and Liltved (2002) report that many fish pathogens are inactivated by UV doses of 30 mJ/cm^2 . However, some pathogens require 5-10 fold higher UV doses to be inactivated, such as infectious pancreatic necrosis virus (Liltved et al., 2006; Yoshimizu et al., 1986), sea bass neuropathy nodavirus (Frerichs et al., 2000) and the protozoan parasite *Costia* (Vlasenko, 1969). Additionally, some pathogenic bacteria in aquaculture systems, such as *Aeromonas salmonicida*, *Vibrio anguillarum* and *Yersinia ruckeri*, are only temporarily inactivated by UV, eventually repairing themselves and regaining infectivity (Liltved and Landfald, 1996). The ability of some pathogens to tolerate recommended UV doses used in aquaculture systems further demonstrates the potential unreliability of UV irradiation.

1.4 *Flavobacterium psychrophilum*

Flavobacterium psychrophilum (formerly *Cytophaga psychrophila* and *Flexibacter psychrophilus*; Bernardet et al., 1996) is a rod-shaped, Gram-negative bacterium that is the causative agent of bacterial cold water disease (BCWD). *F. psychrophilum* has been associated with skin ulcers and fin-rot as well as septicaemia in a number of fish, especially rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*; Nilsen et al., 2011). *F. psychrophilum* has been reported to transmit from fish both horizontally and vertically (Brown et al., 1997; Ekman et al., 1999) and outbreaks have been reported to cause mortality rates up to 90 % in rainbow trout fry (Nilsen et al., 2011). *F. psychrophilum* has caused significant economic losses in salmonid rearing systems and has emerged as an important bacterial pathogen in the aquaculture industry (Michel et al., 1999; Nematollahi et al., 2003b).

BCWD continues to be prevalent in the aquaculture industry because outbreaks are difficult to prevent and treat. To date, there is no commercially available vaccine to prevent BCWD (Barnes and Brown, 2011) and management strategies, such as improvements in water quality, can only reduce the severity of clinical disease (Taylor, 2004). Antibiotics can be effective at treating BCWD, but antibiotic resistance is common and most antibiotics are not registered for use in aquaculture (Barnes and Brown, 2011). The inability to prevent and treat BCWD presents a significant problem for the biosecurity of aquaculture systems.

Due to the ineffectiveness of vaccines and antibiotics, emphasis has been placed on pathogen control technologies, such as UV, to remove *F. psychrophilum* from aquaculture systems (Barnes and Brown, 2011). However, a study by Hedrick et al. (2000) demonstrated that UV doses of 126 mJ/cm² are required to achieve over 5 log reductions of *F. psychrophilum*, which is more than four times the recommended UV dose used in aquaculture systems (i.e. 30 mJ/cm²; Sharrer et al., 2005). The applied UV dose can be enhanced by increasing UV intensity, residence time or UV transmittance (Summerfelt et al., 2003; Wedemeyer, 1996), but these enhancements may require additional pre-treatments or over-sized UV systems that may be economically impractical. In addition, bacteria may bypass UV treatment by embedding themselves inside particulate matter to avoid UV irradiation (Sharrer et al., 2005), which suggests that increasing the applied UV dose may not increase the inactivation of *F. psychrophilum*. Therefore, alternative pathogen control technologies need to be implemented to reduce the prevalence of *F. psychrophilum* in RAS and the impact of BCWD on aquacultural production.

1.5 Membrane Filtration

Membrane filtration (MF) is a pressure driven process that physically separates solids from fluid using semi-permeable membranes (Madaeni, 1999; Peters, 2010). MF processes include microfiltration, ultrafiltration, nanofiltration and reverse osmosis, which are classified based on membrane pore size (Madaeni, 1999; Peters, 2010). The pore size of ultrafiltration membranes ranges between 0.005 to 0.02 µm (Madaeni, 1999; Peters, 2010), which allows the diffusion of dissolved components and water (permeate) while retaining suspended particles, protozoa, bacteria, viruses and other waste components (retentate) larger than the applied pore size (Guo et

al., 2009). Ultrafiltration is advantageous compared to other MF processes because it can achieve high pathogen removal efficiencies at high water production rates (permeate flux; Crozes et al., 1997; Madaeni, 1999). In addition, ultrafiltration membranes are operated at very low pressure differentials, which can reduce pumping costs and increase long-term operation (Madaeni, 1999; Zhou and Smith, 2002). Therefore, potential exists to use ultrafiltration as an effective pathogen removal treatment in RAS. Note that ultrafiltration will be addressed as membrane filtration (MF) for the remainder of this thesis.

MF technologies are increasingly relied upon to meet the demand for high quality water, which continues to increase with the growth of the human population (Gomez et al., 2007). In the past, MF technologies were considered costly, but advances in MF efficiency and its widespread use have increased its affordability (Gomez et al., 2007; Reith and Birkenhead, 1998). The main advantage of MF is its ability to continuously remove waste components from water while tolerating fluctuations in influent quality (Guo et al., 2010; Lazarova et al., 1999). Other advantages of MF include production flexibility, ease of use, compact design and reliable long-term operation (Guo et al., 2010; Jacangelo et al., 1989; Peters, 2010). These advantages have made MF an attractive treatment for many water production and wastewater reuse applications (Gomez et al., 2007; Peters, 2010).

One of the potential disadvantages of using MF as a continuous pathogen removal strategy in RAS is the degree of maintenance required to maintain its long-term operation. Pathogen removal technologies that require a high degree of maintenance can result in higher production costs, such as increases in personnel labour and treatment interruptions (Sharrer et al., 2005;

Summerfelt et al., 2009). MF systems can be automated and typically do not require a high degree of maintenance, unless they experience high rates of membrane fouling (Le-Clech et al., 2006; Madaeni, 1999). Membrane fouling is the deposition or absorption of particulate matter onto or into membranes, which increases transmembrane pressure when a constant permeate flux is maintained (Le-Clech et al., 2006; Madaeni, 1999). Continual fouling increases transmembrane pressure until a critical point where further filtration can damage the membranes (Le-Clech et al., 2006; Madaeni, 1999). At this point, membranes are “recovery cleaned” with high concentrations of chemicals, such as sodium hypochlorite, to remove foulants and restore transmembrane pressure (Le-Clech et al., 2006; Madaeni, 1999). Recovery cleanings allow filtration to continue, but these events require interruptions in wastewater treatment and chemical exposure shortens membrane lifespan (Crozes et al., 1997; Madaeni, 1999). Treatment interruptions and membrane degradation increase maintenance costs and reduce long-term operation of MF systems (Crozes et al., 1997; Madaeni, 1999). The interval between recovery cleanings can be increased by implementing “maintenance cleanings” that use lower doses of chemicals, but treatment is still interrupted to perform these events (Le-Clech et al., 2006). Therefore, membrane fouling rates should be minimized to extend the interval between recovery cleanings and allow MF systems to maintain long-term operation in RAS.

Physical cleaning strategies can be applied, directly or indirectly, to MF systems to reduce the rate of membrane fouling without interrupting treatment or using chemicals (Le-Clech et al., 2006). For example, air-scouring can be directly applied to MF systems to remove foulants from the surface of membranes via air bubbles (Le-Clech et al., 2006). Influent wastewater pre-treatments, such as micro-screen filtration, can indirectly reduce membrane fouling by removing

large solids from wastewater and preventing them from depositing onto or into membranes (Gomez et al., 2007; Le-Clech et al., 2006). In RAS, micro-screen filtration is typically used to remove large solids from wastewater to improve water quality and reduce UV particle shielding (Patterson et al., 1999), which suggests that MF systems may experience low fouling rates when implemented after this pre-treatment. In addition, fouling rates experienced by MF systems in RAS may be further reduced by increasing the frequency, combination and duration of physical cleaning strategies (Le-Clech et al., 2006). Therefore, MF systems may experience low fouling rates and maintain long-term operation in RAS due to the opportunity to implement wastewater pre-treatments and other cleaning strategies.

1.6 Wastewater Treatment using MF and UV

MF technologies have not yet been evaluated as a pathogen removal treatment in aquaculture systems, but its ability to remove suspended solids and other wastes from fish effluent has been investigated. Viadero and Noblet (2002) removed between 69-94 % of suspended solids from synthetic fish wastewater using MF systems with pore sizes that ranged from 1.2 to 0.05 μm (micro to ultrafiltration). Yang et al. (2006) removed 99.9 % of turbidity from wastewater from a RAS using a MF system with a nominal pore size of 0.22 μm (microfiltration) coupled with alum precipitation. In addition, Castaing et al. (2010) removed approximately 99 % of micro-algae and turbidity from surface waters using MF systems with pore sizes ranging from 0.2 to 0.001 μm (micro to ultrafiltration). Therefore, MF may exhibit an additional benefit of removing high levels of suspended solids and other wastes when used as a pathogen control treatment in RAS.

Other studies have also demonstrated that MF systems can remove other wastes from fish effluent when coupled with a bioreaction process, termed membrane bioreactor. Membrane bioreactors have been reported to retain high levels of bacteria in order to biologically treat wastewater from RAS (Gemende et al., 2008; Pulefou et al., 2007; Sharrer et al., 2007; Sharrer et al., 2009; Visvanathan et al., 2008). This suggests that MF systems without the bioreaction process may also retain high levels of bacteria when used in RAS. Membrane bioreactors are not used to continuously remove pathogens from wastewater because the bioreaction process requires long residence times (Le-Clech et al., 2006). However, non-integrated MF systems can continuously separate solids from fluid at high flow rates (Zhou and Smith, 2002). Therefore, the ability of membrane bioreactors to retain bacteria suggests that MF systems may achieve high bacterial removal efficiencies when used to continuously treat wastewater in RAS.

The pathogen control process is very different between MF and UV technologies (Table 1.1, Appendix 1), which may influence their ability to remove pathogens from wastewater in RAS. MF may achieve higher pathogen removal efficiency than UV because MF can physically remove pathogens from wastewater (Lazarova et al., 1999; Madaeni, 1999). UV irradiation cannot physically remove pathogens, which can result in pathogen regrowth after treatment and the recirculation of infectious pathogens to cultured fish (Kudson, 1985; Lazarova et al., 1999; Sharrer and Summerfelt, 2007). However, MF is not classified as a disinfection treatment because it cannot inactivate pathogens, thus retained wastewater (retentate) may have to be disinfected after MF treatment if required (Madaeni, 1999; Peters, 2010). Nevertheless, MF may reduce pathogen loads in RAS by physically removing pathogens from filtered water (permeate) and may achieve higher pathogen removal efficiency than UV irradiation.

Table 1.1. General comparison of wastewater treatment characteristics between UV and MF (modified from Lazarova et al., 1999).

Characteristic	UV	MF
Solids removal	-	+++
Protozoa removal	+	+++
Bacterial removal	++	+++
Virus removal	++	+++
Bacterial re-growth	+	-
By-products	+	-
Operation flexibility	+	+++

"-" none; "+" low; "++" moderate; "+++" high

MF may also achieve higher pathogen removal efficiency than UV when treating wastewater in RAS because MF is less impacted by fluctuations in water quality and fouling (Gomez et al., 2007; Lazarova et al., 1999). Influent containing high levels of suspended solids and thick fouling layers on UV sleeves can reduce UV transmittance, the applied UV dose and overall UV disinfection efficiency (Liltved and Cripps, 1999; Wedemeyer, 1996). In comparison, influent quality and fouling does not reduce the pathogen removal efficiency of MF, whereas fouling can increase particle retention by restricting membrane pore size (Madaeni, 1999; Peters, 2010; Zhou and Smith, 2002). A study by Gomez et al. (2007) demonstrated that MF can achieve more consistent pathogen removal efficiency than UV when treating municipal wastewater and suggested that fluctuations in UV transmittance reduced the disinfection efficiency of UV. Therefore, MF may achieve higher pathogen removal efficiency than UV when treating wastewater in RAS because pathogen removal by MF is less impacted by fluctuations in fouling and water quality.

This thesis project attempts to evaluate the efficacy of using MF as a bacterial removal technology in RAS. As demonstrated above, investigation of alternative bacterial removal technologies is needed in order to reduce the prevalence of bacteria in RAS and potential incidences of clinical disease. More specifically, this thesis project also examines the ability of MF to remove *F. psychrophilum* in order to determine if MF can be used in RAS to reduce the incidence of BCWD. To accomplish the above objectives, this research project was divided in two parts:

- 1) Evaluation of membrane filtration and UV irradiation for the control of bacteria in recirculation aquaculture systems.
- 2) Control of *Flavobacterium psychrophilum* using membrane filtration.

Chapter 2: Evaluation of Membrane Filtration and UV Irradiation for the Control of Bacteria in Recirculation Aquaculture Systems

2.1 Introduction

The ability of pathogens to avoid or tolerate UV irradiation may increase pathogen loads in RAS, potentially increasing the incidence of infection and disease (Sharrer and Summerfelt, 2007; Wedemeyer, 1996). MF has been well documented as an effective wastewater treatment in many industries (Gomez et al., 2007), but its ability to continuously remove pathogens from wastewater in RAS has yet to be evaluated. MF may achieve higher bacterial removal efficiency than UV when treating wastewater in RAS because MF is less affected by variations in water quality and fouling (Gomez et al., 2007; Lazarova et al., 1999). However, MF systems may experience high rates of membrane fouling that requires frequent recovery cleaning, consequently reducing the long-term operation of MF in RAS (Crozes et al., 1997; Le-Clech et al., 2006; Madaeni et al., 1999). Conversely, MF systems have potential to experience low rates of membrane fouling in RAS since micro-screen filtration is commonly used to remove wastes that reduce UV disinfection efficiency and water quality (Gomez et al., 2007; Patterson et al., 1999). Therefore, MF has potential to achieve higher bacterial removal efficiencies than UV and maintain long-term operation when used to treat wastewater in RAS.

In this chapter, the efficacy of using MF as a bacterial removal technology in RAS was evaluated. It was hypothesized that the bacterial removal efficiency of MF is not impacted by fluctuations in water quality and fouling. If this hypothesis is true, then MF should achieve

higher bacterial removal efficiency than UV when treating wastewater in RAS. The following objectives were set out to test this hypothesis and prediction:

- 1) Measure and compare bacterial removal efficiencies achieved by MF and UV systems when treating wastewater in RAS.
- 2) Determine the impact of MF and UV systems on water quality when treating wastewater in RAS.
- 3) Measure the membrane fouling rates experienced by the MF system when treating wastewater in RAS and estimate the interval until recovery cleaning is required.

2.2 Methodology

2.2.1 Recirculation Aquaculture System

Research was performed at the Alma Aquaculture Research Station, Ontario, Canada, using a warm-water (i.e. 20-23°C) RAS that raised Nile tilapia (*Oreochromis niloticus*) broodstock. The 5,600 L system was composed of twelve 1-m circular fibreglass tanks that reared 271 fish (1.3 ± 0.003 kg, mean weight \pm S.E.) at a density of 96.8 ± 0.3 kg/m³. The animals were fed commercial feed on alternate days at a rate of 0.21 ± 0.02 % body weight to meet their maintenance energy requirements. The trial was carried out in accordance with the criteria set out by the Canadian Council of Animal Care (CCAC, 2005), under Animal Utilization Protocol #09G001.

A series of technologies were used in the RAS to remove solids, nitrogen, dissolved gasses and pathogens from wastewater and recirculate the treated water to cultured fish (Figure 2.1). Wastewater from twelve fish tanks was collected in an equalization sump and then treated by a 60 μ m rotating micro-screen drum filter (PR Aqua Supplies Ltd., BC, Canada). Treated wastewater was discharged into the collection sump, then side-streams were treated by a foam fractionator and a fluidized micro-bead biofilter coupled with a CO₂ stripper (PR Aqua Supplies Ltd., BC, Canada). Lastly, a pressurized wastewater stream from the collection sump was super-oxygenated in an oxygen cone (PR Aqua Supplies Ltd., BC, Canada) and then disinfected by a low-pressure, UV reactor (Trojan UV LogicTM midflow model 02AM20, Trojan Technologies Inc., ON, Canada) before it was recirculated to the fish tanks. The system recycled

approximately 98-99 % of the total flow and water lost from the drum filter, foam fractionator and sump overflow was replaced with well water (8.5°C).

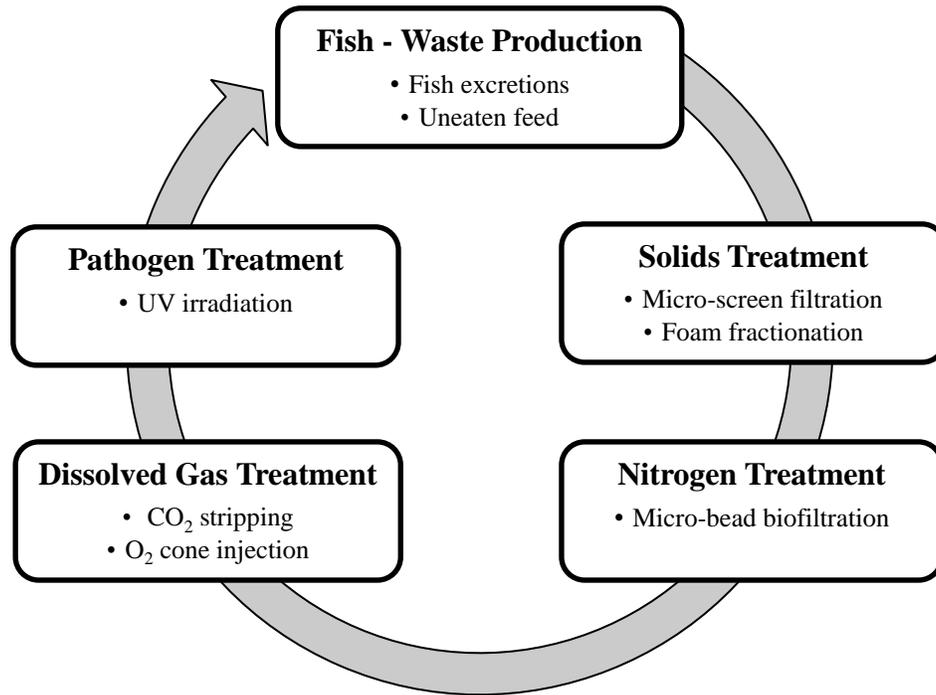


Figure 2.1. Schematic of the RAS illustrating the general wastewater production and treatment process.

2.2.2 UV Irradiation System

The UV system was composed of a 20 L closed-channel reactor that contained two UV lamps housed inside individual quartz sleeves. The UV system was designed to achieve a minimum UV dose of 60 mJ/cm² at a flow rate of 227 L/min and a UV intensity of 7.5 mW/cm². Before the study, two new UV lamps were installed into the reactor and the quartz sleeves were cleaned with a weak acid to maximize UV disinfection efficiency (Summerfelt et al., 2003; Wedemeyer,

1996). A UV sensor, positioned perpendicular to the UV lamps, was also cleaned to ensure accurate measurements of UV intensity were taken. Appendix 1 summarizes the manufacturer's specifications, operating conditions and configuration of the UV system.

