

AN EVALUATION OF WASTEWATER TREATMENT TECHNOLOGIES FOR THE
PURPOSE OF RECIRCULATION IN AQUACULTURE FACILITIES WITH A FOCUS ON
AN UNSUBMERGED BIOREACTOR

By

STEPHANIE COLLINS

B.Sc., University of Guelph, 2002

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

ENVIRONMENT AND MANAGEMENT

We accept this thesis as conforming
to the required standard

.....
Dr. Brent Wootton, Thesis Supervisor
Sir Sandford Fleming College

.....
Dr. Audrey Dallimore, Thesis Coordinator
School of Environment and Sustainability

.....
Dr. Chris Ling, Director
School of Environment and Sustainability

ROYAL ROADS UNIVERSITY
May 2017

© Stephanie Lorraine Collins, 2017

Abstract

As aquaculture continues to grow worldwide, land-based aquaculture facilities can be ideal because a large volume of fish can be produced in a small area. With growth comes the concern of water usage and wastewater management. New technologies are being utilized to address these issues, including those to lead to a recirculating aquaculture system (RAS) which can allow for almost complete reuse of hatchery waters. A cost-effective, efficient, and sustainable system is still needed. This study evaluated one potential RAS technology, an unsubmerged bioreactor, for: its effectiveness at removing wastewater contaminants from a rainbow trout hatchery in Ontario; and the effect operating conditions such as influent temperature, influent concentration, and treatment time have on contaminant removal. The results indicate that for this hatchery, the technology was effective at reducing or maintaining contaminant levels to those appropriate for recirculation, and that treatment time will be dependent on wastewater influent characteristics.

Table of Contents

Abstract	2
List of Figures	5
List of Tables	8
Introduction.....	10
Literature Review.....	13
Aquaculture Around the World.....	13
Aquaculture in Ontario.....	14
Water and Environmental Concerns	17
Recirculation Aquaculture Systems	19
RAS Challenges	20
The Unsubmerged Bioreactor Technology	20
Successful Biogill® Installations	25
Research Objectives and Hypotheses	26
Research Methodology	28
Operations	29
Results.....	31
Fleming College Salmon Hatchery	31
Coldwater Fisheries Inc.....	32
Discussion.....	46

Technology Comparisons..... 49

Economic reality 55

Conclusions and Recommendations 56

List of Figures

<i>Figure 1.</i> Photo of the Biogill® bioreactor membrane suspended in the housing unit.	21
<i>Figure 2.</i> The wastewater dispersal piping located on top of the membrane in the bioreactor housing unit.....	22
<i>Figure 3.</i> Bioreactor technology configuration for Fleming College salmon hatchery experiment. The green metal housing contains the membrane placed atop the 1000L tote containing the wastewater.....	23
<i>Figure 4.</i> Averaged cBOD ₅ concentrations of collected water samples during 24 hr batch treatments. The triangle marker is the averaged cBOD ₅ value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum cBOD ₅ values that were tested.	33
<i>Figure 5.</i> Averaged total suspended solids (TSS) concentrations in water samples collected during 24hr batch treatments. The triangle marker is the averaged TSS value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum TSS values that were tested.	34
<i>Figure 6.</i> Averaged NH ₃ -N concentration of water samples collected over 24 hr batch treatments. The triangle marker is the averaged NH ₃ - N value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum NH ₃ -N values that were tested.	35
<i>Figure 7.</i> Averaged NO ₃ -N concentrations in water samples collected over 24 hr batch treatments. The triangle marker is the averaged NO ₃ -N value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum NO ₃ -N values that were tested.	35

- Figure 8.* Averaged $\text{NO}_2\text{-N}$ concentrations in water samples collected over 24 hr batch treatments. The triangle marker is the averaged $\text{NO}_2\text{-N}$ value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum $\text{NO}_2\text{-N}$ values that were tested. . 36
- Figure 9.* Relationship between percent daily cBOD_5 reduction and wastewater influent temperature. The data has a correlation value of 0.43 indicating a low positive correlation. 37
- Figure 10.* Relationship between TSS reduction and wastewater influent temperature. The data has a correlation value of 0.11 indicating no correlation..... 38
- Figure 11.* Relationship between $\text{NH}_3\text{-N}$ reduction and wastewater influent temperature. The data has a correlation value of -0.45 indicating a low negative correlation. 38
- Figure 12.* Relationship between $\text{NO}_3\text{-N}$ increase and wastewater influent temperature. The data has a correlation value of -0.28 indicating a very low negative correlation. 39
- Figure 13.* Relationship between $\text{NO}_2\text{-N}$ reduction and wastewater influent temperature. The data has a correlation value of 0.39 indicating a low positive correlation. 39
- Figure 14.* Relationship between T-0 cBOD_5 influent concentrations on daily $\text{NH}_3\text{-N}$ reduction rates per 24 hr batch treatment. The data has a correlation value of 0.001 indicating no correlation. 40
- Figure 15.* Effect of T-0 cBOD_5 influent concentrations on daily $\text{NO}_3\text{-N}$ increase rates per 24 hr batch treatment. The data has a correlation value of 0.09 indicating no correlation. 41
- Figure 16.* Effect of T-0 cBOD_5 influent concentrations on daily $\text{NO}_2\text{-N}$ increase rates per 24 hr batch treatment. The data has a correlation value of 0.52 indicating a low positive correlation.. 41
- Figure 17.* Averaged percent cBOD_5 concentration reductions during the sample interval times during 24 hr batch treatments. 43

<i>Figure 18.</i> Averaged percent TSS concentration reductions during the sample interval times during 24 hr batch treatments.	43
<i>Figure 19.</i> Averaged percent NH ₃ -N concentration reductions during the sample interval times during 24 hr batch treatments.	44
<i>Figure 20.</i> Averaged percent NO ₃ -N concentration increase rates during the sample interval times during 24 hr batch treatments. The NO ₃ -N concentration increases substantially during the first 3 hrs of treatment, where all other treatment times have only a minimal increase in NO ₃ -N concentration.	45
<i>Figure 21.</i> Averaged percent NO ₂ -N concentration reductions during the sample interval times during 24 hr batch treatments.	46

List of Tables

Table 1. Summary of data results from the study conducted using the unsubmerged bioreactor technology to treat Fleming College salmon hatchery wastewater.	32
-----------------------------------------------------------------------------------------------------------------------------------------------------------------	----

Acknowledgements

I would first like to thank my thesis supervisor Dr. Brent Wootton for his expertise and guidance throughout this process, as well as Dr. Gordon Balch who offered his knowledge and friendship to help me through the years of working on this thesis. Without their support, this work would not have been completed.

I would also like to acknowledge the exceptional laboratory staff at the Centre for Alternative Wastewater Treatment at Fleming College, who I had the privilege to work beside during this project.

Finally, I must express my gratitude to my family for providing me with unfailing support and continuous encouragement throughout this seemingly never-ending task. This accomplishment would not have been possible without them. Thank you.