2.2.3 Membrane Filtration System

The MF system (Figure 2.2) was composed of a membrane filter (submerged type element LSU-1515, Toray Membrane Inc., CA, USA) submerged in a vertical PVC tank that was capped at the bottom, termed "membrane tank". The membrane tank, dimensions 1.8 m height x 0.2 m diameter, provided 40 L of operating capacity with an overflow port located 0.02 m below the top of the tank. The filter consisted of approximately 6,500 hollow-fibre membranes made of polyvinylidene fluoride (PVDF), which provided a total membrane surface area of 25 m². The membranes had a nominal pore size of 0.01 µm or a molecular weight cut off (MWCO) of 150 kDa. The membranes were potted at the bottom and open at the top to allow filtered water to collect in the manifold at the top of the filter. A protective cylinder surrounded the membranes with openings at the top and bottom to allow cross-flow of influent wastewater. Appendix 1 summarizes the manufacturer's specifications, operating conditions and configuration of the membrane filter.

A pressurized side stream of wastewater was diverted, before UV treatment, into the membrane tank to supply both MF and UV systems with the same quality of influent wastewater. The MF system was operated in a cross-flow configuration, which produced two streams; water that diffused through the membranes, termed "permeate", and wastewater that was retained by the

membranes, termed “retentate” (Madaeni, 1999). Vacuum pressure was applied to the permeate side of the membranes using a 1/15 hp centrifugal pump (Cole-Parmer, QB, Canada), which drew permeate through the membranes and discharged it back into the sump. Influent wastewater entered the membrane tank at a constant rate of approximately 16 L/min, permeate water (effluent) discharged from the membrane filter at 15 L/min (permeate flux of 36 L/m²/hr) and retentate wastewater overflowed from the membrane tank at 1 L/min. Influent and effluent flow rates were measured, in triplicate, using a stopwatch and graduated cylinder and the rates were adjusted every two or three days using back-pressure from a gate valve installed after the influent and effluent pumps (Figure 2.2).

Transmembrane pressure was measured and recorded every minute using a pressure transducer (Cole-Parmer, QB, Canada) connected to a datalogger (Lascar Electronics Inc., PA, USA), which was used to calculate membrane fouling. In addition, a negative pressure gauge (Ashcroft Inc., CT, USA) was installed between the transducer and effluent pump to improve control over the MF system. Three direct cleaning (anti-fouling) strategies were applied to the MF system in this study: continuous air-scouring, relaxation cycles and maintenance cleanings. Air was generated at a rate of approximately 25 L/min by a ½ hp aerator (GAST Manufacturing Inc., MI, USA) connected to an air stone placed at the bottom of the membrane tank. A repeat cycle timer (ART-DNe, Custom Automated Products Inc., CA, USA) was used to turn off the effluent pump for one minute every 30 minutes, to relax the membranes and enhance the fouling removal efficiency of continuous air-scouring. The permeate discharge point was elevated to the same height as the water level in the membrane tank (i.e. 1.7 m) to avoid continual filtration, due to suction, when the timer turned off the effluent pump during relaxation cycles.

Maintenance cleanings were performed on the MF system every two or three days, as recommended by the manufacturer (Toray Membrane Inc., CA, USA), to disinfect the membrane filter and prevent bacterial contamination. However, maintenance cleanings were not scheduled to occur unless the filter was contaminated with bacteria since frequent chemical cleaning events reduce long-term operation of MF systems (Le-Clech et al., 2006). Maintenance cleanings involved taking the MF system offline, recirculating a solution of 300 mg/L (0.03 %) sodium hypochlorite through the system for ten minutes, soaking the system in the solution for twenty minutes and then rinsing the system with well water for 90 minutes (Toray Membrane Inc., CA, USA). During the MF system soak, the solution was aerated for 30 seconds every five minutes to ensure the solution was properly mixed and then both the solution and rinse water were drained from the MF system for the remainder of the cleaning event (Toray Membrane Inc., CA, USA). After the MF system was rinsed, it was re-installed into the RAS after no free chlorine was detected in the permeate samples (Hach Method 4500-Cl) to ensure no residual chlorine was transferred to the fish rearing system. Recovery cleanings were also required when the transmembrane pressure exceeded 70 kPa, which involved the same procedure as maintenance cleaning, except a stronger solution of sodium hypochlorite (ie. 3,000 mg/L or 0.3 %) was used to remove more foulants and restore the transmembrane pressure (Toray Membrane Inc., CA, USA). In addition, a pressure decay test (also called integrity test) was performed on the membrane filter before and after the trial to determine if any membranes were broken (Toray Membrane Inc., CA, USA). The membrane filter used in this study was new, except for a few days of conditioning.

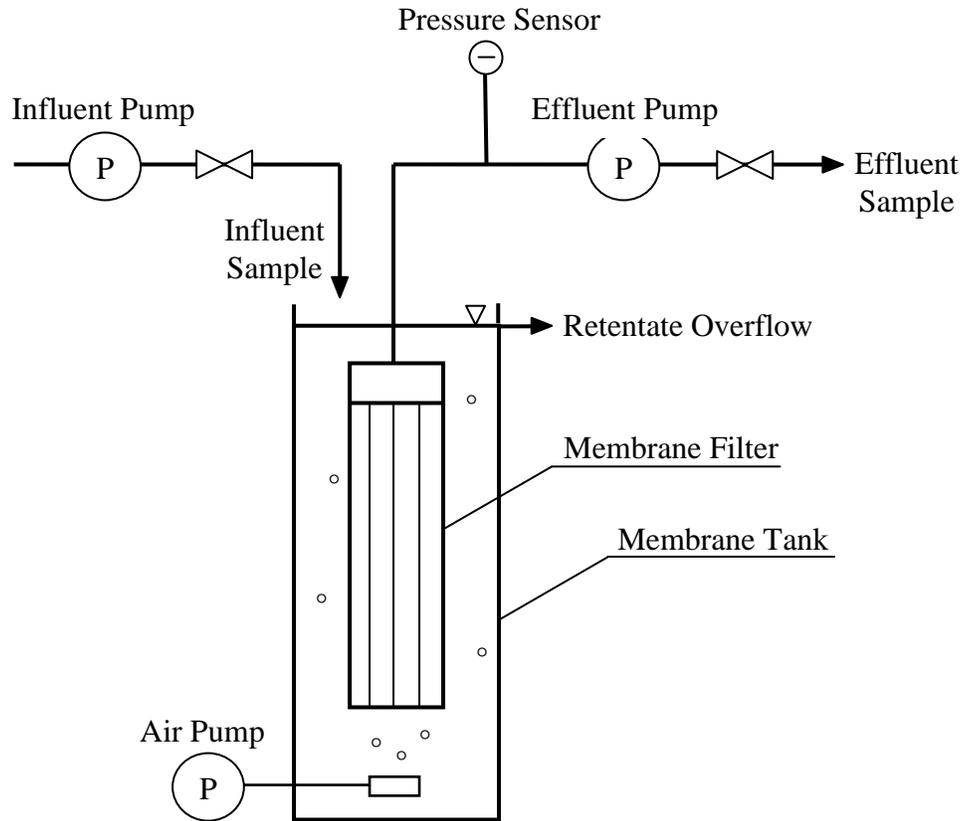


Figure 2.2. Schematic of the MF system illustrating wastewater from the RAS pumped into the membrane tank (influent) and permeate pumped through the membrane filter (effluent), which was recirculated to the rearing system. The membrane filter was continuously air-scoured as retentate overflowed from the membrane tank.

2.2.4 Sampling Regimen

The MF system processed wastewater from the RAS, in parallel with the UV system, for 30 days. Grab samples were collected every two or three days from wastewater streams at three sample locations within the RAS: before MF/UV treatment (influent), after MF treatment (effluent) and after UV treatment (effluent; Figure 2.3). Water samples were collected from each site at approximately the same time every day of sampling to maintain consistency. Experimental conditions were repeated as a function of time, as samples were collected and measured every two or three days over a 30 day period.

For bacterial analyses, 250 mL water samples were collected in sterile bottles, in triplicate, from the same three sample locations and then placed on ice to slow bacterial growth. Additional samples were collected from the MF effluent site before each maintenance cleaning event to determine the bacterial removal efficiency of MF before the system was disinfected. For chemical/physical analyses, 1 L water samples were collected, in duplicate, from the three sample locations. For both bacterial and chemical/physical analyses, samples were taken five minutes apart from each other to account for fluctuations in water quality. In addition, extra samples were collected once every seven days, placed on ice and submitted to the Agriculture and Food Laboratory at the University of Guelph to measure additional water quality parameters.

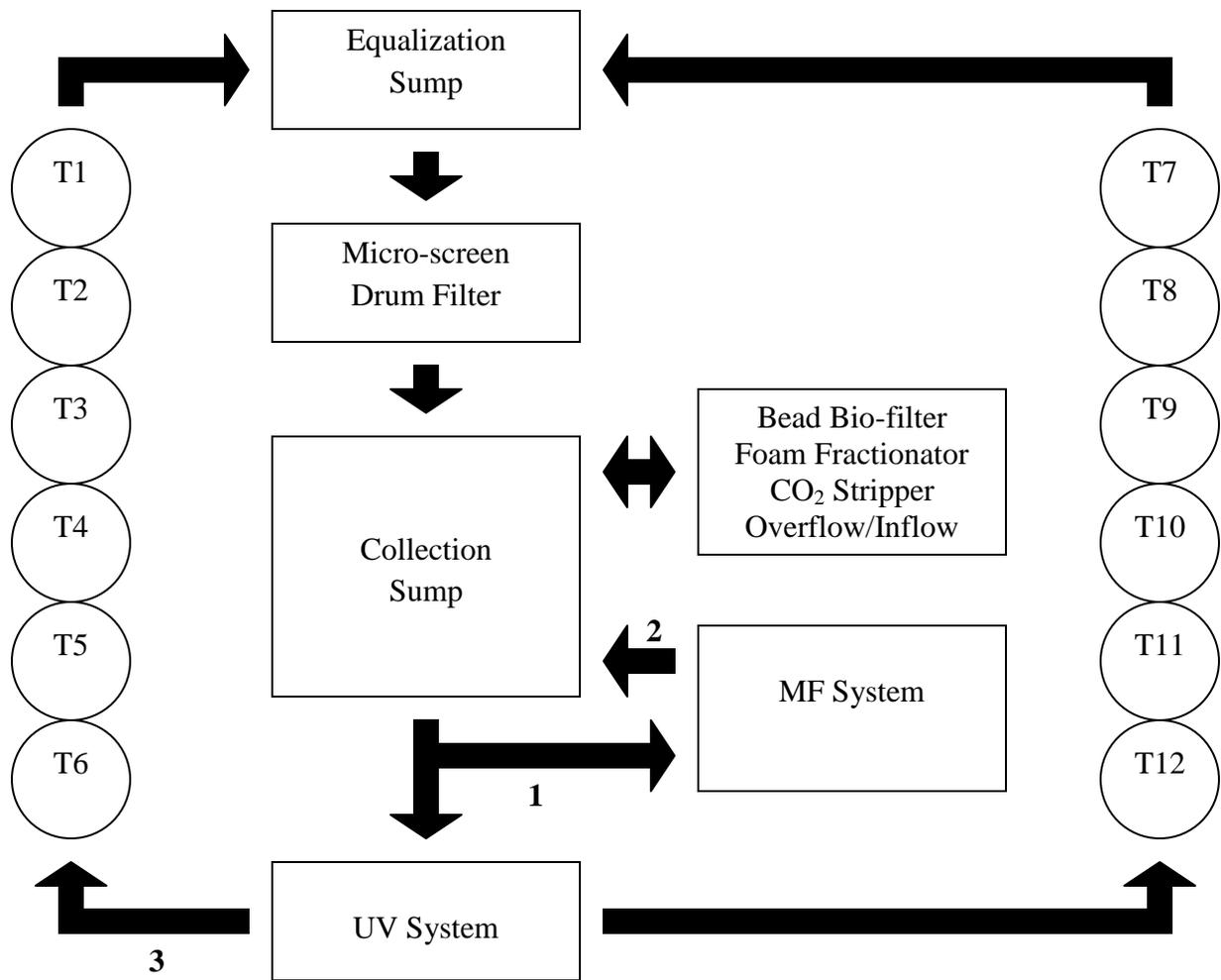


Figure 2.3. Schematic of the RAS illustrating wastewater production and treatment, with the addition of the MF system and sampling locations. Wastewater from the fish tanks (T1-T12) was collected in the equalization sump, treated by multiple processes and then discharged into the collection sump. Treated wastewater was supplied to both MF and UV systems, which was processed and recirculated to the collection sump and fish tanks. Bold numbers indicate the three sample locations: 1) wastewater before MF/UV treatment (influent), 2) after MF treatment (effluent) and 3) after UV treatment (effluent).

2.2.5 Water Quality Analyses

2.2.5.1 Bacterial Analyses

Total bacterial counts of each water sample were determined using the NEO-GRID/ISO-GRID membrane filtration system (Neogen, MI, USA) as described in the Official Methods of Analysis method 986.32 (AOAC, 2007). Aseptically, an aliquot of each sample was individually vacuum-filtered, in duplicate, on an iso-grid and then placed on tryptic soy agar (Sigma-Aldrich, MO, USA). Agar plates were incubated for approximately three days at 18°C. After incubation, bacterial colonies that developed on the grids were enumerated and converted to its corresponding Most Probable Number (MPN) and then multiplied by the dilution factor to obtain cfu/mL of total bacteria (AOAC, 2007). Additional water samples were submitted to the Agriculture and Food Laboratory at the University of Guelph to perform heterotrophic plate counts according to the Standard Methods (APHA, 1998).

2.2.5.2 Chemical/Physical Analyses

Dissolved oxygen and temperature were measured at each sample site during collection using a handheld probe (OxyGuard, Denmark). All the other water quality parameters were measured from grab samples after collection. UV absorbance was measured at a 1 cm path length and a wavelength of 254 nm using a UV spectrophotometer with Chemstation[®] software (Hewlett-Packard, CA, USA), which was used to calculate UV transmittance (i.e. $UVT = 10^{-(UV \text{ absorbance})} \times 100\%$). Additional water samples were submitted to the Agriculture and Food

Laboratory at the University of Guelph to measure levels of total Kjeldahl nitrogen and total organic carbon according to the Standard Methods (APHA, 1998). Table 2.1 summarizes the analytical methods, units and sample frequency of each water quality parameter measured throughout the 30 day trial.

Table 2.1. Analytical methods, units and sample frequency of each water quality parameter measured throughout the 30 day trial. Water samples were collected from three locations in the RAS and analyzed every 2-3 or 7 days.

Parameter	Method	Units	Sample Frequency (days)
Dissolved Oxygen	OxyGuard DO243C meter	mg/L	2-3
Temperature	OxyGuard DO243C meter	°C	2-3
pH	YSI pH100 meter		2-3
Total Suspended Solids	Standard Methods 2560	mg/L	2-3
Total Dissolved Solids	Hach 44600 meter	mg/L	2-3
Conductivity	Hach 44600 meter	mS/cm	2-3
Turbidity	HF Micro 100 turbidimeter	NTU	2-3
UV Transmittance (254nm)	HP UV-Vis 8453 spec.	%	2-3
Total Hardness	Hach Method 8213	mg CaCO ₃ /L	2-3
Nitrite Nitrogen	Hach Method 8507	mg/L	2-3
Nitrate Nitrogen	Hach Method 8039	mg/L	2-3
Total Ammonia Nitrogen	Hach Method 8038	mg/L	2-3
Total Kjeldahl Nitrogen	Standard Methods 4500	mg/L	7
Total Organic Carbon	Standard Methods 5310	mg/L	7
Organic Nitrogen	Calculated*	mg/L	7
Total Nitrogen	Calculated**	mg/L	7
Heterotrophic Plate Count	Standard Methods 9215	cfu/mL	7
Total Bacterial Count	AOAC Method 986.32	cfu/mL	2-3

* Organic nitrogen = total Kjeldahl nitrogen – total ammonia nitrogen.

** Total nitrogen = total Kjeldahl nitrogen + total ammonia nitrogen + nitrate nitrogen + nitrite nitrogen.

2.2.6 Data Analyses

2.2.6.1 Calculating Removal Efficiencies

Removal efficiencies of MF and UV treatments were calculated as the percentage difference between the water quality values measured from the influent and effluent water samples, defined by the equation:

$$\% \text{ Removal} = \frac{\text{value}_{\text{influent}} - \text{value}_{\text{effluent}}}{\text{value}_{\text{influent}}} \times 100$$

Log₁₀ reductions of bacteria achieved by MF and UV treatments were based on the percent removal values calculated above, defined by the equation:

$$\text{Log}_{10} \text{ reduction} = - \log_{10} \left(1 - \frac{\% \text{ removal}}{100} \right)$$

2.2.6.2 Calculating Operational Parameters

Residence time (τ) for each system was calculated by dividing the volume (V) of the treatment system by its effluent flow rate (Q_e), defined by the equation (Metcalf and Eddy, 2003):

$$\tau = \frac{V}{Q_e}$$

Permeate flux (J) was calculated by dividing permeate flow rate (Q_p) by membrane surface area (A), defined by the equation (Metcalf and Eddy, 2003):

$$J = \frac{Q_p}{A}$$

UV dose was calculated by multiplying UV intensity values measured during the trial by the residence time (τ) calculated above, defined by the equation (Metcalf and Eddy, 2003):

$$\text{UV dose} = \text{UV intensity} \times \tau$$

2.2.6.3 Calculating Membrane Fouling

Transmembrane pressure (TMP) was corrected for by subtracting the hydrostatic pressure loss from the MF system by the pressure values recorded by the datalogger during the 30 day trial, defined by the equation (Freeze and Cherry, 1979):

$$\text{TMP} = \rho gh - p_0$$

where;

ρ = fluid density (kg/m^3);

g = gravitational acceleration (m/s^2);

h = height (m) between the effluent pump and point of discharge;

p_0 = initial pressure (kPa).

Fouling resistance (R_f) was calculated based on the TMP value calculated above and the initial membrane resistance (R_m) recorded at the start of the 30 day trial ($R_f = 0$), defined by Darcy's equation (Belfort et al., 1994):

$$J = \frac{\text{TMP}}{\eta(R_m + R_f)}$$

where;

J = permeate flux ($\text{L/m}^2/\text{hr}$);

TMP = transmembrane pressure (kPa);

η = viscosity (kPa·min);

R_m = membrane resistance (/m);

R_f = fouling resistance (/m).

Fouling rate (F) was calculated as the difference between the final and initial fouling resistances (R_f), which was divided by the duration (t) of each filtration cycle (i.e. interval between each maintenance cleaning event), defined by the equation (Fan and Zhou, 2007):

$$F = \frac{R_{f,t2} - R_{f,t1}}{t_2 - t_1}$$

Mean values, efficiencies and rates achieved by MF and UV treatments were reported with \pm standard error and calculated using Microsoft[®] Office Excel[®] 2007 software.

2.2.7 Statistical Analyses

A Shapiro-Wilk test was performed on each water quality data set to determine data normality (Field et al., 2012). A two-sample Student's t-test was performed on data sets that were normally distributed and a two-sample Wilcoxon signed-rank test was performed on data sets that were not normally distributed (Field et al., 2012). Statistical analyses were performed to determine if significant differences ($p < 0.05$) existed between removal efficiencies of total and heterotrophic bacteria for MF and UV treatments. In addition, differences between removal efficiencies of total bacteria for each MF and UV treatment from the first and second half of the trial were also analyzed to determine if each treatment was consistent throughout the 30 day trial. Mean values

of chemical/physical water quality parameters were compared between influent and effluent samples to determine if significant differences existed ($p < 0.05$) for each treatment. All statistical analyses were completed using R[®] version 2.11.1 software (R Core Development Team, 2011).

2.3 Results

2.3.1 Bacterial Removal by MF and UV Treatments

Both MF and UV systems achieved high removal efficiencies of total and heterotrophic bacteria throughout the 30 day trial (Table 2.2 and 2.3). The MF and UV treatments removed 98.6 ± 0.4 % and 99.6 ± 0.1 % of total bacteria and 93.2 ± 5.2 % and 92.5 ± 4.1 % of heterotrophic bacteria, respectively. Data sets for each MF and UV treatment were not normally distributed for total ($W = 0.873$, $n = 12$, $p = 0.071$; $W = 0.551$, $n = 14$, $p = 0.00002$) and heterotrophic bacteria ($W = 0.664$, $n = 5$, $p = 0.004$; $W = 0.773$, $n = 5$, $p = 0.048$). The removal efficiencies of total and heterotrophic bacteria between MF and UV treatments were not significantly different ($W = 50$, $n = 12$, $p = 0.085$; $W = 12$, $n = 5$, $p = 1$). In addition, removal efficiencies of total bacteria between the first (i.e. days 1-15) and second half (i.e. days 16-30) of the trial were not significantly different ($W = 29$, $n = 6$, $p = 0.073$; $W = 19$, $n = 7$, $p = 0.535$) for each MF and UV treatment (Table 2.4).

Two water samples collected after MF treatment, on day four and seven of the trial, were not included in the analyses of total bacteria because the effluent bacterial counts were higher than influent bacterial counts (Figure 2.4), which indicated that the permeate side of the membrane filter was contaminated with bacteria. After this occurrence, maintenance cleanings were implemented every two or three days, which started on the ninth day and prevented bacterial contamination for the remainder of the trial. Appendix 2 summarizes the total and heterotrophic counts measured throughout the 30 day trial.

Low levels of total and heterotrophic bacteria bypassed both MF and UV treatments (Table 2.2 and 2.3). Total bacterial counts after MF and UV treatments ranged between 0 to 10 cfu/mL and 0 to 6 cfu/mL. Heterotrophic bacterial counts after MF and UV treatments ranged between 0 to 15 cfu/mL and 0 to 20 cfu/mL, respectively. Appendix 3 illustrates the variation in effluent bacterial counts between the MF and UV treatments.

Table 2.2. Mean (\pm S.E.) counts and removal efficiencies of total bacteria achieved by MF and UV systems when used to treat wastewater in the RAS over the 30 day trial (n = 14).

Treatment	Influent (cfu/mL)	Effluent (cfu/mL)	Removal (%)	Log ₁₀ Reduction
MF	263.5 \pm 18.2	3.6 \pm 1.0*	98.5 \pm 0.4	2.4 \pm 0.1
UV	263.5 \pm 18.2	1.0 \pm 0.4	99.6 \pm 0.1	2.7 \pm 0.1

* Two bacterial counts were not included in the analysis due to filter contamination.

Table 2.3. Mean (\pm S.E.) counts and removal efficiencies of heterotrophic bacteria achieved by MF and UV systems when used to treat wastewater in the RAS over the 30 day trial (n = 5).

Treatment	Influent (cfu/mL)	Effluent (cfu/mL)	Removal (%)	Log ₁₀ Reduction
MF	343.0 \pm 129.6	7.0 \pm 2.5	93.2 \pm 5.2	1.4 \pm 0.3
UV	343.0 \pm 129.6	8.0 \pm 3.7	92.5 \pm 4.1	1.0 \pm 0.3

Table 2.4. Mean (\pm S.E.) counts and removal efficiencies of total bacteria achieved by MF and UV systems in the RAS between the first and second half of the 30 day trial (n = 7).

Treatment	Days	Influent (cfu/mL)	Effluent (cfu/mL)	Removal (%)	Log ₁₀ Reduction
MF	1-15	293.2 \pm 27.7	1.7 \pm 1.2*	99.4 \pm 0.4	3.1 \pm 0.6
MF	16-30	233.8 \pm 19.2	5.0 \pm 1.4	97.9 \pm 0.6	1.9 \pm 0.2
UV	1-15	293.2 \pm 27.7	0.7 \pm 0.1	99.7 \pm 0.1	2.6 \pm 0.1
UV	16-30	233.8 \pm 19.2	1.2 \pm 0.8	99.6 \pm 0.3	2.8 \pm 0.2

* Two bacterial counts were not included in the first half due to filter contamination.

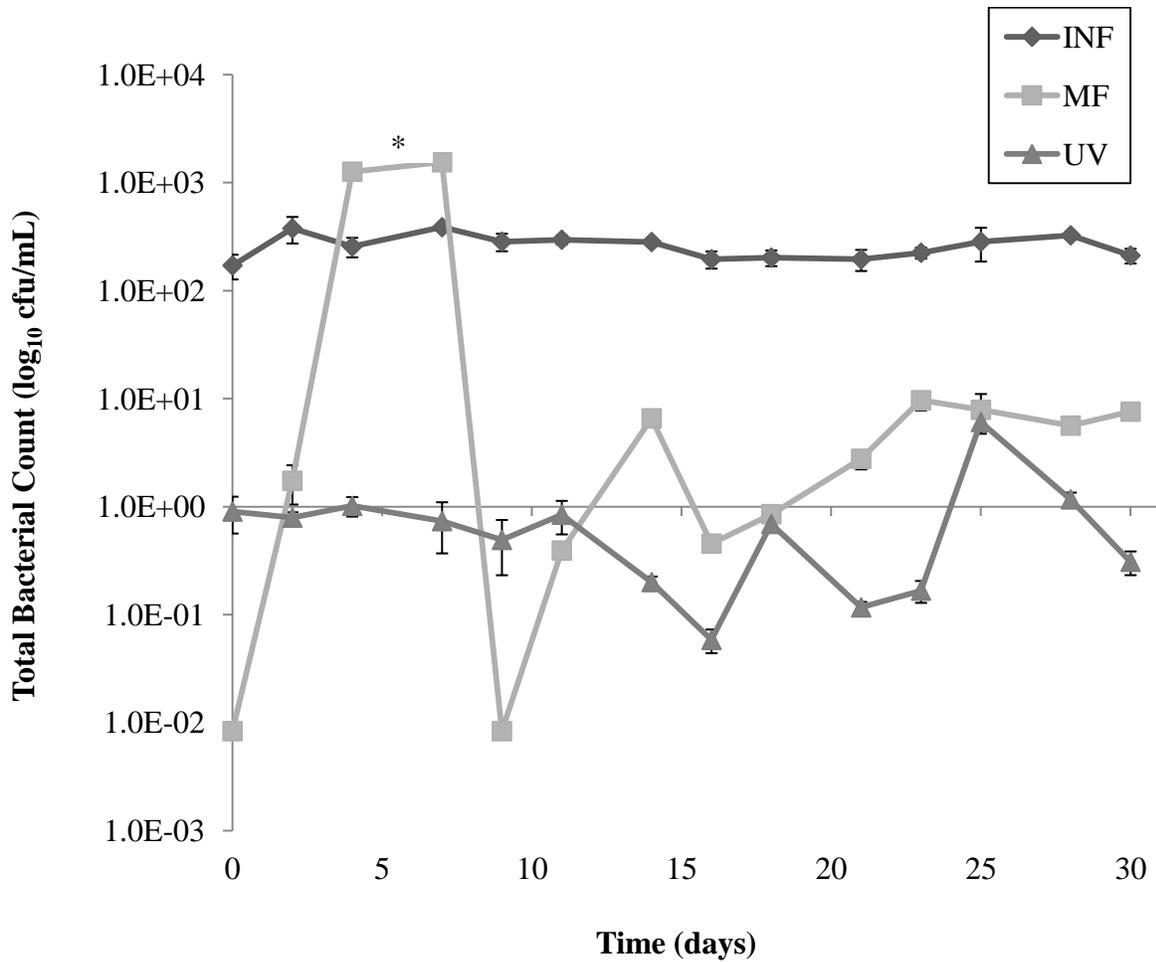


Figure 2.4. Total bacterial counts (\pm S.D.) measured from water samples collected throughout the 30 day trial ($n = 14$) from three sample sites within the RAS; MF/UV influent (INF), MF effluent (MF) and UV effluent (UV).

* Two total bacterial counts from MF effluent samples collected on day four and seven were not included in the bacterial analyses due to filter contamination.

2.3.2 Chemical/Physical Impact by MF Treatment

MF influent and effluent levels were significantly different ($p < 0.05$) from each other for water quality parameters of turbidity, total suspended solids (TSS), UV transmittance (UVT), pH and dissolved oxygen (DO; Table 2.5). The MF system achieved 93.6 ± 1.0 % and 95.4 ± 1.0 % removal of turbidity and TSS, which increased UVT by 3.0 ± 0.4 %. No significant differences ($P < 0.05$) existed between the MF influent and effluent levels for temperature, total dissolved solids, conductivity, total hardness, total organic carbon, nitrite nitrogen, nitrate nitrogen, total ammonia nitrogen, total Kjeldahl nitrogen, organic nitrogen and total nitrogen (Appendix 4).

Table 2.5. Mean (\pm S.E.) influent and effluent values, that were statistically significant ($p < 0.05$), and removal efficiencies achieved by the MF system when used to treat wastewater in the RAS throughout the 30 day trial ($n = 13$).

Parameter	Units	Influent	Effluent	% Removal/Change	P-value
Turbidity	NTU	0.88 \pm 0.07	0.06 \pm 0.01	93.60 \pm 1.03	<0.0001 ^b
TSS	mg/L	1.33 \pm 0.12	0.06 \pm 0.01	95.35 \pm 1.04	<0.0001 ^b
UVT	%	92.05 \pm 0.66	94.83 \pm 0.56	-3.04 \pm 0.37	0.0038 ^a
pH		8.23 \pm 0.02	8.35 \pm 0.03	-1.39 \pm 0.18	0.0110 ^b
DO	mg/L	9.10 \pm 0.04	8.78 \pm 0.05	3.45 \pm 0.63	0.0001 ^b

^a Analyzed using a two-sample Student's t-test.

^b Analyzed using a two-sample Wilcoxon signed-rank test.

2.3.3 MF and UV Operation

This trial was designed to supply both MF and UV treatments with the same quality of wastewater in order to evaluate bacterial removal efficiencies, but the operational performance between MF and UV systems was not equal due to differences in scale and treatment (Table 2.6). Notably, the MF system produced effluent at a rate of 14.9 ± 0.1 L/min compared to 193.5 ± 1.2 produced by the UV system. In addition, the MF system required a residence time of 159.8 ± 0.8 seconds compared to 6.2 ± 0.04 seconds required by the UV system.

Large fluctuations in the influent level of TSS were not observed throughout the 30 day trial, except on day 25 when the level of TSS was highest (i.e. 2.7 mg/L; Figure 2.5). In comparison, large fluctuations in the applied UV dose were observed throughout the trial. As expected, one of the lowest UV doses was recorded on day 25 (i.e. 101 mJ/cm^2) when the TSS level was the highest and UV transmittance level was the lowest (i.e. 88.5 %). However, sleeves of the UV system did not require cleaning throughout the 30 day trial despite fluctuations in the applied UV dose.

Table 2.6. Mean (\pm S.E.) values of water flow rates and treatment performance achieved by MF and UV systems when used to treat wastewater in the RAS throughout the 30 day trial ($n = 13$).

Parameter	Units	MF	UV
Influent Flow Rate	L/min	15.8 \pm 0.1	193.5 \pm 1.2
Effluent Flow Rate	L/min	14.9 \pm 0.1	193.5 \pm 1.2
Retentate Overflow Rate	L/min	0.9 \pm 0.1	0 \pm 0
System Recovery	%	94.2 \pm 0.5	100.0 \pm 0
Residence Time	s	159.8 \pm 0.8	6.2 \pm 0.04
Permeate Flux	L/m ² /hr	35.8 \pm 0.1	n/a \pm n/a
UV Intensity	mW/cm ²	n/a \pm n/a	17.4 \pm 0.2
UV Dose	mJ/cm ²	n/a \pm n/a	108.5 \pm 1.5

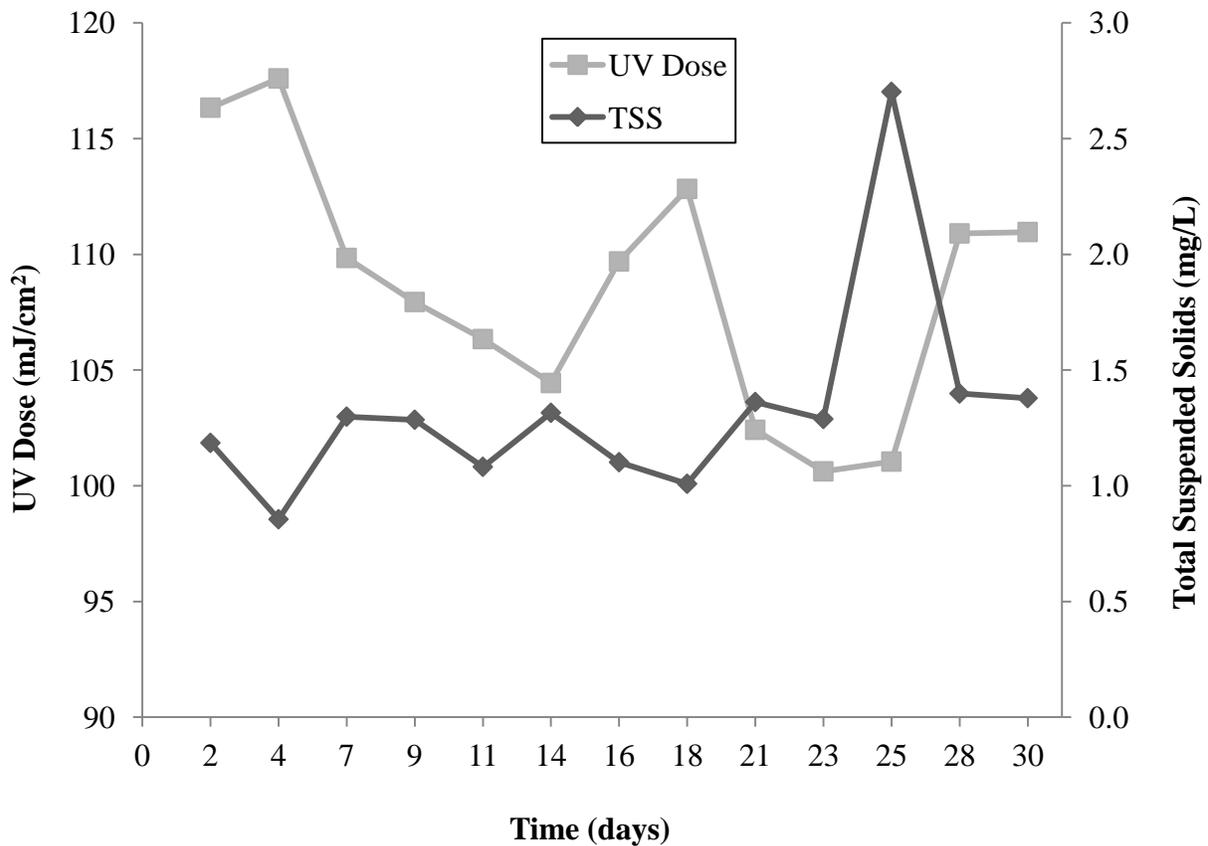


Figure 2.5. UV doses achieved by the UV system and total suspended solids (TSS) values measured from influent wastewater samples collected from the RAS throughout the 30 day trial ($n = 13$).

2.3.4 Membrane Fouling

Transmembrane pressure (TMP) of the MF system increased from 3.1 kPa to 22.9 kPa over the 30 day trial. Recovery cleaning was not performed on the MF system throughout the trial because it is not required until the level of TMP exceeds 70 kPa (Toray Membrane Inc., CA, USA). Based on the rate of TMP experienced by the MF system over 30 days and assuming a linear relationship thereafter, recovery cleaning was estimated to be required after 134 days or 4.5 months of MF operation ($R^2 = 0.648$; Figure 2.6).

Permeate flux was maintained at a constant rate throughout the trial, thus increases in TMP indicated increases in fouling resistance across the MF system. Fouling resistance increased throughout the 30 day trial, especially during the first nine days of the trial when maintenance cleanings were not yet implemented (Figure 2.7). Once maintenance cleanings were implemented (i.e. after day nine), the fouling resistance decreased and did not surpass the level of resistance recorded on day nine until the twenty third day of filtration. Also, the first three cleaning events reduced the fouling resistance the most compared to the other events performed later in the trial. Appendix 5 summarizes the operational parameters achieved by the MF system, which was used to calculate the fouling resistances and rates.

Maintenance cleanings were performed ten times on the MF system throughout the 30 day trial, thus ten filtration cycles occurred (i.e. interval between each maintenance cleaning). The first filtration cycle was the longest since the first maintenance cleaning was performed on the ninth day of filtration (Figure 2.7). In addition, the fouling rate was highest during the first filtration

cycle at 2.3×10^5 /m/day or a change in TMP of 2.1 kPa/day, but the following fouling rates for each cycle were lower after maintenance cleanings were implemented (Figure 2.8). The average fouling rate over the 30 day trial (10 cycles) was $8.59 \pm 1.9 \times 10^4$ /m/day (change in TMP of 0.8 ± 0.2 kPa/day). The initial and final fouling resistances used to calculate the fouling rates were averaged from the first and last 30 minutes of each cycle to account for TMP fluctuations. However, initial fouling resistances of cycles two, three and four were calculated after five hours of filtration when TMP stabilized in order to avoid negative membrane fouling rates after maintenance cleaning events.