Introduction

Aquaculture is a quickly growing industry across the globe. The aquaculture industry has grown steadily over the last five decades and now provides almost half of all fish for human consumption (Food and Agriculture Organization of the United Nations [FAO], 2014). This trend is not slowing down, having an increasing annual rate of 3.2% (FAO, 2014). As the population continues to grow, this reliance on aquaculture for food production is also expected to increase to meet the demand. In a world where population is expected to reach 9.6 billion by 2050, aquaculture can play a significant role in combating hunger and poverty, and promoting health (FAO, 2014). This increased demand for fish production and its subsequent growth is due partly to the large volume of product that can be harvested in a small area (Pulefou, Jegatheesan, Steicke, & Kim, 2008). Nevertheless, with this rapid growth come environmental concerns regarding water quantity used and wastewater quality. The main concerns with wastewater discharge streams of aquaculture facilities are the potential for introduction of pathogenic organisms to native fish populations, and the high nutrient and solids content which could lead to eutrophication of receiving waters (Pulefou et al., 2008). In many countries, including Canada, Denmark, and Norway, government entities are searching for solutions to aid with the quickly growing aquaculture industry and dealing with the emerging problems of water use and wastewater management (Badiola, Mendiola, & Bostock, 2012). For this growing industry to be sustainable, it needs to be able to create a high level of production coupled with an environmentally sustainable technology that has low operating costs (Pulefou et al., 2008). Recirculated aquaculture systems (RAS) are considered to be part of the solution to aquaculture facilities sustainability issues (Badiola et al., 2012).

A RAS is a “closed” system that allows the aquaculture facilities to manage, collect, treat, and reuse their produced wastewater onsite with a less than 10% total water volume replaced per day (Pulefou et al., 2008; Schreier, Mirzoyan, & Saito, 2010). By achieving a RAS, this allows for production in areas with limited water supply, improves biosecurity, increases the ability to operate closer to markets, and achieves greater environmental sustainability (Pulefou et al., 2008); however, the costs to establishing and running a RAS can be limiting. Many technologies are being tested to be used in the aquaculture industry with varying degrees of success. Some are very efficient at removing contaminants, such as membrane filtration, but can have high operating costs. Others are more economically viable, but tend not to have the desired contaminant removal capacity for recirculation. For a technology to be very competitive in this field it will need to have low operating/infrastructure costs, it must be efficient and effective at removing contaminants and disease, and it must address local issues such as quantity of water or land restrictions (Pulefou et al., 2008).

This thesis evaluates some wastewater technology options for the aquaculture industry with a specific focus on an unsubmerged trickle bioreactor with patented ceramic membrane technology that supports the growth of endemic microorganisms (Biogill®, 2014); a technology that has the potential to be promising to the aquaculture industry as it has shown to be effective for treating other sources of wastewater in Canada. The ceramic membrane fabric is suspended as loops in a vertical housing container and wastewater trickles over the membrane where a biomass is established and continues to grow. The biomass contains the microorganisms that consume and degrade the wastewater nutrients, effectively removing cBOD₅, nitrogen, and solids (Biogill®, 2014). As a relatively new technology in Canada, little research has been completed on the mechanisms of action or what the logistical limitations are for deployment at a Canadian

aquaculture facility. This study assessed the bioreactor technology treatment parameters by examining its capacity for reduction of nutrients, temperature influences on nutrient removal, and efficient cycling time for batch treatment. This was determined by evaluating data collected from the bioreactor experiments designed to test the technology effectiveness for the treatment of aquaculture wastewater under two different wastewater regimes (e.g., low nutrient / low temperature versus moderate nutrient / moderate temperatures) at two separate land-based, flow-through aquaculture facilities in Ontario. This study evaluated the performance of the bioreactor for removal of cBOD_5 , TSS, ammonia (NH_3), nitrate (NO_3) and nitrite (NO_2) in relation to temperature, and also investigated the treatment efficiency over a time series to optimize operating conditions. To expand on treatment options for the aquaculture industry, a comparison to other current and potential wastewater technologies, such as submerged membrane bioreactors and trickle filters, was developed to outline some of the existing technologies available and how this unsubmerged bioreactor may compare to them.

This research has the possibility to be significant to environmental management in the aquaculture industry as the unsubmerged bioreactor technology has the potential to be a solution to the aquaculture wastewater management issue. As an effective, efficient, and economically feasible technology it could have widespread application to aquaculture facilities in Ontario and the rest of Canada. It also has the potential to be a long term solution, with wide applications to other waste streams. The research completed will lead to a greater understanding of the technology for the industry partner which could potentially assist in optimizing their technology, expanding their market, and increasing their revenues.

Literature Review

Aquaculture Around the World

Aquaculture is defined by the National Oceanic and Atmospheric Administration as the farming of aquatic plants and animals. This includes fish (both for food and for sport), crustaceans, mollusks, algae, sea vegetables, and fish eggs (“What is Aquaculture?”, n.d.). Fish is the most traded food commodity globally and for the last fifty years, fish production (both capture and aquaculture combined) has grown steadily at an annual rate of 3.2% across the globe (FAO, 2014). This increase in production coincides with a substantial increase in consumption, logically due in part to an increase in global population. More specifically, world food fish aquaculture during 2000-2012 grew at a rate of 6.2% annually (FAO, 2014). Along with this increased demand for food fish for consumption is a rise in employment for this industry (FAO, 2014), contributing to this industry being a large global economic player (FAO, 2014). However, with these continued increases, there is the risk and evidence of overfishing and concerns of environmental sustainability have arisen. Aquaculture can be carried out by land-based fish farming as well as open water cage culturing. Cage culturing involves rearing fish from fingerlings to market size in net pens in an open water habitat (Moccia, 1997). The drawbacks of this rearing practice are the addition of nutrients to the environment from uneaten food and fish waste, and also the escape of reared fish into native populations. Land-based fish farming rears fish utilizing a variety of technologies such as rectangular raceway tanks and circular tanks in a building requiring a reliable water source and some level of primary wastewater treatment technology. While the land-based fish farms require additional land and water resources over the cage culturing, it allows the ability to have a greater number of farming operations and allows for

high fish stocking densities (Moccia & Bevan, 2005). The drawback of land-based fish farms is the wastewater discharge.

Aquaculture in Ontario

Aquaculture and more specifically fish culturing, has been taking place in Canada since the early 1900s (Moccia, Naylor, & Reid, 1997). In Ontario, fish culturing by the provincial government started off mainly as a means to stock lakes and streams (Moccia et al., 1997). Until the early 1960's, fish culturing remained a predominantly government endeavour (Moccia et al., 1997) but today there are many commercial facilities across Canada. Ontario has shown to be well suited for aquaculture growth for both provincial and commercial venues because the province has an abundant supply of water resources, adequate domestic demand for its products, as well as having the appropriate and well developed infrastructure, equipment, and expertise (Moccia et al., 1997). Under provincial legislation, Ontario has over 40 species of fish which can be raised including trout, Arctic char, and yellow perch (Urban Agriculture Business Information Bundle, 2016). Rainbow trout accounts for the majority of the fish culturing in Ontario, but the type of species reared is based on a number of things: legislation, availability of domestic stocks, good quality feed, and demand for the species (Moccia et al., 1997). For this research, data were collected from two land-based, flow-through aquaculture facilities, one that reared Atlantic salmon and the other that reared predominantly rainbow trout.

The industry operations in Ontario are governed by both the provincial and federal governments. Aquaculture falls as a sub-sector under the Ontario Ministry of Agriculture, Food, and Rural Affairs, but the Ontario Ministry of Natural Resources and Forestry (MNR) administers the licences to culture and sell fish under the Game and Fish Act, and the Ontario Ministry of the Environment and Climate Change (MOECC) administers the Ontario Water

Resources Act (OWRA) which states the requirements of users who take over 50,000 l/day to obtain a permit to take water (Moccia et al., 1997). The MOECC is also the governing body that oversees discharges of wastewater to the receiving environment and issues approvals as an Environmental Compliance Approval for the facility to discharge, which usually mandates some level of wastewater treatment (Moccia et al., 1997).

Water is a key aspect to a profitable and sustainable aquaculture operation. The facility will require a high quality and sufficient supply year round, so location is top priority. Aquaculture facilities depending on location and fish species will use groundwater, spring water, and/or surface waters (Moccia et al., 1997). The quantity to be used depends on the size of the operation but typically ranges between 36 LPM to 45000 LPM (51840 litres per day and 6.48×10^7 litres per day respectively) (Moccia et al., 1997). In 2003, Ontario aquaculture produced 4375 tonnes of fish for human consumption, 1175 tonnes coming from land-based aquaculture facilities (Moccia & Bevan, 2005). It is estimated that there are approximately 188 land-based aquaculture facilities in Ontario, mostly located in the central and southwestern regions of the province (Moccia & Bevan, 2005). While it may seem that land-based facilities are fairly low in production in comparison to open-cage aquaculture, it can have a significant environmental impact due to water demands. These facilities tend to be located near high population areas and therefore can be competing for surface and ground water, having the potential to cause issues further down the watershed (Moccia & Bevan, 2005). The land-based aquaculture facilities in Ontario tend to be predominantly flow-through, but while this can result in a large volume of water taken, aquaculture is not seen as a net user since most of the water is returned to the watershed (Moccia & Bevan, 2005). However, with a large amount of water being used daily, and with an expectation of continued growth, it is important that solutions to decrease net water

usage and decrease nutrients disposed from aquaculture operations into receiving waters are implemented.

Since the early days of aquaculture in Ontario, the industry states that it has put environmental sustainability and accountability at the forefront (Northern Ontario Aquaculture Association [NOAA], 2006). The industry is seen as a sector capable of generating and maintaining substantial employment and economic prosperity in the province and therefore is of high priority to maintain a sustainable industry (NOAA, 2006). The basis for environmental management and regulation of aquaculture in Ontario works off of three protocols:

1. International agreements to which Canada is obligated to adhere to (e.g.: U.N Food and Agriculture Organization's Code of Conduct for Sustainable Aquaculture)
2. National and provincial environmental laws and regulations
3. Codes of Conduct and/or Best Management Practices at production (NOAA, 2006)

In Ontario, the "Best Management Practices for Sustainable Aquaculture In Ontario" was produced by the Northern Ontario Aquaculture Association (NOAA, 2006) and the purpose of this document is so that the facility can demonstrate its operations are in compliance with regulatory requirements and can be productive and environmentally sustainable (NOAA, 2006). Aquaculture wastewater discharge regulations differ significantly from country to country so there are no overarching regulations. Some regulations are based on effluent; others regulated based on water consumption and feed usage. Therefore the general tendency is rather than imposing effluent standards, best management practices are provided instead (van Rijn, 2013).

The decision to utilize RAS in Ontario would be based on location, fish species being reared, production capacity, and costs of operating. In many aquaculture facilities in Ontario, there is plenty of freshwater available and so it may be more economical to use a flow-through system rather than incorporate wastewater technologies to complete a RAS. In some countries the decision to move to a RAS facility may be dictated by regulatory requirements, lack of availability of land and water resources, whereas in Ontario the decisions to become RAS may largely be dependent on company economics and environmental stewardship rather than imposed standards.

Water and Environmental Concerns

There are a number of ecological concerns that can arise from a quickly growing aquaculture industry. These can differ country by country due to their own individual natural restrictions, but usually revolve around quality and safety of the produced species, and environmental issues due to the high rate of water consumption and discharging of untreated wastewaters (Zhang et al., 2011). For example, China is the largest consumer and producer of aquaculture products, accounting for 67% global quantity in 2006 (FAO, 2008), and they have had to deal with a number of ecological concerns including: land and water resource limitations, degradation of aquatic ecosystems, disease outbreaks, and difficulties with sediment and wastewater treatment (Zhang et al., 2011).

One of the large concerns about aquaculture's environmental impact is waste management. Effluent characteristics between different styles of aquaculture facilities can vary widely due to the species being reared, the facility size, and the ownership (U.S. Environmental Protection Agency, Office of Science and Technology, 2004). Effluent waters are mainly comprised of solids and nutrients arising from fish urine and excrement, and uneaten food. Some

aquaculture facilities have the ability to discharge waste streams directly into local receiving waters; in Ontario this would require an Environmental Compliance Approval (ECA) issued by the Ministry of Environment and Climate Change (MOECC) and likely require some primary physical treatment such as a drum filter, and an effluent monitoring plan (Moccia & Bevan, 2005). Although some aquaculture facilities such as land-based flow-through systems use large volumes of water and therefore typically have effluents with dilute waste concentrations compared to an RAS, the cumulative waste loadings can be significant. Therefore, some environmental regulations cover both mass loading and concentration of waste characteristics for aquaculture effluents (Davidson, Helwig, & Summerfelt, 2008), however current guidelines in Ontario do not address this (Ontario Animal Research and Services Committee (OARSC), 2005). Excess nutrients and solids deposited into these receiving waters can have significant environmental concerns; both physiochemical and biological impacts. Physiochemical impacts are those that show immediate changes in environmental conditions while biological impacts are those changes that will occur over a longer period of time (Camargo, 1994).

Physiochemical impacts include the settling of solids onto stream beds as well as the increase of five-day biochemical oxygen demand (BOD_5) of receiving waters (Snow, Anderson, & Wootton, 2011). Another physiochemical impact is that created by the discharge of nitrogen and phosphorus to receiving waters, leading to the concern of eutrophication (Camargo, 1994). Eutrophication is the process of excessive nutrients such as phosphorus and nitrogen entering into a body of water, stimulating plant and algae growth, resulting in a depletion of oxygen and this process can be intensified in areas where land based aquaculture facilities release their facility effluents (Eutrophication, n.d.). This increased production of algae is of particular concern if it is blue-green algae or cyanobacteria. This algae has the ability to produce and

release powerful toxins into the water body rendering some locations as potentially deadly to animals and humans if enough water is ingested or absorbed through the skin (Health and Ecological Effects, n.d.). The presence of algae blooms can also significantly reduce oxygen concentrations in water bodies. This depletion of oxygen can lead to fish and invertebrate deaths (United Nations Environment Programme, Division of Technology, Industry and Economics, n.d.).