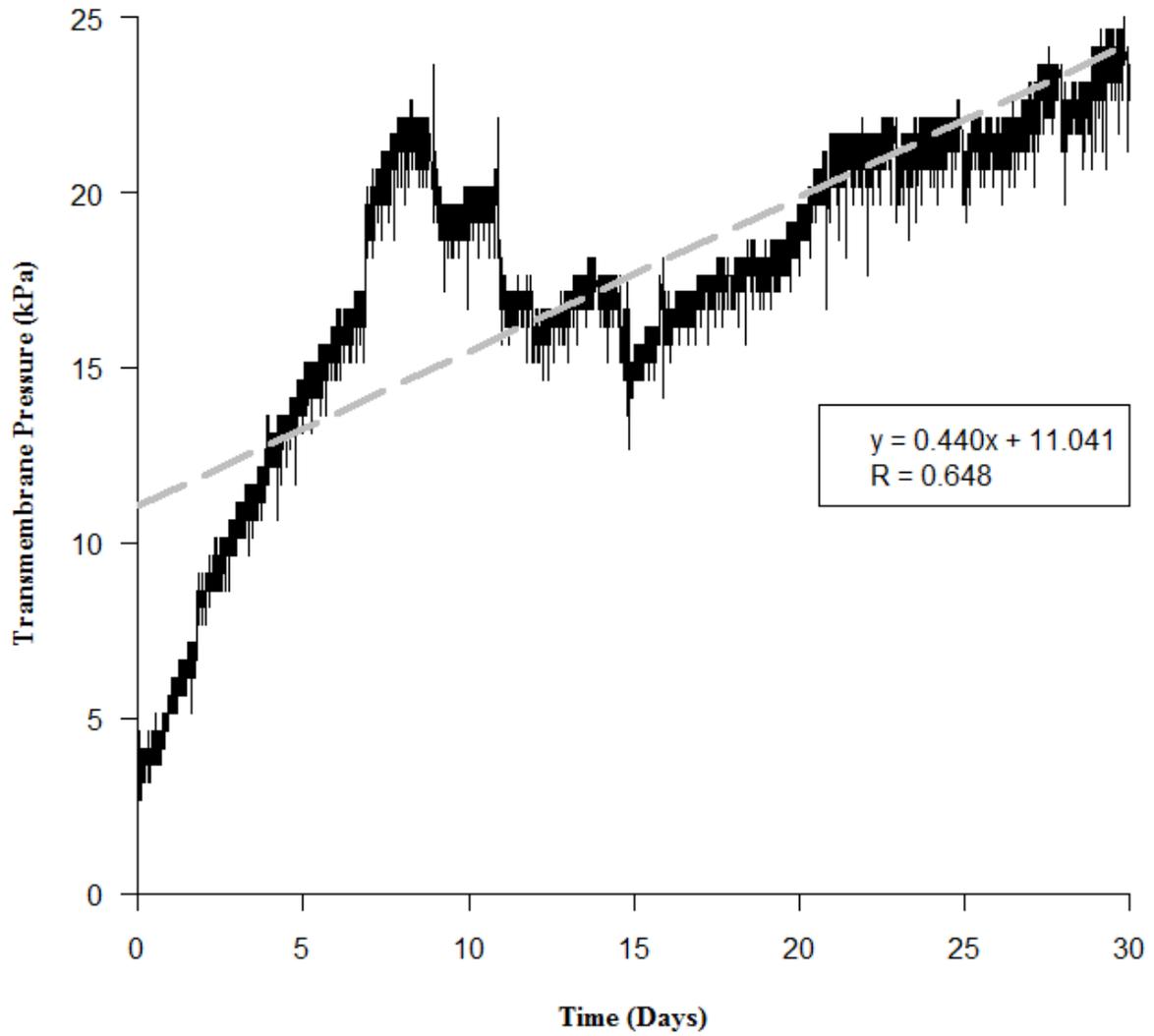


Figure 2.6. Transmembrane pressure (TMP) experienced by the MF system when used to treat wastewater in the RAS over 30 days.

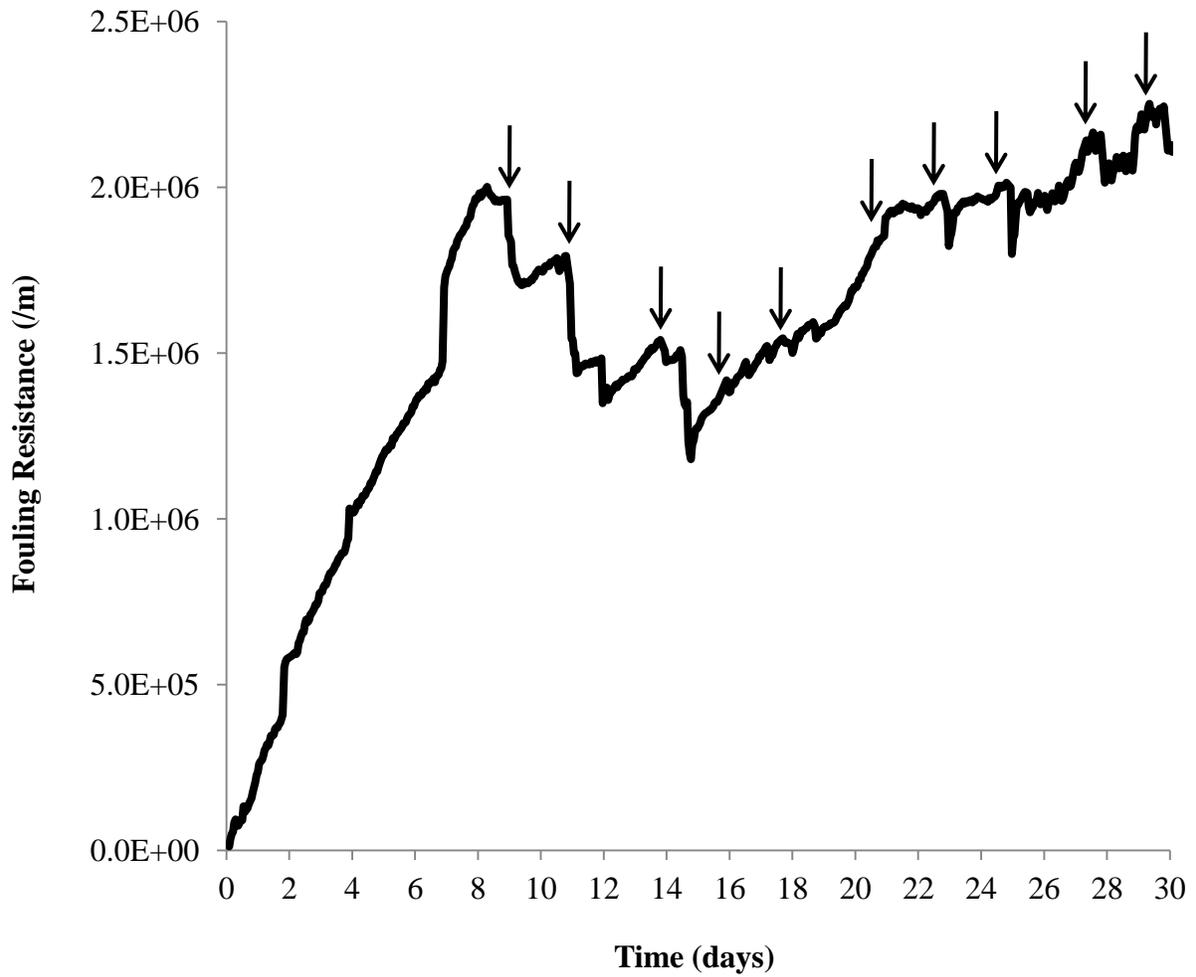


Figure 2.7. Fouling resistance experienced by the MF system when used to treat wastewater in the RAS over 30 days. Arrows indicate when maintenance cleanings were performed.

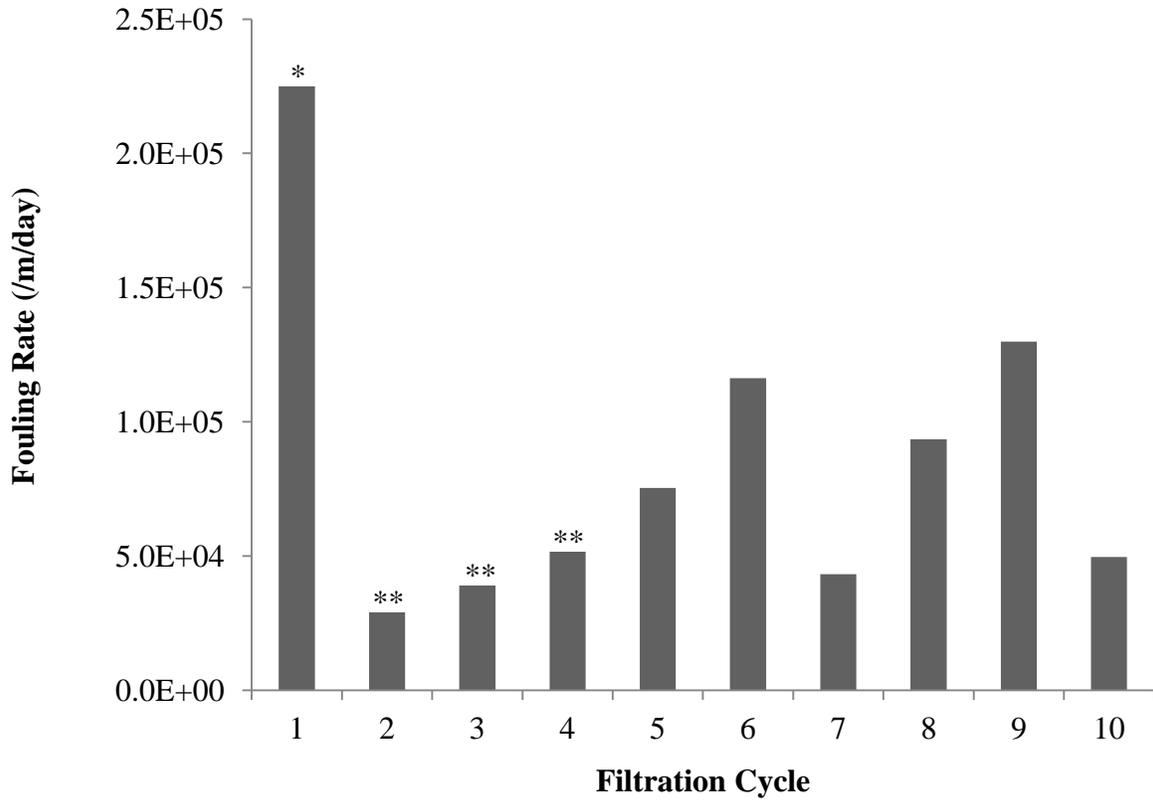


Figure 2.8. Fouling rates, per filtration cycle (2-3 days), experienced by the MF system when used to treat wastewater in the RAS over 30 days.

* The first filtration cycle was nine days in duration.

** Initial fouling resistances were taken five hours after the start of the filtration cycle to avoid negative fouling rates after maintenance cleaning events.

2.4 Discussion

2.4.1 Bacterial Removal by MF and UV Treatments

These results supported the hypothesis because the removal efficiency of total and heterotrophic bacteria by MF was not impacted by fluctuations in water quality and fouling. Additionally, the results did not support the prediction because the MF treatment did not achieve higher bacterial removal than UV. On the other hand, bacterial removal by MF was indistinguishable from removal by UV, which demonstrated the potential applicability of MF as an alternative to UV irradiation in RAS.

The results from this study were consistent with findings from other studies that have evaluated the bacterial removal efficiency of MF when treating various types of wastewater. For example, MF systems (0.005 and 0.01 μm pore size) in studies by Guo et al. (2009) and Nakatsuka et al. (1996) achieved over 2 and 3 log reductions of total bacteria from surface waters. In contrast, a MF system (0.01 μm pore size) in a study by Glucina et al. (2000) achieved over 4 log reductions of heterotrophic bacteria from surface waters. However, the MF system in Glucina et al. (2000) was challenged with higher levels of bacteria than the MF system in this study, which may have allowed the MF system to achieve higher bacterial removal efficiency. Therefore, the results from this study were consistent with the bacterial removal efficiencies achieved by MF in other studies.

MF and UV systems may have achieved the same bacterial removal efficiencies in this study because the high quality of influent wastewater may not have reduced the efficiency of UV. Wastewater high in suspended solids reduces UV transmittance and the applied UV dose (Liltved and Cripps, 1999; Wedemeyer, 1996), but wastewater in this study was low in suspended solids and high in UV transmittance. UV transmittance of influent wastewater was high in this study because the brood fish reared in the RAS were fed low amounts of feed (i.e. 0.2 % body weight) only to meet their maintenance energy requirements, consequently reducing the level of suspended solids in their wastewater. Thus, fewer bacteria should have been shielded by particles, consequently increasing UV transmittance and maximizing the applied UV dose. Therefore, the high quality wastewater in the RAS may have allowed the UV system to achieve maximum bacterial removal efficiency in this study, consequently matching the efficiency of MF.

MF achieved equivalent bacterial removal efficiency compared to UV in this study, but potential exists for MF to achieve higher removal efficiency in RAS when treating more concentrated wastewater, specifically low in UV transmittance. A study by Gomez et al. (2007) achieved more consistent pathogen removal efficiency using a MF system (0.05 μm membrane pore size) compared to a UV system (20-37 mJ/cm^2 applied UV dose) when treating municipal wastewater. Gomez et al. (2007) suggested that variations in water quality, specifically those which reduced UV transmittance, reduced the efficiency of UV disinfection. In addition, other studies have demonstrated that reductions in UV transmittance, due to particle shielding, reduce UV disinfection efficiency (Lazarova et al., 1999; Liltved and Cripps, 1999; Sharrer et al., 2005). On the other hand, MF can tolerate fluctuations in influent wastewater quality (Gomez et al., 2007;

Guo et al., 2009, Lazarova et al., 1999). Therefore, MF may potentially achieve higher pathogen removal efficiency than UV in RAS when challenged with concentrated wastewater, specifically low in UV transmittance, but more research is required to determine this possibility.

2.4.2 Solids Removal by MF Treatment

The high solids removal efficiency achieved by the MF system in this study (Table 2.5) was similar to other studies that used MF to treat different types of wastewater. For example, a MF system (0.05 μm pore size) in a study by Gomez et al. (2007) achieved approximately 91 and 99 % removal of suspended solids and turbidity, respectively, from municipal wastewater. MF systems (both 0.01 μm pore size) in Glucina et al. (2000) and Nakatsuka et al. (1996) achieved approximately 99 % removal of turbidity from surface waters. Viadero and Noblet (2002) also demonstrated that a MF system (0.05 μm pore size) can remove approximately 94 % of suspended solids from synthetic fish wastewater. Therefore, the results from this study were consistent with the solids removal efficiencies achieved by MF systems in these studies.

The additional ability of MF to achieve high removal efficiencies of suspended solids and turbidity in this study suggests that its use may positively impact rearing conditions in RAS. The MF system removed fine solids that bypassed micro-screen filtration, which would have accumulated and degraded in the rearing system. Chen et al. (1993) reported that 48 % of solids mass in RAS contained particles less than 10 μm , which would not be removed by micro-screens with pore sizes greater than 50 μm typically used in RAS (Patterson et al., 1999). If these fine solids are not removed by other treatments in RAS they would degrade in the rearing system,

resulting in the secondary production of ammonia and other wastes that can negatively impact fish health (Patterson et al., 1999; Piedrahita et al., 2003; Sharrer and Summerfelt, 2007). Therefore, using MF to remove fine solids from RAS may improve water quality and rearing conditions for cultured fish.

In this study, the ability of MF to remove fine solids from the rearing system suggests that MF can also improve fish health by removing potential pathogens associated with those solids (Bullock et al., 1997; Sharrer and Summerfelt, 2007). The addition of organic carbon rich substrates to aquaculture rearing systems has been reported to increase populations of heterotrophic bacteria (Hari et al., 2004) as their growth is directly related to the flux of organic carbon (Moriarty, 1997). MF has been reported to remove high levels of organic carbon from wastewater (Castaing et al., 2010; Nakatsuka et al., 1996), which implies that the use of MF in RAS may indirectly reduce heterotrophic populations by removing their food source. However, the MF system in this study did not remove significant levels of organic carbon, but influent levels of organic carbon may have been too low to be impacted. The MF system in Castaing et al. (2010) treated influent wastewater with an organic carbon level of 22.2 ± 1.8 , compared to 2.0 ± 0.3 mg/L treated in this study (Appendix 4). Further research should determine if MF can remove significant levels of organic carbon from wastewater and indirectly reduce populations of heterotrophic bacteria in RAS.

The ability of MF to remove suspended solids and increase UV transmittance in this study also demonstrated its potential as a UV pre-treatment in RAS. UV disinfection requires pre-treatments in many industries, including aquaculture, to maintain high UV disinfection

efficiencies by removing suspended solids (Gomez et al., 2007; Liltved and Cripps, 1999; Wedemeyer, 1996). In comparison, MF is used in the drinking water industry as a reverse osmosis pre-treatment to reduce membrane fouling (Glucina et al., 2000). The pre-treatment requirement for UV disinfection and the successful use of MF as a pre-treatment suggests that combining both strategies may be beneficial in RAS. Using MF to enhance UV disinfection may reduce the amount of pathogens that accumulate in the rearing system, reducing the potential for infection and disease. Therefore, MF may increase the efficiency of UV disinfection when used as a pre-treatment in RAS.

2.4.3 Membrane Fouling

In this study, the MF system was estimated to operate for 4.5 months until recovery cleaning would be required, which may imply that MF systems can maintain long-term operation in RAS. This interval is consistent with the manufacturer's recommendation of performing recovery cleanings, using sodium hypochlorite, between 1-6 months of MF operation (Toray Membrane Inc., CA, USA). In contrast, Le-Clech et al. (2006) recommended that recovery cleanings, using sodium hypochlorite, should be performed between 6-12 months of MF operation. The interval estimated in this study should be taken with caution because it was an extrapolation based on the linear rate of TMP (fouling resistance), which may have increased, plateaued or decreased after 30 days of filtration. However, the fouling rate did not increase exponentially over the 30 day trial, which indicates the MF system experienced sustainable rates of fouling. Therefore, the low rate of membrane fouling indicates that MF may maintain long-term operation in RAS, but more

research is needed to determine if MF systems can maintain operation in RAS over several months or years.

In general, it is difficult to compare membrane fouling rates between studies because the rate is largely dependent on water quality and the duration and frequency of cleaning strategies (Le-Clech et al., 2006). The average fouling rate of the MF system in this study was less than the rate experienced by the MF system (0.008 μm pore size) in the study by Di Profio et al. (2011), who achieved 1.75×10^{11} /m/day (16.4 kPa/day) based on a 63 hour lab-scale filtration of surface seawater using permeate backwashing and air-scouring strategies. In contrast, a MF system (0.005 μm pore size) in a study by Guo et al. (2009) did not experience significant increases in fouling resistance over three months when treating surface water using permeate backwashing, flushing and maintenance cleaning strategies. However, the maintenance cleaning interval was shorter in Guo et al. (2009) compared to this study, which suggests that fouling rates may have been reduced if more frequent maintenance cleanings were performed. In addition, increasing the duration, frequency and combination of other cleaning strategies can also reduce membrane fouling (Le-Clech et al., 2006). For example, permeate back-washing can be applied to the MF system periodically to dislodge particulates deposited in the membrane pores (Le-Clech et al., 2006). Therefore, more efficient cleaning strategies should be applied to MF systems, which may reduce membrane fouling rates and increase the interval between recovery cleanings, potentially improving the long-term operation of MF in RAS.

This research demonstrated that frequent maintenance cleanings, using sodium hypochlorite, are essential in maintaining MF operation because they prevented the filter from further bacterial

contamination. Originally, maintenance cleanings were not scheduled to occur throughout the trial because the MF system experienced a low rate of fouling at the start of the trial, which indicated that additional cleaning was not necessary. However, the MF system was contaminated with bacteria after four days of filtration, thus maintenance cleanings were performed periodically and prevented further contamination for the remainder of the 30 day trial (Figure 2.4). Low levels of bacteria were able to continuously bypass MF throughout the 30 day trial (Table 2.2 and 2.3), which can imply that bacteria need four days to travel through or proliferate within the membrane filter to high enough levels to cause contamination. Maintenance cleaning events with sodium hypochlorite inactivated the bacteria inside the membrane filter, which also hydrolyzed and removed organic foulants from the surface of the membrane filter (Le-Clech et al., 2006). Therefore, this study demonstrated that frequent maintenance cleanings, using sodium hypochlorite, are essential in preventing bacterial contamination of membrane filters and maintaining MF operation.

Conversely, chlorine used in the maintenance cleanings may recirculate and harm cultured fish in the rearing system if chlorine is not properly rinsed from the MF system. This risk is unique to aquaculture because permeate is directly supplied to animals that are sensitive to chlorine, whereas chlorine sensitivity is not an issue in other MF applications, e.g. grey water reuse for boiler heaters (Peters, 2010). However, MF operations are able to avoid this risk by automating cleaning procedures that flush the system until chlorine is removed from the permeate (Le-Clech et al., 2006). In this study, the MF system was thoroughly rinsed for approximately 90 minutes until no chlorine was detected. No mortalities were reported during the weeks throughout and following the 30 day trial, which implies that the flushing procedure was effective at removing

chlorine from the MF system and preventing its transfer to the rearing system. Therefore, this study demonstrated that the risk of chlorine exposure to fish can be avoided by implementing proper flushing procedures during maintenance cleanings. However, more research is needed to reduce the duration and frequency of maintenance cleanings in order to reduce treatment interruptions and the risk of exposing chlorine to fish.

2.5 Conclusions

The MF system used in this study achieved equivalent bacterial removal efficiency compared to UV, demonstrating the potential applicability of using MF as an alternative to UV irradiation in RAS. The high influent level of UV transmittance may have allowed the UV treatment to achieve maximum bacterial removal efficiency in this study, thus MF has potential to achieve higher efficiency when challenged with concentrated wastewater, specifically lower in UV transmittance. The MF system also exhibited an additional benefit of achieving high removal efficiency of suspended solids and turbidity, which may improve rearing conditions in RAS. Lastly, the rate of membrane fouling did not limit the bacterial removal efficiency or the long-term operation of the MF system in RAS. Therefore, the results from this study demonstrated that MF could potentially be used as an effective bacterial removal treatment in RAS. However, more research is needed to determine the economical and operational efficacy of using MF on a larger scale in RAS before it can be confidently used as a pathogen control treatment in commercial aquaculture rearing systems.

This research showed that MF can achieve high removal efficiency of total and heterotrophic bacteria under a single set of conditions, but the ability of MF to remove specific fish pathogenic bacteria that can tolerate high doses of UV irradiation has yet to be evaluated. A laboratory trial is thus necessary to determine whether MF can achieve high removal efficiency of *Flavobacterium psychrophilum*.

Chapter 3: Control of *Flavobacterium psychrophilum* using Membrane Filtration

3.1 Introduction

F. psychrophilum, the causative agent of bacterial coldwater disease (BCWD), has been reported to cause mortalities up to 90 % in fish rearing systems (Nilsen et al., 2011), which has caused substantial economical losses in the aquaculture industry (Michel et al., 1999). Wastewater treatment technologies, such as UV, have been relied upon to remove *F. psychrophilum* from RAS since current antibiotics and vaccines are ineffective at controlling the pathogen (Barnes and Brown, 2011). However, *F. psychrophilum* has been reported to tolerate recommended UV doses used in aquaculture systems and bypass micro-screen pores as small as 2 µm (Hedrick et al., 2000). For that reason, alternative pathogen removal technologies must be implemented in RAS to reduce the prevalence of *F. psychrophilum* and the impact of BCWD on the aquaculture industry. The diameter of *F. psychrophilum* ranges between 0.3 to 0.75 µm (Holt et al., 1993; Pacha, 1968), which suggests that MF may be able to remove *F. psychrophilum* because ultrafiltration pore sizes range between 0.005 to 0.02 µm (Madaeni, 1999; Peters, 2010). MF has been well documented as an effective pathogen removal technology in many industries (Gomez et al., 2007), but its ability to remove *F. psychrophilum* has not yet been evaluated.

In this chapter, the ability of MF to remove *F. psychrophilum* from treated water was evaluated. It was hypothesized that the membrane pore size of the MF system is small enough to remove *F. psychrophilum*. If this hypothesis is true, then the MF system should achieve over 5 log

reductions of *F. psychrophilum*. The following objectives were set out to test this hypothesis and prediction:

- 1) Isolate and sequence *F. psychrophilum* from an infected fish and culture the pathogen to high concentrations.
- 2) Challenge the MF system with high concentrations of *F. psychrophilum* and determine the bacterial removal efficiency of MF.

3.2 Methodology

3.2.1 Isolation and Sequencing of *Flavobacterium psychrophilum*

F. psychrophilum strain ONT 6179 was isolated from the epithelium of an Atlantic salmon submitted to the Fish Health Laboratory at the University of Guelph. Aseptically, *F. psychrophilum* was streaked and cultured on modified cytophaga agar [tryptone (Becton, Dickinson and Company, MD, USA), 0.5g/L; yeast extract (Becton, Dickinson and Company, MD, USA), 0.5g/L; sodium acetate (Fisher Scientific, NJ, USA), 0.2g/L; beef extract (Becton, Dickinson and Company, MD, USA), 0.2g/L; supplemented with KCl (Fisher Scientific, NJ, USA), 0.05g/L; MgCl (Fisher Scientific, NJ, USA), 0.05g/L; CaCl₂ (Fisher Scientific, NJ, USA), 0.05g/L; 15g/L agar (Bio Basic Inc, ON, Canada); adjusted pH to 7.5], which was based on the study by Anacker and Ordal (1959), as used in the Fish Health Laboratory (M. Raymond, personal communication, March 12, 2012). Agar plates of bacteria were incubated at 15°C for approximately six days. Bacterial DNA was isolated and then PCR amplified using a thermocycler (Biometra, LS, Germany), PCR reagent kit (Life Technologies Co., ON, Canada) and a pair of universal primers: 20F (5'-AGAGTTTGATCATGGCTCAG-3') and 1500R (5'-CGGTTACCTTGTTACGACTT-3') designed and amplified according to Weisburg et al. (1991). Amplified DNA was purified on a 1 % agarose gel, then extracted and submitted to the Genomics Facility at the Advanced Analysis Centre of the University of Guelph, where they were sequenced using a DNA Analyzer (model 3730, Applied Biosystems, CA, USA). The search similarity software program BLAST[®] (Altschul et al., 1990), associated with the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/BLAST>),

was used to confirm that the DNA sequence matched the sequence of *F. psychrophilum* found in the GenBank database.

3.2.2 Culture of Flavobacterium psychrophilum

Bacteria, derived from samples confirmed to be *F. psychrophilum*, were inoculated into 50 mL of modified cytophaga broth and incubated for 48 hours in an orbital shaker adjusted to 15°C and 200 rpm. Aseptically, a 5-10 mL aliquot of cultured broth was inoculated into 1 L of cytophaga broth and then incubated, as previously described. After incubation, the cultured broth was adjusted to concentrations between 10^7 - 10^8 cfu/mL based on a standard curve, which was prepared using absorbances of serially diluted cultured broth measured at a wavelength of 600 nm by a spectrophotometer (SmartSpec Plus, Bio-Rad Laboratories, Inc., CA, USA). An aliquot from the adjusted cultured broth was serially diluted using 0.9 % saline and then pipetted on modified cytophaga agar, according to the droplet technique of Miles & Misra (1938). Agar plates of bacteria were incubated for approximately six days at 15°C and then enumerated. Droplets containing between 20-100 colonies were counted and multiplied by the dilution factor to obtain cfu/mL of *F. psychrophilum*.

3.2.3 Membrane Filtration System

The exact same MF system was used as described in Section 2.2.3, except permeate was recirculated back into the membrane tank at a rate of 15 L/min with no retentate overflow (dead-end configuration) to maintain a constant level of water and concentration of bacteria in the

membrane tank. The MF system (Figure 3.1) was composed of a hollow-fibre membrane filter (Appendix 1; LSU-1515, Toray Membrane Inc., CA, USA) submerged in a 40 L membrane tank, which was connected to a permeate hose leading to a permeate pump (Cole-Parmer, QB, Canada). The permeate pump was turned off every 30 minutes for one minute by a repeat cycle timer (Custom Automated Products Inc., CA, USA) and an aerator (GAST Manufacturing Inc., MI, USA) continuously produced air under the filter at a rate of 25 L/min. The maintenance cleaning procedure was also modified, being performed before and after each challenge test to disinfect the MF system. In addition, the MF system was rinsed for approximately four hours and then soaked in well water to allow chlorine to dissipate out of the filter. The MF system was rinsed again for ten minutes and filled with 39 L of well water, which was left for approximately five hours to allow the water to equilibrate to room temperature. The level of total and free chlorine in permeate water was measured using water quality test strips (Hach Company, CO, USA) before each test to ensure no residual chlorine remained in the MF system. Lastly, pressure decay tests were performed on the membrane filter before and after each challenge test to determine if any membranes were broken (Toray Membrane Inc., CA, USA).

3.2.4 Sampling Regimen

Grab samples were taken from two sample locations within the MF system: the retentate, i.e. water retained by the membranes, and the permeate, i.e. water diffused through the membranes (Figure 3.1). 250 mL water samples were collected in sterile bottles before, during and after the three hour filtration, and then placed on ice to slow bacterial growth. Before each challenge test, 1 L of adjusted culture broth of *F. psychrophilum* was added to the 39 L of well water in the

membrane tank, and then aerated for one minute to ensure adequate mixing. Retentate samples were collected before and after each filtration, as well as 5 minutes after the start of filtration. Permeate samples were collected at 0, 5, 15, 30, 60, 120 and 180 minutes after the start of filtration.

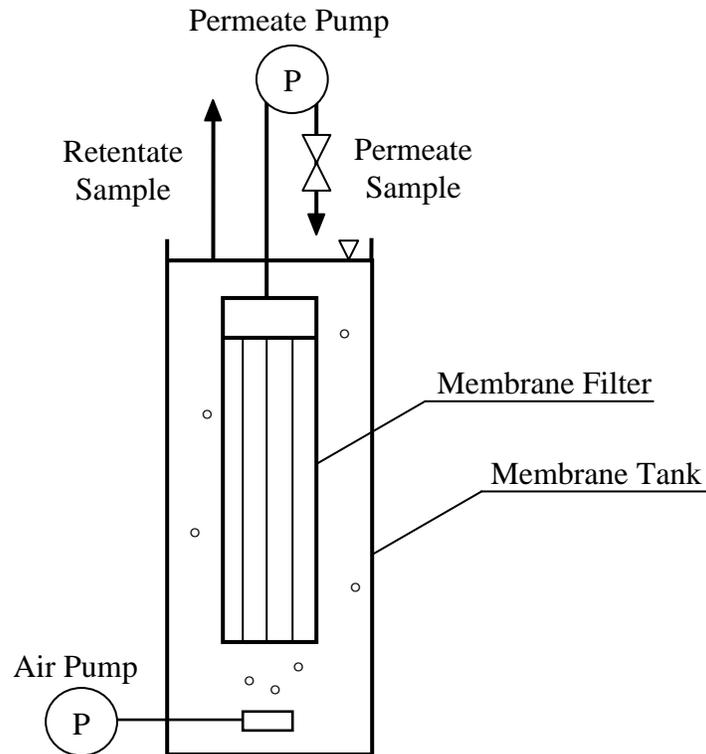


Figure 3.1. Schematic of the MF system illustrating water that diffused through the membrane filter and recirculated back into the membrane tank via permeate pump and valve, where permeate samples were collected. Retentate samples were collected from the retained water at the top of the membrane tank as it is continually mixed via aeration.

3.2.5 Water Quality Analyses

3.2.5.1 Bacterial Analyses

Bacterial analyses were performed immediately after each challenge test to avoid bacterial growth or inactivation. Aseptically, retentate samples were serially diluted using 0.9 % saline and then pipetted, in triplicate, on modified cytophaga agar according to the droplet technique of Miles & Misra (1938). Agar plates with droplets were incubated at 15°C for six days. Aliquots of both retentate and permeate samples were individually vacuum filtered, in triplicate, on gridded membrane disc filters (0.47 µm pore size; Pall Corporation, NY, USA) and then the filters were placed on modified cytophaga agar. Agar plates with filters were incubated at 15°C for six and twelve days. Droplets and filters containing between 20-100 and 20-200 colonies were counted and multiplied by the dilution factor to obtain cfu/mL.

3.2.5.2 Chemical/Physical Analyses

Before each challenge test, retentate was measured for dissolved oxygen and temperature using a handheld probe (YSI Inc., OH, USA). After each challenge test, retentate samples were measured for total hardness using a digital titrator (Hach Company, CO, USA), UV transmittance (254 nm) using a UV spectrophotometer (Hewlett-Packard, CA, USA) and pH using a handheld probe (YSI Inc., OH, USA). In addition, all retentate and permeate samples were measured for turbidity using a turbidimeter (Micro 100, HF Scientific, FL, USA).

3.2.6 Scanning Electron Microscopy

Scanning electronic microscopy (SEM) was performed on a hollow-fibre membrane and *F. psychrophilum* at the Food Science Department at the University of Guelph. SEM images were taken to illustrate the size and structure of the membrane and *F. psychrophilum*. Before SEM imaging, colonies of *F. psychrophilum* were removed from an agar plate, adhered to carbon planchets and then fixed by soaking them in 2 % glutaraldehyde (Canemco, QC, Canada) with 0.07 M Sorensen's phosphate buffer (pH 6.8; Fisher Scientific, NJ, USA) for one hour. The fixative was removed by rinsing the samples with buffer. The samples were post-fixed in 1 % osmic acid anhydride (Fisher Scientific, NJ, USA), prepared in the phosphate buffer, for 30 minutes. The samples were dehydrated in a series of ten minute ethanol rinses (50, 70, 80, 90 and 100 % three times), and then dried in a critical point dryer. Both samples of the hollow-fibre membrane and *F. psychrophilum* were sputter coated with 2 nm of gold-palladium and images were taken at 10 kV using a scanning electron microscope (model S-570, Hitachi High Technologies Co., Tokyo, Japan).

3.2.7 Data Analysis

Bacterial and turbidity removal efficiencies of MF were calculated as the percentage difference between the influent and effluent values, defined by the equation:

$$\% \text{ Removal} = \frac{\text{value}_{\text{influent}} - \text{value}_{\text{effluent}}}{\text{value}_{\text{influent}}} \times 100$$

Log₁₀ reductions of bacteria by MF treatment were based on the percent removal values calculated above, defined by the equation:

$$\text{Log}_{10} \text{ reduction} = -\log_{10} \left(1 - \frac{\% \text{ removal}}{100} \right)$$

Bacterial counts were estimated based on the mass-balance approach for the pulse input of bacteria (tracer) into a batch MF system that experienced an ideal flow pattern, defined by the equation (Metcalf and Eddy, 2003):

$$C = C_0 e^{-t/\tau}$$

where;

C = concentration of bacteria at time t (cfu/mL);

C₀ = initial concentration of bacteria (cfu/mL);

t = filtration time (min);

τ = residence time, (min).

Mean values for permeate, retentate and removal efficiencies achieved by the MF system were reported with ± standard error and calculated using Microsoft[®] Office Excel[®] 2007 software.

3.3 Results

3.3.1 *Flavobacterium psychrophilum* Removal

The MF system achieved, on average, 5.8 ± 0.2 log reductions of bacteria when challenged with an average retentate dose of $1.7 \pm 1.0 \times 10^6$ cfu/mL of *F. psychrophilum* (Table 3.1). The removal efficiencies and retentate bacterial counts were calculated based on the retentate water samples collected at the start of filtration (i.e. 0 minutes) for each challenge test because the retentate bacterial count decreased rapidly after the start of filtration.

Permeate samples contained, on average, 2.6 ± 0.2 cfu/mL of bacteria, with a range of 0 to 6 cfu/mL. However, bacterial colonies that developed on the lab filters from permeate samples did not visually resemble colonies of *F. psychrophilum* from control samples (Figure 3.2). Colonies from control samples were raised and yellow-pigmented, whereas, colonies from permeate samples were not raised or yellow-pigmented. In addition, colonies from permeate and well water samples also required twice the incubation period compared to control samples of *F. psychrophilum* (i.e. twelve days compared to six days).

Table 3.1. Mean (\pm S.E.) bacterial counts and removal efficiencies achieved by the MF system when challenged with *F. psychrophilum* throughout three challenge tests (n = 3).

Test	Retentate (cfu/mL)	Permeate (cfu/mL)	Removal (%)	Log ₁₀ Reduction
1	1.1E+06 \pm 3.0E+04*	3.0 \pm 0.9	99.9997 \pm 0.00008	5.69 \pm 0.14
2	3.6E+06 \pm 3.6E+05*	2.9 \pm 0.4	99.9999 \pm 0.00001	6.12 \pm 0.06
3	4.7E+05 \pm 2.0E+05*	1.8 \pm 0.4	99.9996 \pm 0.00008	5.46 \pm 0.09
Mean	1.7E+06 \pm 9.6E+05	2.5 \pm 0.4	99.9998 \pm 0.00009	5.76 \pm 0.19

* Measured from samples collected at 0 minutes of filtration.

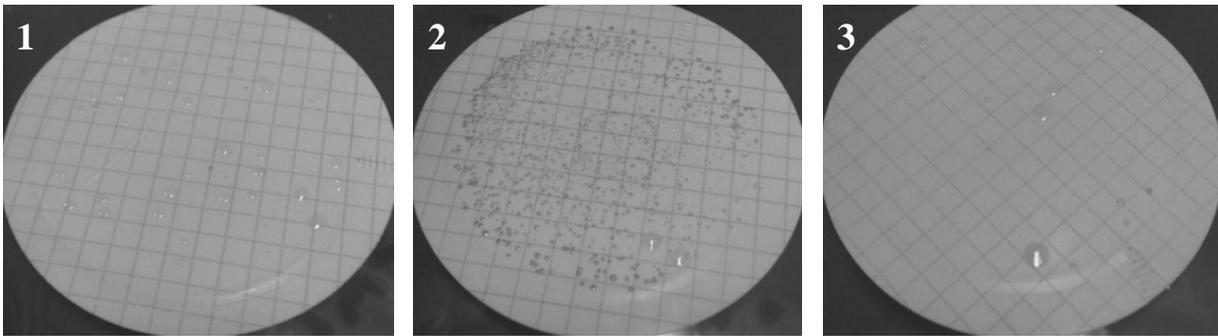


Figure 3.2. Photos of disc filters (2.5 cm diameter) containing bacterial colonies cultured from water samples collected during the third challenge test: 1) retentate collected before dosage (bacteria derived from well water), 2) retentate collected after dosage (*Flavobacterium psychrophilum*), and 3) permeate collected after fifteen minutes of filtration (unknown bacteria).