Biological impacts include such changes as a decrease in invertebrate groups sensitive to organic pollution; structural and functional changes in native fish communities; an increase in periphyton; and an increase of heterotrophic bacteria and sewage fungi (Snow et al., 2011). Overall these changes can dramatically change the native composition of a water body.

The other main concern for aquaculture facilities is the amount of water and/or electricity required to run the facility. Water volumes will range between species, but in Ontario this could be anywhere from 35 litres per minute (LPM), to upwards of 45, 000 LPM (Moccia et al., 1997). While water has been said to be “abundant” in Ontario, as the number of aquaculture facilities grow, it shouldn’t be assumed that existing water reserves could meet the demand. Therefore strategies should be developed now in order to prepare for this.

Recirculation Aquaculture Systems

Recirculation aquaculture systems (RAS), which aim to use less water and improve waste management and nutrient release, can be a key component to sustainable aquaculture, which must have a focus on creating a minimal ecological impact (Martins et al., 2010). Recirculation aquaculture systems are defined as systems where the facility water is partially re-used after treatment, both mechanical and biological, in an effort to reduce total water consumption and

release of nutrients into the environment (Zhang et al., 2011). RASs were developed in an effort to address the concerns of sustainability both economically for aquaculture facilities, but also environmentally as concerns of nutrient loading in receiving waters becomes more of an issue (Martins et al., 2010). These systems are also a solution for areas that have water and land use restrictions to address (Martins et al., 2010). Other advantages to implementing RAS are better control over the facility's hygiene and disease management (Summerfelt et al., 2009; Tal et al., 2009), biological pollution control (Zohar et al., 2005), and the ability to produce a wide range of seafood products in close physical location to their markets thereby reducing GHG emissions from transportation of goods (Masser et al., 1999; Schneider et al., 2010; Martins et al., 2010).

RAS Challenges

Despite the numerous benefits to the incorporation of a RAS, the development of RAS facilities across the globe are slow. This is mainly due to the high capital costs associated with implementing the technologies (Martins et al., 2010). There are also concerns of managing disease outbreaks since water is re-used, and overall proper system management (Martins et al., 2010; Badiola et al., 2012). RAS has been slowly growing in European countries, but there have been issues with implementation of the systems mainly due to poor initial designs of the systems and poor management (Badiola et al., 2012). For successful implementation of a RAS, more attention is needed on finding the right technologies for the specific facility and having properly skilled individuals maintaining the system (Badiola et al., 2012).

The Unsubmerged Bioreactor Technology

The bioreactor technology used in this research is in summary an above ground, non-submerged bioreactor and the specific unit used was the Biogill® technology. It is composed of

a housing unit in which patented, flexible, ceramic membranes are looped over rods at the top as shown below.



Figure 1. Photo of the Biogill® bioreactor membrane suspended in the housing unit. (Photo credit: Centre for Alternative Wastewater Treatment, 2013).



Figure 2. The wastewater dispersal piping located on top of the membrane in the bioreactor housing unit. (Photo credit: Centre for Alternative Wastewater Treatment, 2013).

The source wastewater is pumped from its location, usually a holding tank or cell, to the top of the housing unit where it is dispersed over the membranes (Figure 2) and flows down the membrane to the same holding tank. In these experiments the housing unit was placed on top of a 1000L tote which contained the influent wastewater which was recirculated over the membrane and back into the tote as shown in Figure 3.



Figure 3. Bioreactor technology configuration for Fleming College salmon hatchery experiment. The green metal housing contains the membrane placed atop the 1000L tote containing the wastewater. (Photo credit: Centre for Alternative Wastewater Treatment, 2013).

Other configurations are available for different scenarios. Since the bioreactor is above ground and un-submerged, convective air moves up between the gill loops providing oxygen transfer to the biofilm that grows on the membrane (Biogill®, 2014).

The nutrient and biochemical oxygen demand (BOD_5) rich wastewater flowing over the membrane creates a biofilm which is a layer of microorganism growth that can form on a surface (Biofilm, n.d.). (Biochemical oxygen demand is a standardized laboratory test used to indirectly

evaluate the organic pollution of water by measuring the amount of dissolved oxygen demanded by aerobic biological organisms to degrade organic material and oxidise inorganic material such as sulphides and reduced forms of nitrogen over a specific time and temperature (Liu, 2002)). Due to the set-up of the unsubmerged bioreactor, oxygen is readily available as well as soluble nutrients from the influent wastewaters, for the biofilm functions. Within the biofilm, the larger organic compounds such as sugars and fatty acids are broken down into smaller molecules via catabolism (von Sperling, 2007). Through the nitrification process, ammonia is oxidized to nitrite and ultimately nitrate. These molecules all then feed the anabolism process of the biofilm itself (von Sperling, 2007).

The bioreactor technology literature states that the technology can effectively treat wastewaters with high BOD₅, COD, nitrogen compounds, and to a lower extent phosphorus (Biogill®, 2014). The technology is adaptable to the wastewater characteristics as the capacity of the membranes to remove BOD₅ is calculated in grams/m² of membrane. The membrane reduction capacity relies on factors such as operating temperature and wastewater characteristics, but the company uses the influent BOD₅ concentration as an estimate for reduction capacity. Therefore the technology can be scaled up or down for the waste stream. In terms of this study where BOD₅ concentrations are fairly low, an estimate of reduction capacity for our system would be 10 g BOD₅/m² considering a 150 mg/L influent BOD₅ concentration.

When implementing this technology for wastewater treatment, according to the Biogill® “Technical and Specification Guide” (2014), numerous factors need to be considered:

1. Solids and particulate removal
2. Influent and air temperature

3. Temperature fluctuations
4. pH
5. Contact time of the wastewater over the gills
6. Nutrient quality of the wastewater
7. Sludge removal
8. Sanitizers, detergents, and biocides

All of these factors need to be taken into account when sizing the technology and determining how appropriate the technology will be to the wastewater and the overall effluent goals. In Canada this technology has been used for food and drink wastewaters, and this research was to examine its performance in land based freshwater aquaculture facilities in Ontario.

Successful Biogill® Installations

The Biogill® bioreactor technology has shown effective results in a number of studies. During the time period of May 2012 and May 2013 the bioreactor technology was used in a comparison study against existing recirculation technology at the Port Stephen's Fisheries Institute in New South Wales Australia. The facility was set up so that each circular fish rearing tank had its own small recirculation system and the mean temperature of the aquaculture waters was $17.0^{\circ}\text{C} \pm 1.5$ s.d. This allowed for the swap out of one of the existing systems, an airlift bioreactor (after the drum filter), for the Biogill® bioreactor technology and directly compare it to the standard technology. The results demonstrated that the Biogill® bioreactor system maintained or improved water quality, maintained breeding performance and health of the fish, as well as a reduction in energy usage due to the removal of the air blower used for the airlift bioreactor (Charlton, 2013).