3.3.2 Reduction in Retentate Bacteria

The measured count of retentate bacteria after five minutes of filtration was lower than the predicted count (Table 3.2, Figure 3.3). The average retentate bacterial count was predicted to decrease by 84.67 %, but the measured bacteria count decreased by $99.99 \pm 0.003 \%$.

Table 3.2. Mean (\pm S.E.) retentate bacterial counts and reductions, measured and predicted, when the MF system was challenged with *F. psychrophilum* throughout three challenge tests (n = 3).

Filtration Time (min)	Retentate (cfu/mL)	Reduction (%)	Log ₁₀ Reduction
0	$1.7E+06 \pm 9.6E+05$		
5	$1.7E+02 \pm 9.9E+01$	99.99 ± 0.003	4.00 ± 0.14
5*	$2.6E+05 \pm 1.5E+05$	$84.66 \pm n/a^{**}$	$0.81 \pm n/a^{**}$

* Predicted value based on the retentate bacterial count collected at 0 minutes of filtration.

** No standard error reported since the bacterial count was calculated.

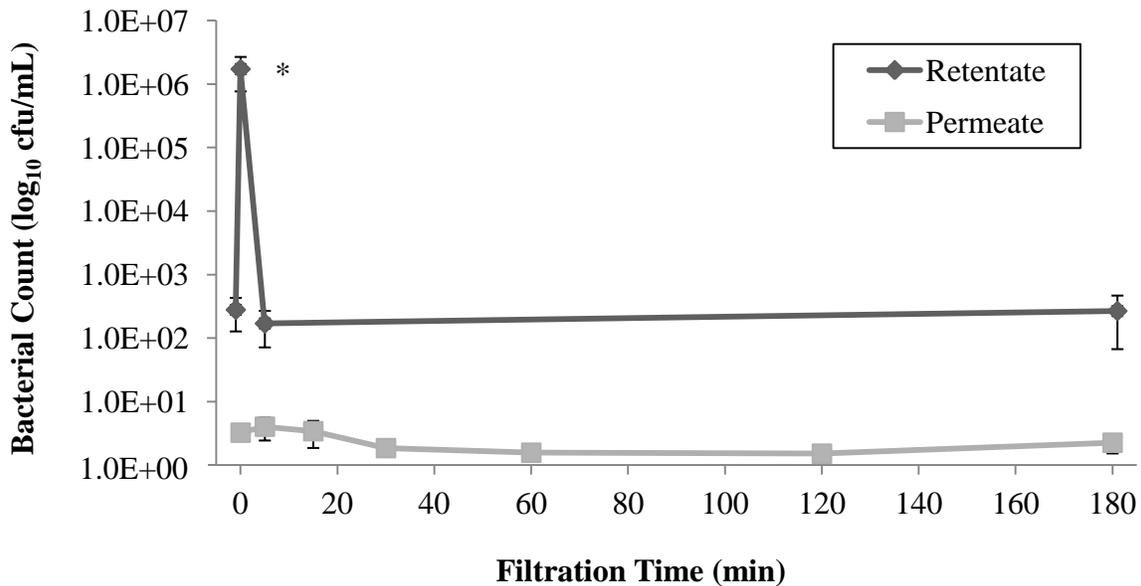


Figure 3.3. Mean (\pm S.E.) bacterial counts from retentate and permeate water samples collected from the MF system when challenged with *F. psychrophilum* over three challenge tests (n = 3).

* Count determined via serial dilution method because it was too high for the filtration method.

3.3.3 Turbidity Removal

The MF system achieved, on average, 98.7 ± 0.3 % removal of turbidity over the three challenge tests (Table 3.3). On average, the MF system reduced the level of turbidity from 10.7 ± 1.8 to 0.1 ± 0.02 NTU. The turbidity removal efficiencies achieved by the MF system were calculated based on the retentate samples collected at the start of filtration (i.e. 0 minutes) for each challenge test because the retentate turbidity level decreased rapidly after the start of filtration. The retentate turbidity level was derived from both the 1 L culture of *F. psychrophilum* and the 39 L of well water because the average turbidity level of the well water was 5.6 ± 2.3 NTU.

Table 3.3. Mean (\pm S.E.) turbidity levels and removal efficiencies achieved by the MF system when challenged with *F. psychrophilum* throughout three challenge tests (n = 3).

Test	Retentate (NTU)	Permeate (NTU)	Removal (%)
1	14.10 \pm 0.35*	0.11 \pm 0.02	99.21 \pm 0.17
2	9.64 \pm 0.28*	0.17 \pm 0.03	98.26 \pm 0.31
3	8.24 \pm 0.08*	0.11 \pm 0.01	98.67 \pm 0.11
Mean	10.66 \pm 1.77	0.13 \pm 0.02	98.71 \pm 0.28

* Measured from samples collected at 0 minutes of filtration.

3.3.4 Reduction in Retentate Turbidity

The measured level of retentate turbidity was higher than the predicted level after five minutes of filtration (Table 3.4, Figure 3.4). The average retentate turbidity level was predicted to decrease by 84.7 %, but the measured turbidity level decreased by 63.2 ± 10.8 %.

Table 3.4. Mean (\pm S.E.) retentate turbidity levels and reductions, measured and predicted, when the MF system was challenged with *F. psychrophilum* throughout three challenge tests (n = 3).

Filtration Time (min)	Retentate (NTU)	Reduction (%)
0	10.7 \pm 1.8	
5	3.6 \pm 0.8	63.2 \pm 10.8
5*	1.6 \pm 0.3	84.7 \pm n/a**

* Predicted value based on retentate bacterial count at 0 minutes of filtration.

** No standard error reported since the turbidity level was calculated.

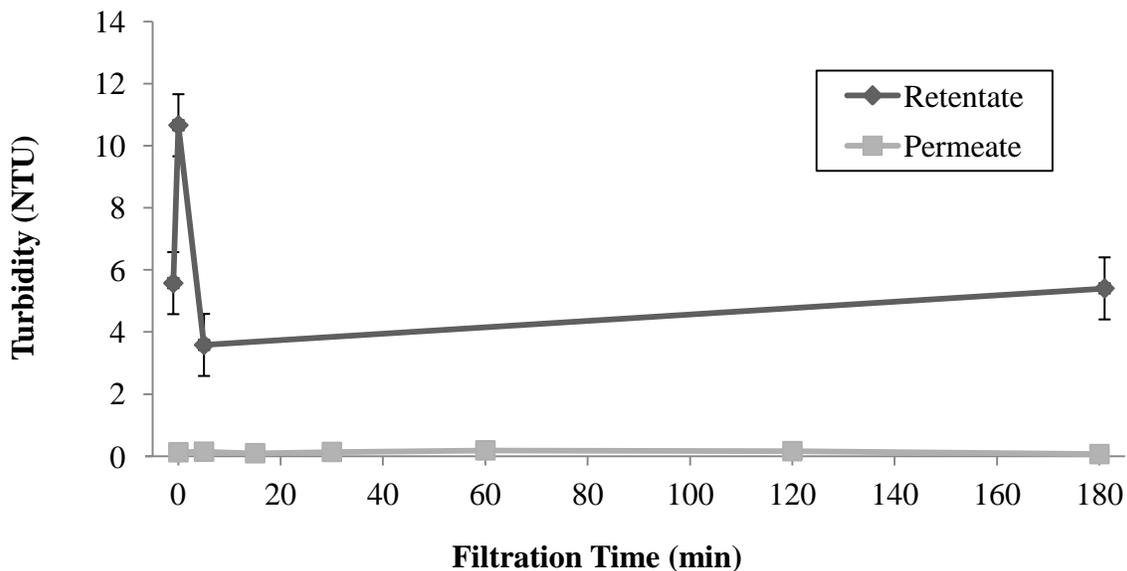


Figure 3.4. Mean (\pm S.E.) turbidity levels from retentate and permeate water samples collected from the MF system when challenged with *F. psychrophilum* over three challenge tests (n = 3).

3.3.5 Scanning Electron Microscope Images

The scanning electron microscope images of the hollow-fibre membrane (Figure 3.5) illustrated the complex barrier that bacteria would have to travel through in order to bypass MF treatment. In addition, images of *F. psychrophilum* (Figure 3.6) illustrated their surface and size variation.

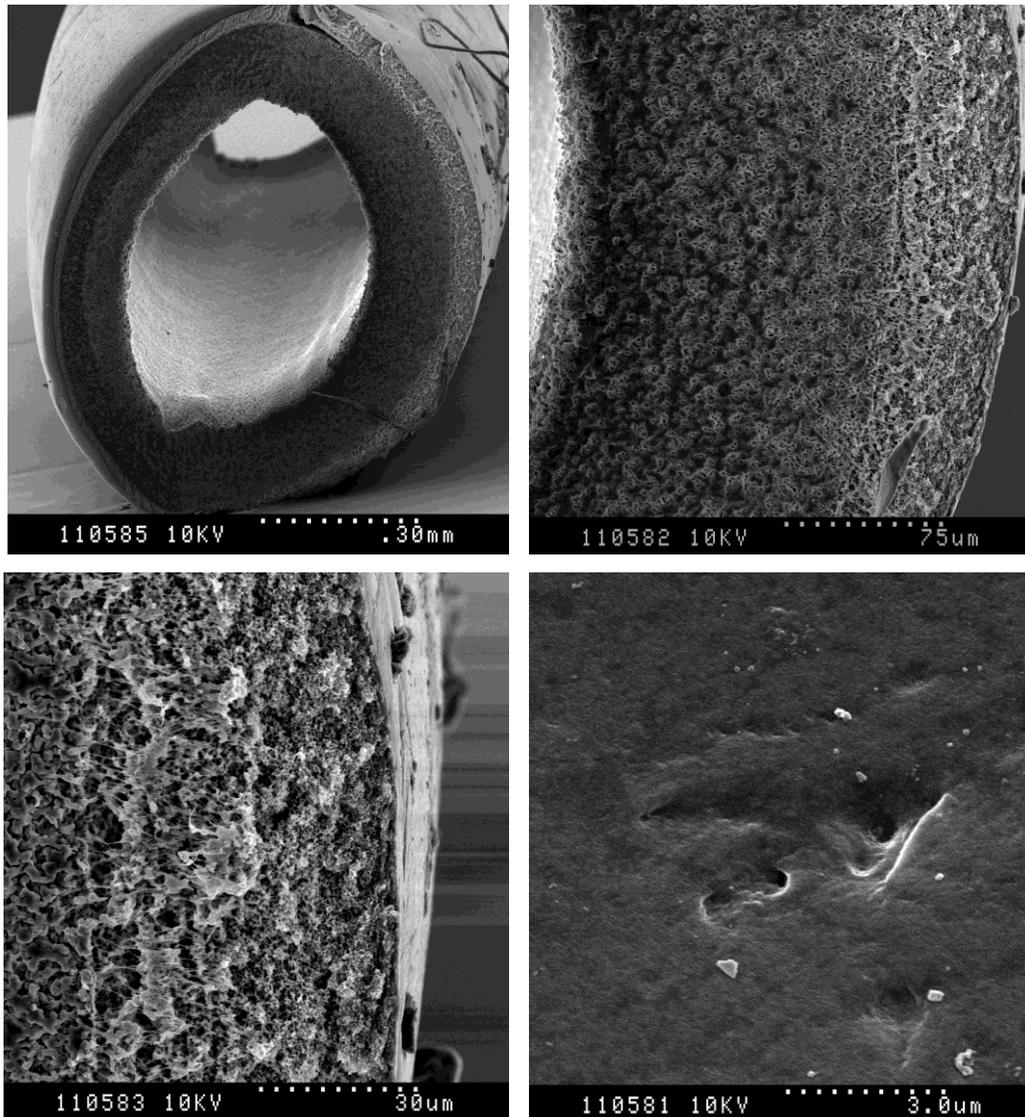


Figure 3.5. Scanning electron microscope images of a cross-section and surface of a hollow-fibre membrane, increasing in magnification from top-left to bottom-right.

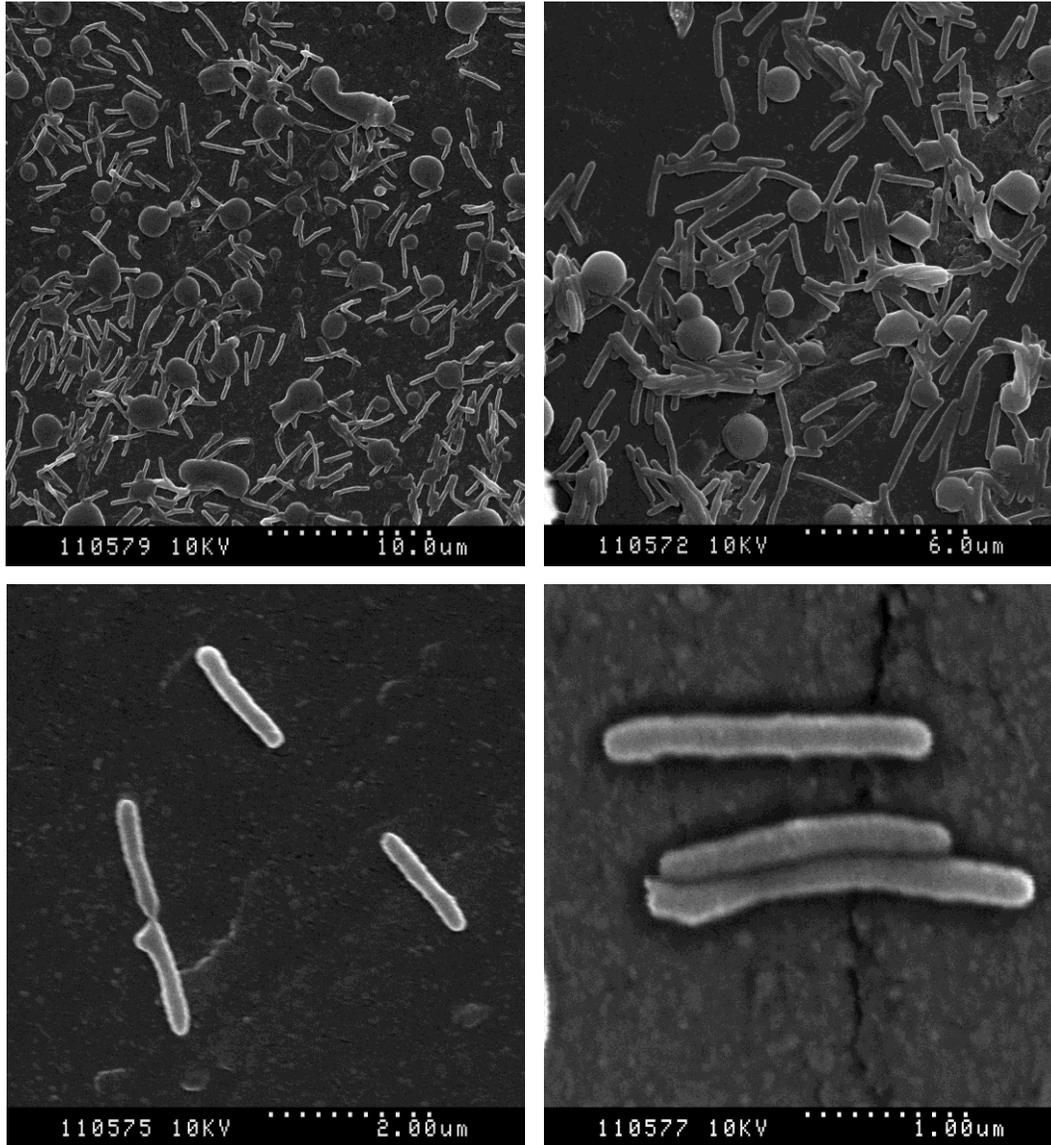


Figure 3.6. Scanning electron microscope images of *Flavobacterium psychrophilum*, increasing in magnification from top-left to bottom-right. Note that the circles in the first two photos are presumed to be drops of nutrient agar.

3.4 Discussion

3.4.1 *Flavobacterium psychrophilum* Removal and BCWD Prevention

Results from this study supported the hypothesis because the membrane pore size of the MF system was small enough to remove *F. psychrophilum* from treated water. The results also supported the prediction because the MF system achieved over 5 log reductions of *F. psychrophilum*. The ability of the MF system to remove high levels of *F. psychrophilum* in this study demonstrated the potential use of MF to reduce the prevalence of this particular pathogen in RAS and reduce the potential impact of BCWD. Removal of *F. psychrophilum* may reduce the potential for clinical disease because reduced exposure of contagions to the host can reduce the probability of infection (Conte, 1992). Cipriano and Holt (2005) suggested that supplying aquaculture rearing systems with pathogen-free water either through natural sources or via disinfection treatment may reduce the presence of *F. psychrophilum* and prevent clinical outbreaks of BCWD. Therefore, MF has the potential to be used in RAS to remove high levels of *F. psychrophilum* and potentially improve fish health by preventing incidences of BCWD.

Results from this study also demonstrated that MF can remove *F. psychrophilum* when challenged with high levels of turbidity (Table 3.1 and 3.3), which further demonstrated that MF is more efficient at removing the pathogen compared to UV irradiation. High levels of turbidity (suspended particles) can reduce the efficiency of UV disinfection by shielding bacteria from UV irradiation (Liltved and Cripps, 1999; Wedemeyer, 1996), whereas high levels of turbidity did not impact the bacterial removal efficiency of MF in this study. In addition, *F. psychrophilum*

has been reported to tolerate UV doses up to 126 mJ/cm² (Hedrick et al., 2000), which further reduces the ability of UV to inactivate the pathogen. Based on this information, the UV system in Chapter 2 would not have inactivated *F. psychrophilum* if it were present in the RAS because the applied UV dose was 108.5 ± 1.5 mJ/cm². Therefore, the inability of UV to inactivate *F. psychrophilum* and the ability of MF to tolerate fluctuations in water quality suggest that MF may be more effective than UV at controlling the pathogen in RAS and potentially reducing the prevalence of BCWD.

The MF system in this study also exhibited an additional ability of removing high levels of turbidity from treated water, which may reduce the prevalence of BCWD by improving the quality of water supplied to cultured fish. Several studies have recommended that the maintenance of optimal water quality can reduce the incidence of BCWD (Garcia et al., 2000; Nematollahi et al., 2003a; Taylor, 2004). Poor water quality can stress fish, which suppresses their immune system and increases their susceptibility to infection (Bullock et al., 1997; Conte, 2004). Nematollahi et al. (2003a) demonstrated that the infectivity of *F. psychrophilum* can be reduced by decreasing the availability of organic carbon and nitrites. Removal of organic carbon was not measured for the challenge tests, but other studies have reported that MF can remove significant levels of organic carbon (Castaing et al., 2010; Nakatsuka et al., 1996). This implies that MF may be used to remove organic carbon from wastewater and indirectly reduce the level of *F. psychrophilum* in RAS, but more research is required to determine this possibility.