Another earlier study completed by the Biogill® company to assess the technology was completed at an island resort in Fiji (Taylor, 2013). The Biogill® bioreactor was retrofitted in two treatment stages into an existing sewage treatment facility that was not performing adequately. The bioreactor was treating wastewaters from the kitchen, showers, and leachate from the composting toilets. The goal of the treatment was to get the wastewater to concentrations levels that were suitable for sub-surface irrigation. The system was tested for BOD₅ concentrations along with nitrogen and phosphorus compounds. The primary results from effluent from the initial bioreactor showed a reduction of BOD₅ from 662 mg/L to 320 mg/L during an HRT (hydraulic retention time) of 14 hrs, along with some nitrogen removal. The second bioreactor system treated the settled influent from the first bioreactor and was able to reduce BOD₅ to an average of 25 mg/L with an HRT of 15 hours, achieving a 96% BOD₅ removal through the treatment chain (Taylor, 2013).

The Canadian distributor of the Biogill® bioreactor approached the Centre for Alternative Wastewater Treatment believing that the technology could have applications in Canada and further to determine the extent of the applicability of the Biogill® technology in various wastewater streams in Ontario. Since the technology has shown effective treatment results in warm climates for aquaculture facilities, it was thought that it could be beneficial to Ontario's aquaculture and potentially other cold weather locations.

Research Objectives and Hypotheses

The objectives of this study were to determine if the unsubmerged bioreactor technology is an effective and economical aquaculture wastewater treatment technology for use in recirculating aquaculture system facilities. My expectation of this study was that the bioreactor

would reduce five-day carbonaceous biochemical oxygen demand (cBOD₅), TSS, ammonia, nitrate, and nitrite from aquaculture wastewater from two aquaculture facilities with different waste concentrations and temperature regimes (cBOD₅ is the BOD₅ test with the addition of an inhibitor to prevent the oxidization of nitrogen compounds to allow for the test to more accurately measure the oxygen consumption of just the organic material in the water sample (Liu, 2002)).

Testable Hypotheses (H₀):

- i. cBOD₅, TSS, ammonia, nitrate, and nitrite will not be reduced (or maintained) to levels required for aquaculture recirculation (cBOD₅- < 10 mg/L; TSS- < 80 mg/L; Ammonia (NH₃-N)- < 0.05 mg/L; Nitrate (NO₃-N)- < 10 mg/L; Nitrite (NO₂-N)- < 1.0 mg/L (Molleda, 2007) in medium strength hatchery wastewater within a 24 h period.
- ii. The ambient temperature of the source wastewater and of the technology membranes (5-20°C) does not significantly influence nutrient reduction rates achieved by the bioreactor technology.
- iii. cBOD₅ concentration of influent wastewater does not negatively correlate with influent nutrient reduction rates.
- iv. Batch treatment time (1-24 hrs) of influent wastewater recirculated over the membrane of the system does not influence nutrient reduction rates per hour.

Research Methodology

The research questions posed above were evaluated by investigating the following objectives of the research project:

1. Using the Biogill® technology for the bioreactor, the technology was evaluated to determine if it effectively and efficiently reduces cBOD₅, TSS, ammonia, nitrite, and nitrate from wastewater from a small salmon aquaculture facility and a rainbow trout aquaculture facility to a level that meets the requirements for recirculation of hatchery waters.
2. The bioreactor technology was investigated to evaluate relationships affecting the technology's operating conditions, including influent temperature, influent concentrations, and treatment time, for effective removal of cBOD₅, TSS, ammonia, nitrate, and nitrite from aquaculture wastewater to concentrations suitable for a RAS.

To address the research questions and objectives, the Biogill® technology was used for the unsubmerged bioreactor, and was tested under varying conditions in two different land-based, flow-through aquaculture facilities to evaluate its operational effectiveness (low temperature and low nutrients at Fleming College, and moderate temperature and nutrient concentrations at Coldwater). These sites are described more in the operations section below. The experimental research was carried out by collecting wastewater from the wastewater collection areas of the hatcheries which were located downstream of the fish rearing tanks. The researchers and sample collectors at no time had access to the fish in both hatcheries, and all wastewater used for the research was returned to each aquaculture facility's main wastewater discharge site and disposed of according to normal operating procedures for the facility. The

collection of wastewater, the carrying out of the research, and the disposal of wastewater, was all conducted independently from the fish rearing areas and at no time impacted the fish at the hatcheries. Methods used for analyzing wastewater parameters followed or are sufficiently adapted from Standard Methods for the Examination of Water and Wastewater, 22nd Edition (Rice, Baird, Eaton, & Clesceri, 2012). Sampling methods followed the institutional protocols set out by the Centre for Alternative Wastewater Treatment based on Standard Methods for the Examination of Water and Wastewater, 22nd Edition. Parameters evaluated were based on those evaluated under similar research projects (Guerdat, Losordo, Classen, Osborne, & DeLong, 2011; Pulefou, et al., 2008). Due to biological systems being naturally variable, and due to the small sample sizes related to time availability for this study, the variability was too high to state a level of significance, therefore, results were evaluated by examining trends in the data collected.

Operations

1st Phase: Pilot testing of the bioreactor unit at the Fleming College salmon hatchery to determine if the technology can provide adequate waste parameter removal under low nutrient concentration and low ambient temperature.

The bioreactor technology was installed at the Fleming College salmon hatchery located in Lindsay Ontario in December 2013 for the research project. The bioreactor was housed under normal hatchery operating conditions and dosed with standard hatchery wastewater, post drum filter (low nutrient and low temperature). Regular samples of influent and effluent were analyzed. These data were evaluated to determine if the bioreactor effectively treated the existing hatchery wastewater concentrations under the hatchery's normal operating conditions.

Testing: Regular influent and effluent samples were analyzed for pH, ORP, DO, insoluble/soluble cBOD₅, COD, NO₃, NO₂, NH₃, TKN, TP, PO₄, TSS/VSS.

Goals: To establish if the technology can maintain effective removal of cBOD₅, TSS, ammonia, nitrate and nitrite under low influent concentrations and low ambient temperatures.

Time: Sample analysis completed over five months; December 2013– May 2014.

2nd Phase: Pilot testing of the bioreactor unit at the Coldwater Fish Hatchery to establish relationships between length of treatment time and effective removal rates, and temperature and effective treatment.

The bioreactor unit was moved from the Fleming College salmon hatchery to the Coldwater Fisheries aquaculture facility in Coldwater, Ontario. This facility is a commercial aquaculture operation specializing in the production of rainbow trout. The unit was exposed to a warmer ambient temperature than the salmon fish hatchery, but was located outdoors and subjected to the weather. The unit was dosed with aquaculture wastewater from one of the wastewater holding ponds at the facility. Influent and effluent samples were analyzed to determine nutrient removal rates which will be compared to the corresponding temperature data to examine if the temperature plays a role in treatment. Influent and effluent samples were also collected on a time series to determine the duration of ideal treatment.

Testing: Influent and effluent samples from each batch were tested for pH, ORP, DO, insoluble/soluble cBOD₅, NO₃, NO₂, NH₃, TKN, TP, TDP, TSS/VSS, TOC/DOC

Goals: To evaluate if the bioreactor removal rates for cBOD₅, TSS, ammonia, nitrate, and nitrite are affected by temperature, and to evaluate what the ideal length of time for treatment is based

on the results of effluent concentrations of cBOD₅, TSS, ammonia, nitrate, and nitrite over a 24 hour testing period.

Time: Sample collection was completed over two months; September – October 2014.

Results

Fleming College Salmon Hatchery

For the bioreactor installation at the Fleming College salmon hatchery, samples consisted of a time 0 hr influent sample and a time 24 hr effluent sample. At the most, only 15 sample days were completed on some parameters due to poor growth of visual biofilm on the bioreactor membranes. This facility is kept around 10°Celsius, and the aquaculture waters were below this temperature. It is expected that these low temperatures inhibited microbial growth on the membranes which enable the reduction of nutrients. As most aquaculture facilities would have some form of mechanical removal of solids prior to wastewater being discharged, the source water used for this study was taken post drum filter therefore most solids were removed and the nutrient concentrations were quite low. Table 1 displays the results collected from the Fleming College hatchery experiment. The data reveal a high variability within results and no real treatment of the wastewaters. There is a reduction in TSS but this could be due to settling of the solids in the tank. NH₃ was actually higher in the effluent samples and this could be due to a breakdown of existing food particles from organic nitrogen to ammonia, however this was not further investigated. It is possible that the temperatures within the hatchery were too low to encourage biomass growth on the membrane, and the low influent nutrient concentrations would also prevent significant growth of biofilm required to treat the wastewater. Due to the

inconsistent data and large gaps in the data set, it was not used as a comparison to the Coldwater data. From this limited information it was determined that overall, the unsubmerged bioreactor technology would not be appropriate for a facility such as this that consisted of constant high volumes of flow-through water, low water temperatures, and low nutrient concentration in wastewaters.

Table 1

Summary of data results from the study conducted using the unsubmerged bioreactor technology to treat Fleming College salmon hatchery wastewater.

Sample size (n)		<i>n</i> =15		<i>n</i> =13		<i>n</i> =13		<i>n</i> =4	
Parameter		cBOD₅ (mg/L)	std	TSS (mg/L)	std	NH₃-N (mg/L)	std	NO₃-N /NO₂-N (mg/L)	std
	Influent	34	11.14	39.69	35.73	0.36	0.36	0.58	0.07
	Effluent	33.8	48.19	18.55	33.16	1.26	1.94	0.69	0.13

Std = standard deviation

Coldwater Fisheries Inc.

Twenty-six sample sets were collected from the experimental unsubmerged bioreactor system installed at Coldwater Fisheries Inc. starting September 16, 2014 and continuing until October 31, 2014. Wastewater was treated over 24 hrs in 300L batches for the first 8 sample sets from September 16 -30, 2014. The batch treatments were then raised to 1000L. Samples were collected from the batch tote using an automated sampler collecting at 3 hour intervals (0h, 3h, 6h, 9h, 12h, 15h, 18h, 21h, and 24h). The data set collected from Coldwater over the weeks showed a reduction in nutrient concentrations during the 24 hr batch treatments (except for nitrate which increased during treatment cycles).

In Figure 4, carbonaceous biochemical oxygen demand (cBOD₅) over all samples dates have been averaged according to time intervals of collection with error bars illustrating the range of values collected (high and low). The data show an initial averaged cBOD₅ concentration of 20 mg/L being reduced to 9 mg/L over the 24 hr period; an average of 55% reduction of cBOD₅ concentration over the 24 hr treatment period.

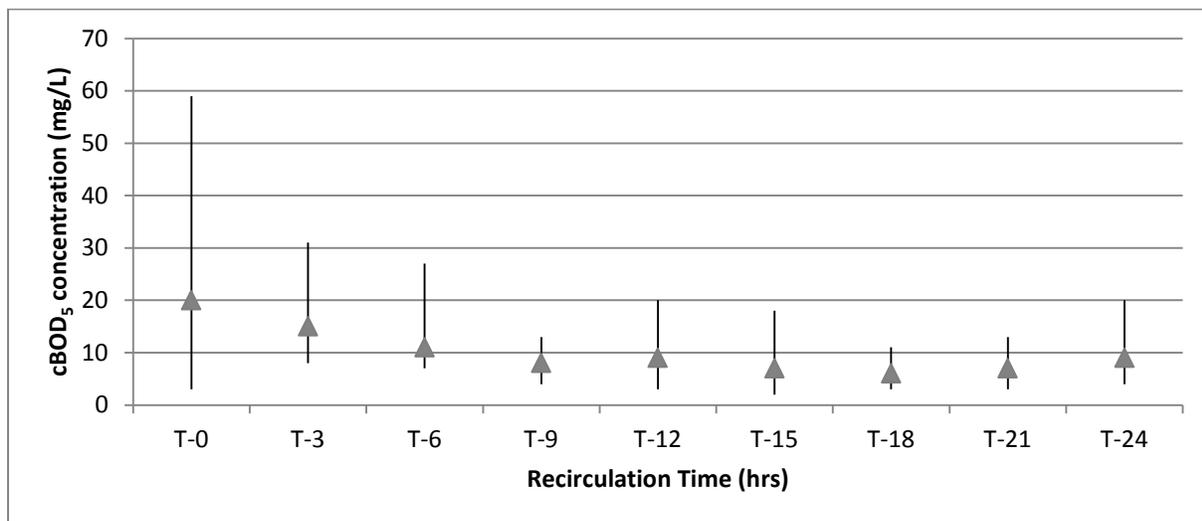


Figure 4. Averaged cBOD₅ concentrations of collected water samples during 24 hr batch treatments. The triangle marker is the averaged cBOD₅ value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum cBOD₅ values that were tested.

In Figure 5, total suspended solids (TSS) values per sample time were averaged over all sample dates for each time interval. Averaged TSS concentrations were 73 mg/L at time 0 hr, and 10 mg/L at time 24 hr treatment, showing an averaged reduction of 86 % over the full 24 hr treatment periods.