3.4.2 Bacteria that Bypassed MF Treatment

Low levels of bacteria were able to bypass MF in this study (Table 3.1), which suggests that MF cannot completely prevent incidences of BCWD because the presence of one bacterium has the potential to cause infection (Barnes and Brown, 2011). However, bacteria that bypassed the MF system in this study did not visually resemble *F. psychrophilum*, in comparison to control samples. Several studies have described colonies of *F. psychrophilum* to be raised and yellow-pigmented when cultured on cytophaga agar (Dalsgaard and Madsen, 2000; Nematollahi et al., 2003a; Pacha, 1968), but the bacteria that bypassed MF did not appear raised or yellow-pigmented (Figure 3.2). The bypass bacteria resembled bacteria from water samples collected before the challenge tests, which may have derived from well water used to rinse the MF system and dilute the cultured broth. In addition, colonies cultured from the well water and permeate samples required twice the incubation period, i.e. twelve days, compared to control samples of *F. psychrophilum*, i.e. six days. These findings can be interpreted to suggest that the MF system may have achieved complete removal of *F. psychrophilum*. However, this interpretation cannot be confirmed because the bacteria that bypassed MF were not identified and further analysis was considered beyond the scope of this project. Also, bacterial colonies may appear differently (i.e. colour and shape) when cultured on different nutrient agar. Nevertheless, reductions of *F. psychrophilum* by MF may reduce the probability of infection and the incidence of BCWD, despite low levels that bypass MF.

In an effort to improve the understanding and operation of MF for future applications, explanations concerning the ability of bacteria to bypass MF were proposed based on the

findings of this study. Jacangelo et al. (1989) suggested that large pores may exist within the membranes due to manufacturing imperfection, which may be large enough to allow bacteria to travel through the membranes. The membrane filter used in this study was reported to have an average membrane pore size of 0.01 μm , but the manufacturer also reported pore sizes up to 0.088 μm (Appendix 1, Toray Membrane Inc., CA, USA). This indicates that the membranes contained a distribution of pore sizes, which may allow bacteria to bypass pores larger than 0.01 μm . However, the diameter of *F. psychrophilum* has been reported to range between 0.3 to 0.75 μm (Holt et al., 1993; Pacha; 1968), which suggests that the bacterium should be too large to travel through the largest reported pore size (i.e. 0.088 μm) of the MF system used in this study. Alternatively, membranes may have torn during filtration and allowed bacteria to travel through them, but pressure decay tests, performed before and after each challenge test, did not indicate that any membranes were broken. Therefore, these findings suggest that large membrane pores caused by manufacturing imperfection or tears during filtration did not adequately explain the ability of bacteria to bypass MF.

The ability of bacteria to reduce their size may be another mechanism that allows them to travel through membranes. Leahy and Sullivan (1978) and Madaeni et al. (1999) suggested that microorganisms may travel through membranes when they become smaller during growth transitions. In addition, Humphrey et al. (1983) suggested that some heterotrophic bacteria undergo a starvation-stress response, consequently reducing their size by 45 to 70 % within a few hours. Both of these mechanisms may have allowed bacteria to become small enough to travel through membrane pores. However, a study by Wang et al. (2008) demonstrated that the filterability of bacteria is more dependent on their flexibility and shape, rather than their size. In

this study, the quantity of bacteria that bypassed MF did not increase throughout each of the three hour challenge tests and bacteria were able to bypass MF within five minutes, which implies that bacteria should not have been able to undergo significant morphological changes. Therefore, these findings can be interpreted to suggest that reductions in the size of bacteria did not adequately explain their ability to bypass MF, but increases in the flexibility of bacteria may explain this phenomenon. However, more research is required to determine the mechanisms that allow bacteria to bypass MF in order to improve the removal efficiency of MF systems for future applications.

*3.4.3 Challenge Test Limitations using *Flavobacterium psychrophilum**

Reductions in retentate bacteria during this study (Table 3.2) demonstrated the difficulty of using *F. psychrophilum* in MF challenge tests. The concentration of retentate bacteria was expected to decrease during each challenge test because bacteria adhere to membranes during filtration (Guo et al., 2010), thus fewer bacteria are collected in the water samples. However, the retentate bacterial count decreased by 99.99 % after five minutes of filtration, compared to the estimated reduction of 84.66 %. Similarly, the retentate turbidity level decreased rapidly during the first five minutes of filtration, but the measured level was lower than the estimate, i.e. 63.19 % reduction. Therefore, the near absence of retentate bacteria after five minutes of filtration implies that the bacteria may have been displaced or inactivated during the challenge tests, but further investigation was beyond the scope of this project.

Increasing the recovery of bacteria after MF challenge tests may improve this analysis for future studies, thus explanations concerning the reduction in retentate bacteria were proposed based on the findings from this study. Bacteria may have been displaced during MF due to their biological characteristics, such as hydrophobicity. Studies by Moller et al. (2003) and Vatsos et al. (2001) demonstrated that *F. psychrophilum* was more prone to reside on fish eggs and plastic surfaces rather than being suspended in water. The hydrophobic characteristic of *F. psychrophilum* suggests that they may have adhered to surfaces within the MF system during the challenge tests, resulting in fewer bacteria collected in the retentate water samples. However, retentate was continuously mixed via coarse aeration throughout each three hour challenge test, which implies that low levels of bacteria should have been re-suspended and collected in the water samples. Therefore, the hydrophobic nature of *F. psychrophilum* did not adequately explain the near absence of retentate bacteria during the challenge tests.

Inactivation of *F. psychrophilum* from physiological stress may also explain the reduction in retentate bacteria during the challenge tests. In this study, the culture of *F. psychrophilum* was diluted by 40 times when it was dosed into the MF system, which may have reduced the level of nutrients available to the bacteria, potentially causing physiological stress and inactivation. In addition, *F. psychrophilum* is known to be difficult to isolate and culture because of its specific nutrient requirements (Nematollahi et al., 2003b), which may imply that the pathogen is sensitive to nutrient shifts. However, high levels of *F. psychrophilum* were cultured from water samples collected after the MF system was dosed, which suggests that the pathogen was able to tolerate the nutrient shift. Therefore, inactivation of *F. psychrophilum* caused by physiological stress did not adequately explain the near absence of retentate bacteria during the challenge tests.

Inactivation of *F. psychrophilum* from physical stress may also explain the reduction in retentate bacteria in this study. During the challenge tests, retentate water was exposed to intense shearing forces caused by vigorous stirring from aeration and pressure changes from filtration, which may have damaged and inactivated *F. psychrophilum*. Michel et al. (1999) recommended that intense forces from stirring, vortexing and centrifugations should be avoided in order to obtain viable cell enumerations of *F. psychrophilum*. Therefore, physical stress caused by shearing forces during MF may be the most probable explanation for the additional reduction of *F. psychrophilum*. However, more empirical research is needed to explain the reduction of retentate bacteria during challenge tests in order to improve this analysis of MF systems for future studies.

3.5 Conclusions

The ability of the MF system to achieve over 5 log reductions of *F. psychrophilum* in this study demonstrated its potential use in RAS for the prevention of BCWD. The ability of MF to remove high levels of *F. psychrophilum* when challenged with high levels of turbidity further demonstrated the advantage of using MF in comparison to UV irradiation. The additional ability of MF to remove high levels of turbidity may also reduce the incidence of BCWD by improving water quality in RAS. However, this study was performed under laboratory conditions, thus more research is needed to determine the ability of large-scale MF operations to remove *F. psychrophilum* and prevent incidences of BCWD in RAS. In the future, MF may be an essential tool in controlling *F. psychrophilum* in RAS and potentially reducing the prevalence of BCWD, especially if vaccines, antibiotics and UV treatments continue to be ineffective.

Chapter 4: General Conclusions and Recommendations

Intensifying livestock production can have negative impacts on animal health (Ilea, 2009; Piedrahita, 2003; Thornton, 2010), thus pathogen control technologies are relied upon to reduce production losses due to clinical disease (Thornton, 2010). Production from RAS may be critical in the future because these systems can be intensified while preserving fish health using pathogen control technologies, e.g. UV irradiation (Summerfelt et al., 2003). However, *F. psychrophilum* has been reported to tolerate UV doses recommended in aquaculture (Hedrick et al., 2000) and may accumulate in the rearing system, consequently increasing the potential for infection and occurrence of BCWD. Therefore, alternative pathogen control technologies need to be implemented in RAS to reduce the prevalence of *F. psychrophilum* and impact of BCWD on aquacultural production.

Here, MF and UV treatments were evaluated based on their ability to remove or inactivate bacteria from wastewater in RAS. In Chapter 2, the bacterial removal efficiency of both MF and UV systems was evaluated over 30 days when treating wastewater in an RAS. MF did not achieve higher bacterial removal efficiency than UV, but both efficiencies were indistinguishable. In addition, the MF system achieved high removal efficiencies of suspended solids and turbidity, which increased UV transmissivity of the treated wastewater. The MF system required maintenance cleanings every two or three days to prevent MF contamination, whereas UV systems did not require any cleaning throughout the 30 day trial. Lastly, the low rates of membrane fouling experienced by the MF system indicated that recovery cleaning would be required after several months of MF operation.

The ability of MF to achieve equivalent bacterial removal efficiency in comparison to UV, suggests that MF has the potential to be used as an alternative to UV irradiation. MF was expected to achieve higher bacterial removal efficiency than UV because fluctuations in water quality, specifically those that affect UV transmittance, reduce the applied UV dose and its disinfection efficiency (Gomez et al., 2007; Wedemeyer, 1996). However, both systems treated wastewater that had a high level of UV transmittance in this study, which may have allowed the UV system to achieve optimal bacterial removal efficiency, consequently matching the efficiency of MF. This suggests that MF has the potential to achieve higher bacterial removal efficiency than UV in RAS when challenged with concentrated wastewater, specifically low in UV transmittance.

This research also demonstrated the ability of MF to remove high levels of suspended solids and turbidity, which may improve rearing conditions in RAS. In Chapter 2, the MF system removed fine solids that bypassed micro-screen filtration, which would have accumulated in the rearing system and may have negatively impacted the cultured fish. Fine solids have been shown to degrade water quality and provide production sites for pathogens (Patterson et al., 1999; Piedrahita et al., 2003; Sharrer and Summerfelt, 2007). Thus, removal of fine solids by MF may indirectly reduce accumulations of pathogens and other wastes in rearing systems. In addition, the ability of MF to remove solids and increase UV transmittance suggests that MF may be used as a pre-treatment to reduce UV particle shielding and improve UV disinfection efficiency. Therefore, the ability of MF to remove both bacteria and fine solids in this study demonstrated the potential benefits of using MF in RAS.

The low rates of membrane fouling experienced by the MF system throughout the 30 day trial may imply that MF can maintain long-term operation in RAS. In Chapter 2, the combination of wastewater pre-treatment and cleaning strategies was effective at reducing membrane fouling rates, consequently increasing the recovery cleaning interval. Less frequent recovery cleaning prolongs the operation of MF because fewer interruptions increase wastewater treatment and less chemical exposure increases membrane lifespan (Crozes et al., 1997; Madaeni, 1999). In addition, fouling rates can be further reduced by increasing the frequency, duration and combination of the cleaning strategies applied to MF systems (Le-Clech et al., 2006). Therefore, results from this study indicate that MF systems may maintain long-term operation in RAS, but more research is required to determine the ability of MF to treat wastewater over longer periods of time.

The performance of the MF system in Chapter 2 led to the conclusion that MF can be used as an effective pathogen control technology in RAS, at least when challenged with dilute wastewater on a small-scale. The ability of MF to achieve equivalent bacterial removal efficiency compared to UV in this study demonstrated its potential as an alternative to UV irradiation. The MF system also exhibited an additional benefit of removing suspended solids and turbidity, which may improve rearing conditions for cultured fish in RAS. Lastly, the ability of the MF system to maintain low fouling rates indicates its potential as a long-term treatment in RAS.

In Chapter 3, the MF system was challenged, in three separate tests, with high concentrations of *F. psychrophilum* to determine its removal efficiency of this bacterium. The MF system

consistently achieved over 5 log reductions of *F. psychrophilum* in each challenge test. In addition, the MF system removed high levels of turbidity, which did not reduce the bacterial removal efficiency of MF. However, the reduction in retentate bacteria after five minutes of filtration was higher than expected.

The removal of high levels of *F. psychrophilum* by MF in this study demonstrated its potential to reduce the prevalence of *F. psychrophilum* in RAS and reduce potential incidences of BCWD. In addition, the ability of MF to tolerate high levels of turbidity further demonstrated that MF may be more effective than UV at controlling *F. psychrophilum* in RAS. MF has potential to improve fish production efficiencies in RAS by preventing incidences of clinical BCWD that may reduce growth rates and increase mortality rates. Therefore, the ability of MF to remove high levels of *F. psychrophilum* increases its potential applicability in RAS as a pathogen control technology.

This study has demonstrated the difficulties of performing MF challenge tests using *F. psychrophilum*. In Chapter 3, a reduction in retentate bacteria after five minutes of filtration was expected due to membrane retention, but the near absence of retentate bacteria was not expected. This discrepancy was not investigated because it was considered beyond the scope of this project. However, three explanations for the additional reduction of *F. psychrophilum* were proposed: 1) *F. psychrophilum* may have been absent from the water samples because it was present on the surfaces of the MF system since the pathogen is hydrophobic, 2) *F. psychrophilum* may have been inactivated due to physiological stress experienced during the shift in nutrient availability when the cultured broth was diluted, or 3) *F. psychrophilum* may have been inactivated due to physical stress experienced during intense aeration and filtration from the MF

system. Previous studies have reported difficulties distinguishing whether the decrease in microbes is due to membrane retention or biological inactivation during challenge tests (Guo et al., 2010), which may imply this difficulty is an inherent problem when performing microbial challenge tests. However, the following recommendations may improve this analysis for future studies:

- 1) Collection of retentate bacteria may be improved by operating the MF system in cross-flow configuration or continuously adding bacteria to the system throughout the test.
- 2) Physiological stress experienced by microbes, caused by shifts in nutrient availability, may be reduced by avoiding or minimizing dilutions of the cultured broth.
- 3) Physical stress experienced by microbes, caused by aeration and filtration, may be reduced by lowering both rates or excluding cleaning strategies.
- 4) Microbial inactivation may be reduced by using surrogate microbes that are non-destructive or can tolerate high levels of physical and physiological stress.
- 5) Detection of inactivated and activated microbes may be improved by using microscopy, various culture conditions (e.g. media) or fluorescent staining techniques.

This is the first study to evaluate MF as a bacterial removal treatment in RAS. In Chapter 2, the ability of MF to match the bacterial removal efficiency of UV, remove fine solids that bypass micro-screen filtration and maintain operation in RAS over 30 days was demonstrated. In Chapter 3, the MF system removed high levels of *F. psychrophilum* while tolerating high levels of turbidity. These results suggest that MF can be an effective treatment in RAS, but more research is required before MF can be confidently applied to commercial aquaculture systems. This research did not demonstrate the performance of MF on a large-scale since a one-unit MF

system was used to process either a side-stream of wastewater from a small-scale RAS or a small microbial bath under laboratory conditions. Large-scale MF operations have been evaluated in other applications, such as drinking water production (Peters, 2010), but the ability of MF to treat wastewater in RAS on a large-scale has yet to be evaluated. Therefore, future research should evaluate the performance of large-scale MF operations in RAS under variable wastewater conditions and specifically investigate:

- 1) The capital and maintenance costs of using MF to treat wastewater in RAS and its benefits to fish production and economical gains (i.e. cost-benefit analysis).
- 2) The ability of MF to remove common fish pathogens, reduce fish stress and prevent incidences of clinical disease in RAS.
- 3) The ability of MF to remove fine solids and its impact on the efficiency of other wastewater treatments, microbial populations and fish health in RAS.
- 4) The ability of MF to maintain long-term operation when treating various qualities of wastewater in RAS for several months to years.

In conclusion, this thesis project demonstrated that MF has potential to be used as a long-term bacterial removal treatment in RAS. In the future, MF may become an essential treatment in reducing the prevalence of *F. psychrophilum* in RAS and preventing incidences of BCWD, especially if vaccines, antibiotics and UV irradiation continue to be ineffective. Improving pathogen control using MF may increase production efficiencies of RAS, further demonstrating the potential of RAS as a sustainable form of livestock production. However, more research is needed to determine the performance of large-scale MF operations in RAS before it can be confidently used as a pathogen removal treatment in commercial aquaculture systems.

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Appendices

Appendix 1: Manufacturer's specifications, operating conditions and photos of the MF and UV systems.

Table A1.1. Manufacturer's specifications for the UV system (Trojan UV Logic™ midflow model 02AM20, Trojan Technologies Inc., ON, Canada).

Parameter	Units	Values
UV Dose (min. at 95% UVT)	mJ/cm ²	60
UV Intensity (min. at 95% UVT)	mW/cm ²	7.5
Design Flow Rate	L/min	227
Chamber Volume	L	20
Chamber Dimensions (length x diameter)	m	0.71 x 0.20
Chamber Configuration		Closed-channel
Lamp Configuration		Horizontal
Lamp Type		Low-pressure
Number of Lamps (254 nm)		2



Figure A1.1. Photo of the UV system (Trojan UV Logic™ midflow model 02AM20, Trojan Technologies Inc., ON, Canada).

Table A1.2. Manufacturer’s specifications for the membrane filter (submerged type element LSU-1515, Toray Membrane Inc., CA, USA).

Parameter	Units	Values
Filter Dimensions (length x diameter)	m	1.331 x 0.147
Filter Configuration		Submerged, Cross-flow
Filtration Method		Outside-to-inside
Membrane Type		Hollow-fibre
Membrane Material		PVDF
Number of Membranes		6500
Membrane Diameter (outer x inner)	mm	1.1 x 0.6
Membrane Length	m	1.092
Molecular Weight Cut-Off (MWCO)	kDa	150
Nominal Membrane Pore Size	µm	0.01
Maximum Membrane Pore Size	µm	0.088
Total Membrane Surface Area	m ²	25

Table A1.3. Manufacturer’s maximum operating conditions for the membrane filter (submerged type element LSU-1515, Toray Membrane Inc., CA, USA).