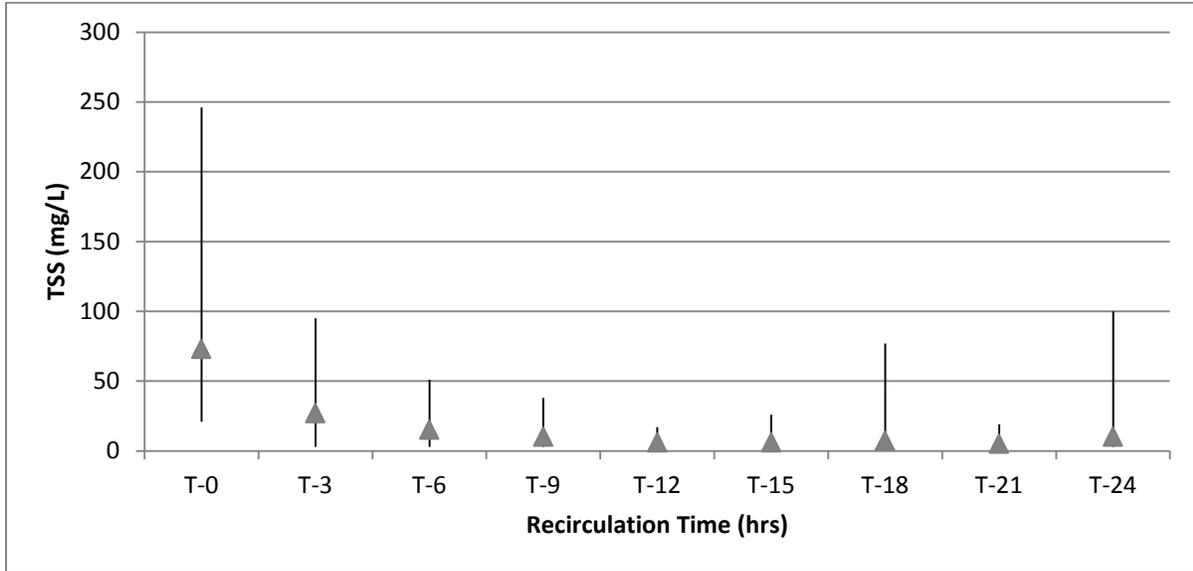


Figure 5. Averaged total suspended solids (TSS) concentrations in water samples collected during 24hr batch treatments. The triangle marker is the averaged TSS value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum TSS values that were tested.

In Figure 6, ammonia ($\text{NH}_3\text{-N}$) values per sample time were averaged over time interval for all sample dates. Averaged $\text{NH}_3\text{-N}$ concentrations were 1.77 mg/L at time 0 hr, and 0.05 mg/L at time 24 hr treatment, showing a reduction of 97 % over the full 24 hr treatment periods.

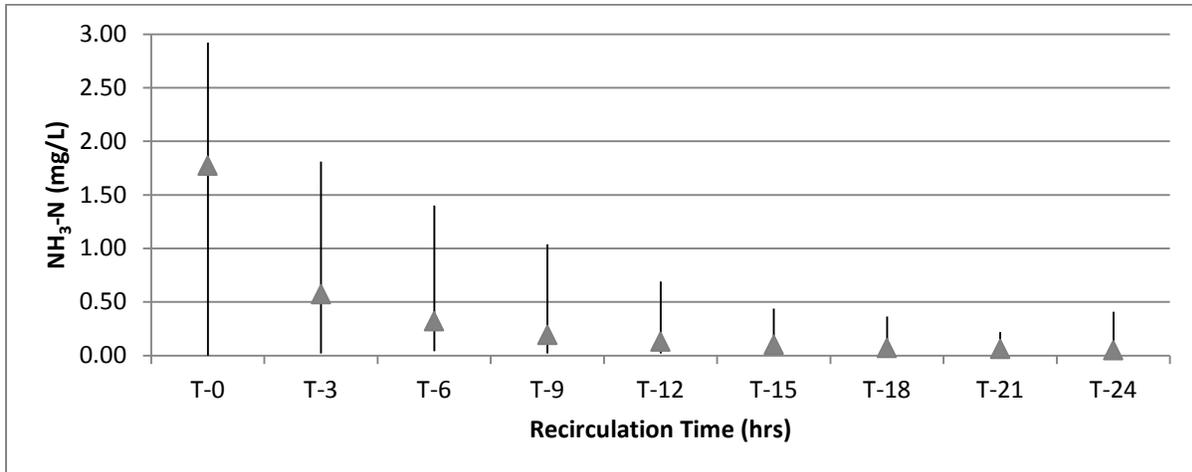


Figure 6. Averaged $\text{NH}_3\text{-N}$ concentration of water samples collected over 24 hr batch treatments. The triangle marker is the averaged $\text{NH}_3\text{-N}$ value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum $\text{NH}_3\text{-N}$ values that were tested.

In Figure 7, nitrate ($\text{NO}_3\text{-N}$) values per interval time were averaged over all sample dates. Averaged $\text{NO}_3\text{-N}$ concentrations were 0.56 mg/L at time 0 hr, and 3.25 mg/L at time 24 hr treatment, showing an increase of 480 % over the full 24 hr treatment period.

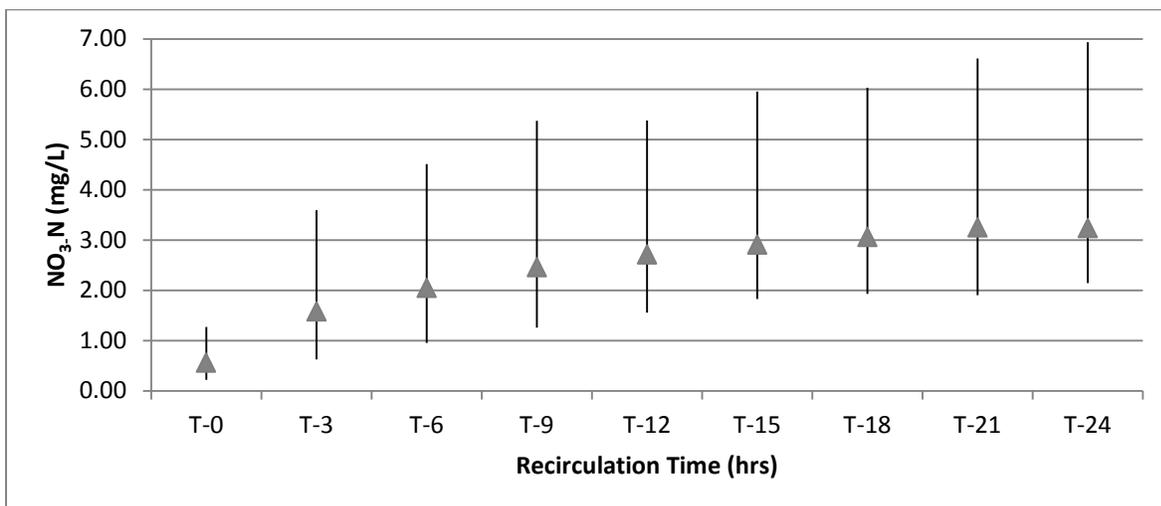


Figure 7. Averaged $\text{NO}_3\text{-N}$ concentrations in water samples collected over 24 hr batch treatments. The triangle marker is the averaged $\text{NO}_3\text{-N}$ value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum $\text{NO}_3\text{-N}$ values that were tested.

In Figure 8, nitrite ($\text{NO}_2\text{-N}$) values per interval time were averaged over all sample dates. Averaged $\text{NO}_2\text{-N}$ concentrations were 0.117mg/L at time 0 hr, and 0.047 mg/L at time 24 hr treatment, showing a reduction of 60 % over the full 24 hr treatment periods.