Parameter	Units	Values
Total Suspended Solids	mg/L	50
Turbidity	NTU	50
Particle Size (Pre-treatment)	mm	0.5
Influent Water Flow	L/min	33.3
Permeate Flux	L/m ² /hr	80
Air Flow	L/min	75
Transmembrane Pressure	kPa	100
Temperature	°C	40

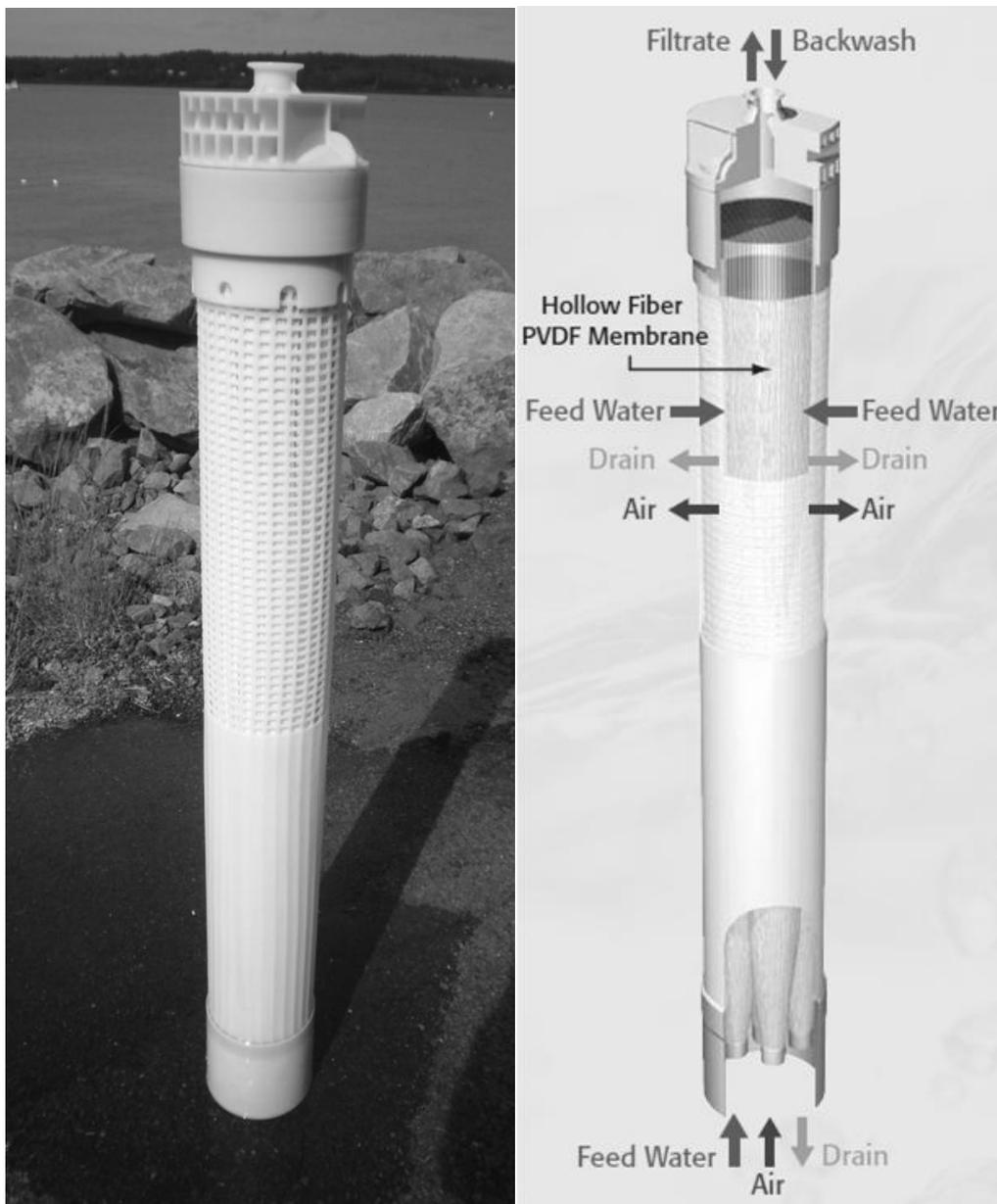


Figure A1.2. Photo and submerged configuration of the membrane filter (submerged type element LSU-1515, Toray Membrane Inc., CA, USA).



Figure A1.3. Photo illustrating the MF and UV systems processing wastewater, in parallel, within the RAS. The MF system (vertical tank on right) received wastewater (black hose) and an effluent pump withdrew water through the membrane filter and discharged the treated water into the collection sump. The UV system (horizontal chamber) irradiated influent wastewater (grey pipe), and then discharged the treated water into the twelve fish tanks.

Appendix 2: Total and heterotrophic bacterial counts measured from water samples collected over 30 days from three locations within the RAS.

Table A2.1. Total bacterial counts (\pm S.D.) measured from water samples collected from three sample locations within the RAS over 30 days (n = 14).

Sample	Day	MF/UV Influent (cfu/mL)	MF Effluent (cfu/mL)	UV Effluent (cfu/mL)
1	0	170.9 \pm 44.1	0.0 \pm 0.01	0.9 \pm 0.3
2	2	377.1 \pm 103.9	1.7 \pm 0.7	0.8 \pm 0.1
3	4	255.8 \pm 52.6	1262.3 \pm 216.2*	1.0 \pm 0.2
4	7	386.3 \pm 25.4	1543.2 \pm 83.7*	0.7 \pm 0.4
5	9	284.1 \pm 52.5	0.01 \pm 0.01	0.5 \pm 0.3
6	11	296.2 \pm 27.3	0.4 \pm 0.5	0.8 \pm 0.3
7	14	281.9 \pm 22.5	6.6 \pm 0.8	0.2 \pm 0.02
8	16	195.2 \pm 35.4	0.5 \pm 0.04	0.1 \pm 0.01
9	18	202.1 \pm 33.7	0.9 \pm 0.1	0.7 \pm 0.1
10	21	195.1 \pm 43.2	2.8 \pm 0.6	0.1 \pm 0.01
11	23	224.1 \pm 26.1	9.7 \pm 1.9	0.2 \pm 0.04
12	25	283.9 \pm 98.0	7.9 \pm 3.2	6.1 \pm 1.1
13	28	325.0 \pm 9.9	5.6 \pm 0.4	1.2 \pm 0.2
14	30	211.3 \pm 32.9	7.6 \pm 0.3	0.3 \pm 0.1

* Bacterial count was not included in the analysis due to filter contamination.

Table A2.2. Heterotrophic bacterial counts from additional water samples collected from three sample locations within the RAS over 30 days (n = 5).

Sample	Day	MF/UV Influent	MF Effluent	UV Effluent
1	2	350	0	0
2	9	790	10	0
3	16	400	5	10
4	23	55	15	10
5	30	120	5	20

Appendix 3: Variation in total and heterotrophic bacterial counts from water samples collected after both MF and UV treatments used in the RAS throughout the 30 day trial.

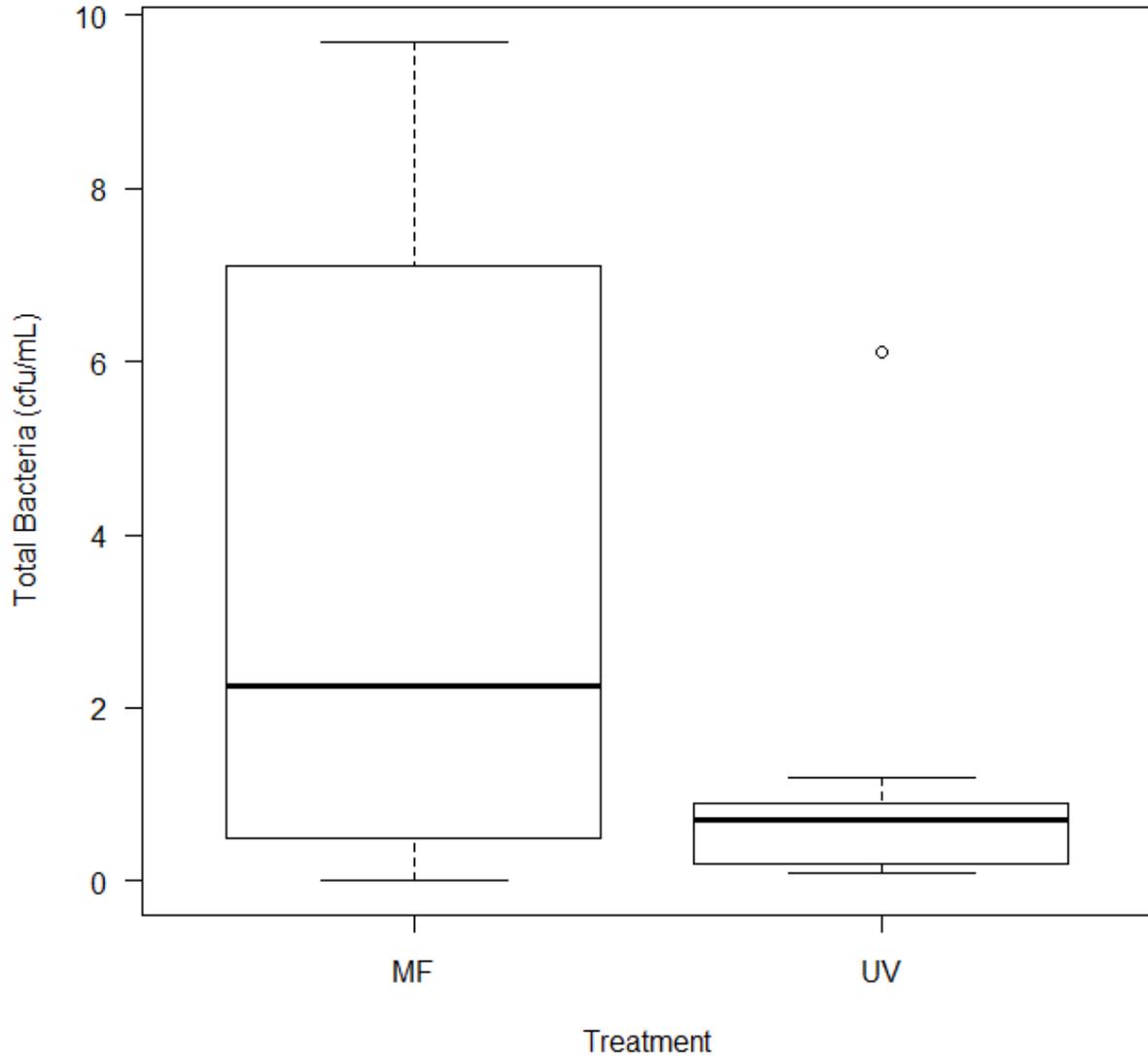


Figure A3.1. Box plot illustrating the variation in total bacterial counts measured from water samples collected after both MF and UV treatments used in the RAS throughout the 30 day trial (n = 14).

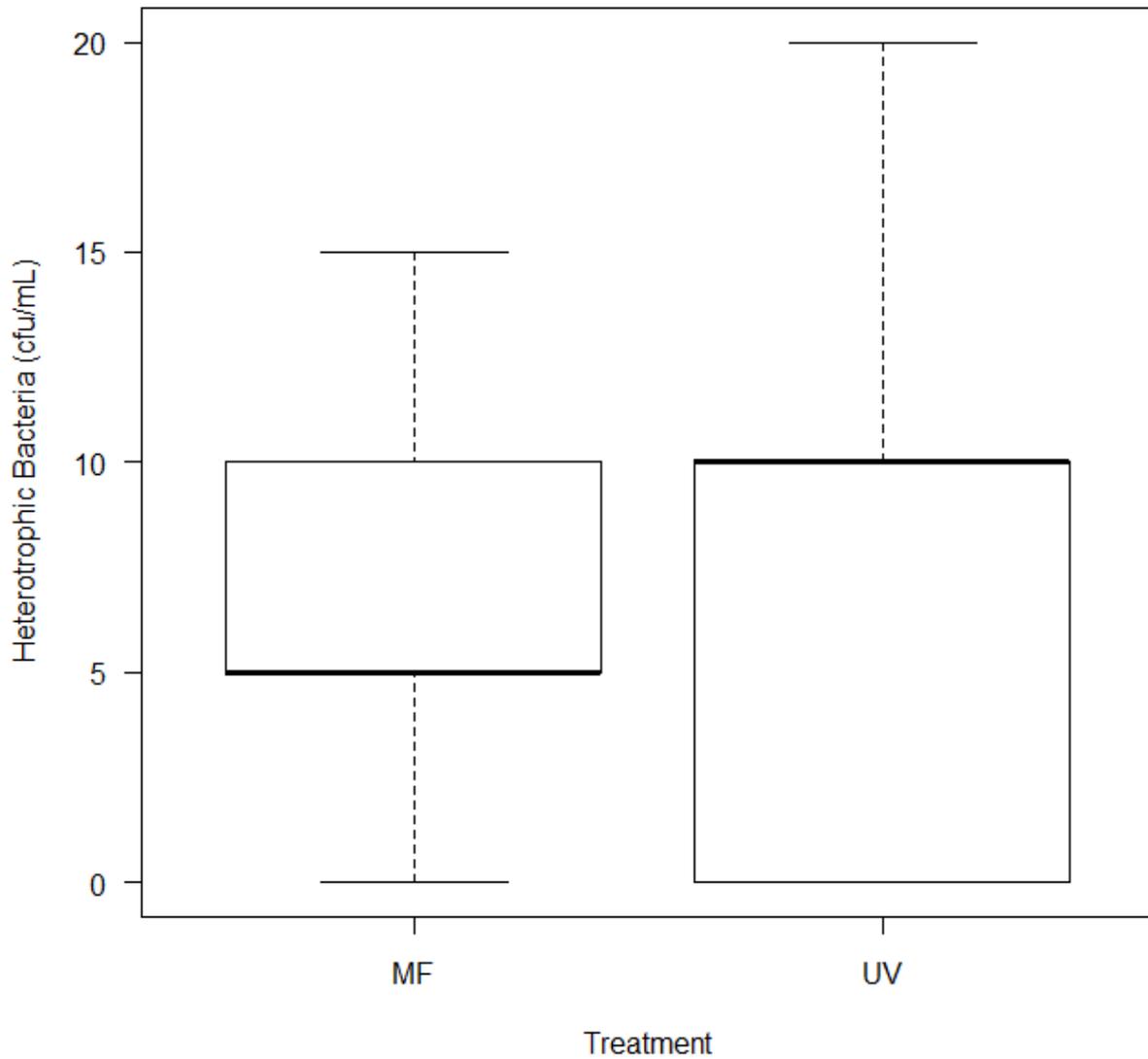


Figure A3.2. Box plot illustrating the variation in heterotrophic bacterial counts measured from water samples collected after both MF and UV treatments used in the RAS throughout the 30 day trial (n = 14).

Appendix 4: Influent, effluent and removal efficiencies achieved by MF for all of the chemical/physical water quality parameters.

Table A4.1. Mean (\pm S.E.) influent, effluent and removal efficiencies for all of the chemical/physical water quality parameters achieved by the MF system when treating wastewater in RAS throughout the 30 day trial (n = 13).

Parameter	Units	Influent	Effluent	% Removal/Change	<i>P</i> -value
Dissolved Oxygen	mg/L	9.10 \pm 0.04	8.78 \pm 0.05	3.45 \pm 0.63	0.0001 ^b
Temperature	°C	21.67 \pm 0.19	21.79 \pm 0.17	-0.58 \pm 0.14	0.6346 ^a
pH		8.23 \pm 0.02	8.35 \pm 0.03	-1.39 \pm 0.18	0.0110 ^b
Total Suspended Solids	mg/L	1.33 \pm 0.12	0.06 \pm 0.01	95.35 \pm 1.04	<0.0001 ^b
Total Dissolved Solids	mg/L NaCl	267.54 \pm 1.00	265.69 \pm 1.11	0.69 \pm 0.20	0.2285 ^a
Conductivity	mS/cm	533.38 \pm 2.04	529.54 \pm 2.23	0.72 \pm 0.22	0.2147 ^a
Turbidity	NTU	0.88 \pm 0.07	0.06 \pm 0.01	93.60 \pm 1.03	<0.0001 ^b
UV Transmissivity	%	92.05 \pm 0.66	94.83 \pm 0.56	-3.04 \pm 0.37	0.0038 ^a
Total Hardness	mg/L CaCO ₃	235.95 \pm 1.09	237.03 \pm 1.52	-0.47 \pm 0.62	0.4381 ^b
Nitrite Nitrogen	mg/L	0.01 \pm 0.00	0.01 \pm 0.00	14.35 \pm 11.14	0.1994 ^a
Nitrate Nitrogen	mg/L	4.37 \pm 0.30	4.27 \pm 0.31	1.33 \pm 4.22	0.5720 ^b
Total Ammonia Nitrogen	mg/L	0.03 \pm 0.01	0.03 \pm 0.01	-13.78 \pm 37.24	0.6499 ^a
Total Kjeldahl Nitrogen	mg/L	0.25 \pm 0.04	0.22 \pm 0.04	6.28 \pm 14.06	0.6254 ^a
Total Organic Carbon	mg/L	2.00 \pm 0.27	1.56 \pm 0.15	15.22 \pm 14.03	0.5982 ^b
Organic Nitrogen	mg/L	0.23 \pm 0.04	0.19 \pm 0.04	15.31 \pm 10.93	0.6004 ^b
Total Nitrogen	mg/L	4.86 \pm 0.69	4.65 \pm 0.66	4.11 \pm 4.77	0.8283 ^a

^a Analyzed using a two-sample Student's t-test.

^b Analyzed using a two-sample Wilcoxon signed-rank test.

Appendix 5: Mean values for operational parameters measured and calculated for the MF treatment used in the RAS throughout the 30 day trial.

Table A5.1. Mean (\pm S.E.) values of transmembrane pressures, resistances and fouling rates achieved by the MF treatment used in the RAS throughout the 30 day trial.

Parameter	Units	Mean
Initial Transmembrane Pressure ^a	kPa	3.13 \pm 0.04
Final Transmembrane Pressure ^b	kPa	22.89 \pm 0.06
Hydrostatic Pressure Loss ^c	kPa	8.85 \pm n/a ^d
Membrane Resistance ^a	/m	3.34E+05 \pm 4.61E+03
Final Fouling Resistance ^b	/m	2.10E+06 \pm 6.47E+03
Average Fouling Rate	/m/day	8.59E+04 \pm 1.88E+04
Average Fouling Rate	kPa/day	0.81 \pm 0.18

^a Calculated based on mean TMP between the first 60 and 90 minutes of filtration.

^b Calculated based on mean TMP during the last 30 minutes of filtration.

^c Calculated based on water viscosity at 22°C and a water level height of 1.7 m.

^d No standard error reported because the value was calculated.

Appendix 6: Retentate water quality characteristics after dosing the MF system with high concentrations of *F. psychrophilum*.

Table A6.1. Mean (\pm S.E.) retentate water quality characteristics after dosing the MF system with *F. psychrophilum* throughout three challenge tests (n = 3).

Parameter	Units	Mean
Dissolved Oxygen	mg/L	8.90 \pm 0.15
Temperature	$^{\circ}$ C	18.57 \pm 0.55
pH		8.14 \pm 0.09
Turbidity	NTU	10.66 \pm 1.77
UV Transmittance	%	78.33 \pm 7.38
Total Hardness	mg/L CaCO ₃	414.50 \pm 7.50