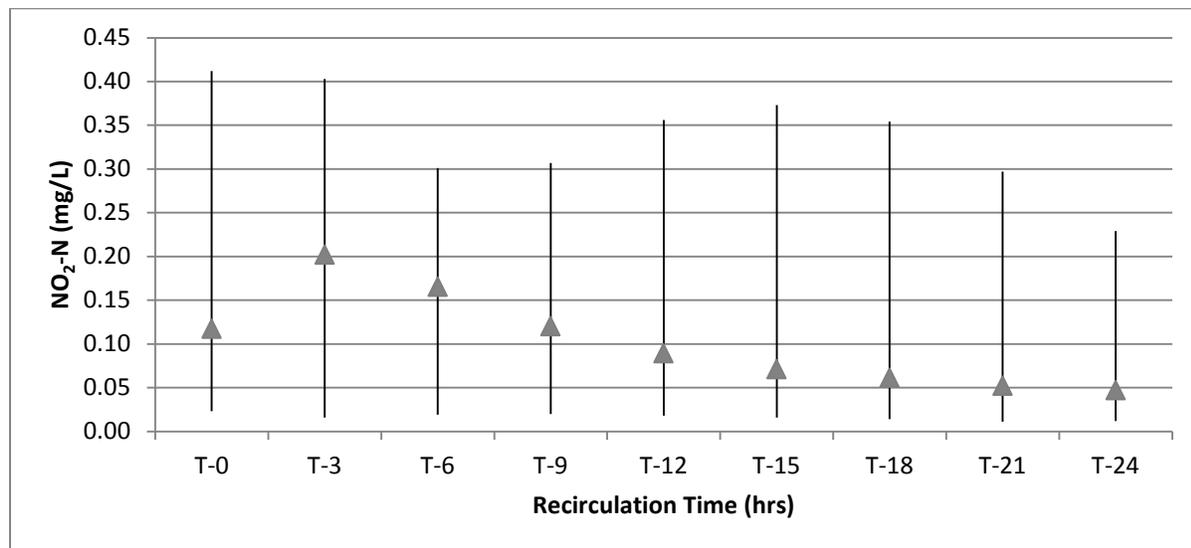


Figure 8. Averaged $\text{NO}_2\text{-N}$ concentrations in water samples collected over 24 hr batch treatments. The triangle marker is the averaged $\text{NO}_2\text{-N}$ value for all T-(x) samples over the course of the study. Error bars represent the maximum and minimum $\text{NO}_2\text{-N}$ values that were tested.

These initial results address my first hypothesis: i) cBOD_5 , TSS, ammonia, nitrate, and nitrite will not be reduced (or maintained) to levels required for aquaculture recirculation (cBOD_5 - < 10 mg/L; TSS- < 80 mg/L; Ammonia ($\text{NH}_3\text{-N}$) - < 0.05 mg/L; Nitrate ($\text{NO}_3\text{-N}$) - < 10 mg/L; Nitrite ($\text{NO}_2\text{-N}$) - < 1.0 mg/L (Molleda, 2007) in medium strength hatchery wastewater within a 24 h period. All results reject this hypothesis as all wastewater parameter levels were reduced (or maintained) to the indicated levels over the 24 hr treatment period.

The second hypothesis: ii) The ambient temperature of the source wastewater and of the technology membrane (5-20°C) does not significantly influence wastewater parameter reduction

rates achieved by the bioreactor technology, was not rejected. Figures 9 - 13 illustrate the temperature of the source influent wastewater over the research period time from September 16 to October 30, 2014. Also on this graph are the reduction rates per interval time period (i.e.: every 3 hours), for the wastewater parameters cBOD₅, TSS, NH₃-N, NO₂-N, NO₃-N. There does not seem to be a clear effect of a decreasing temperature on the reduction rate for all of the wastewater parameters according to these results, and some rates of reduction actually increase with a shown decrease in temperature.

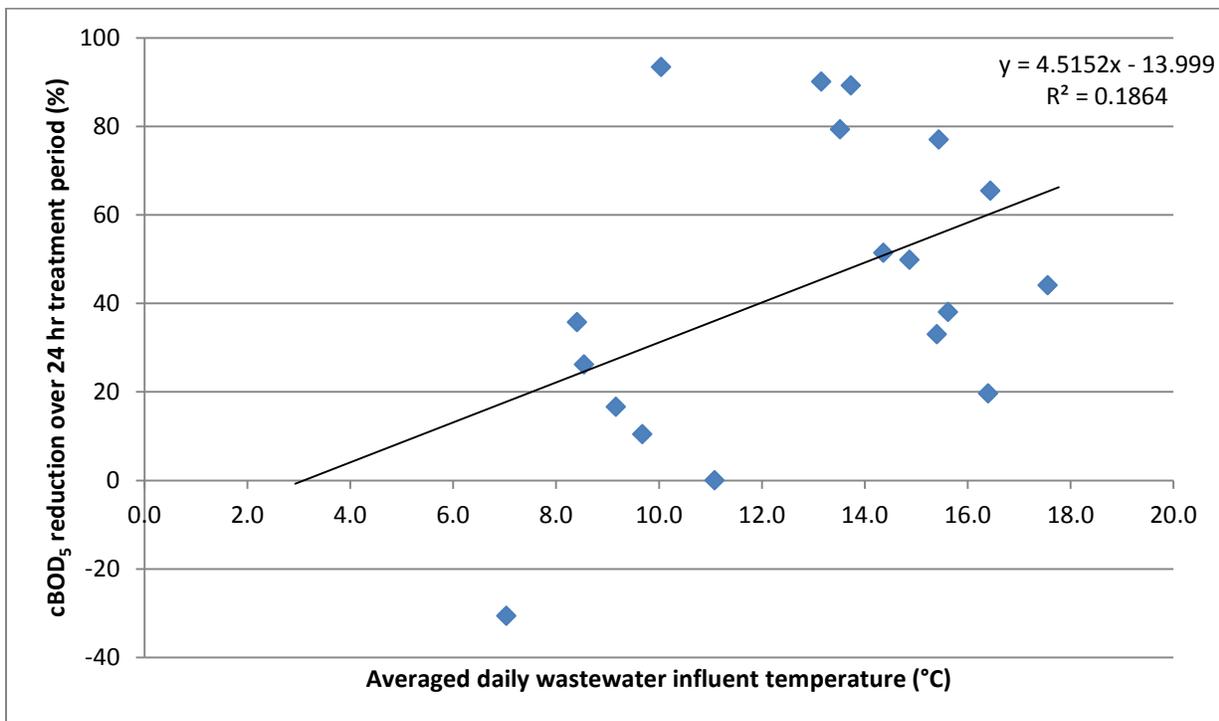


Figure 9. Relationship between percent daily cBOD₅ reduction and wastewater influent temperature. The data has a correlation value of 0.43 indicating a low positive correlation.

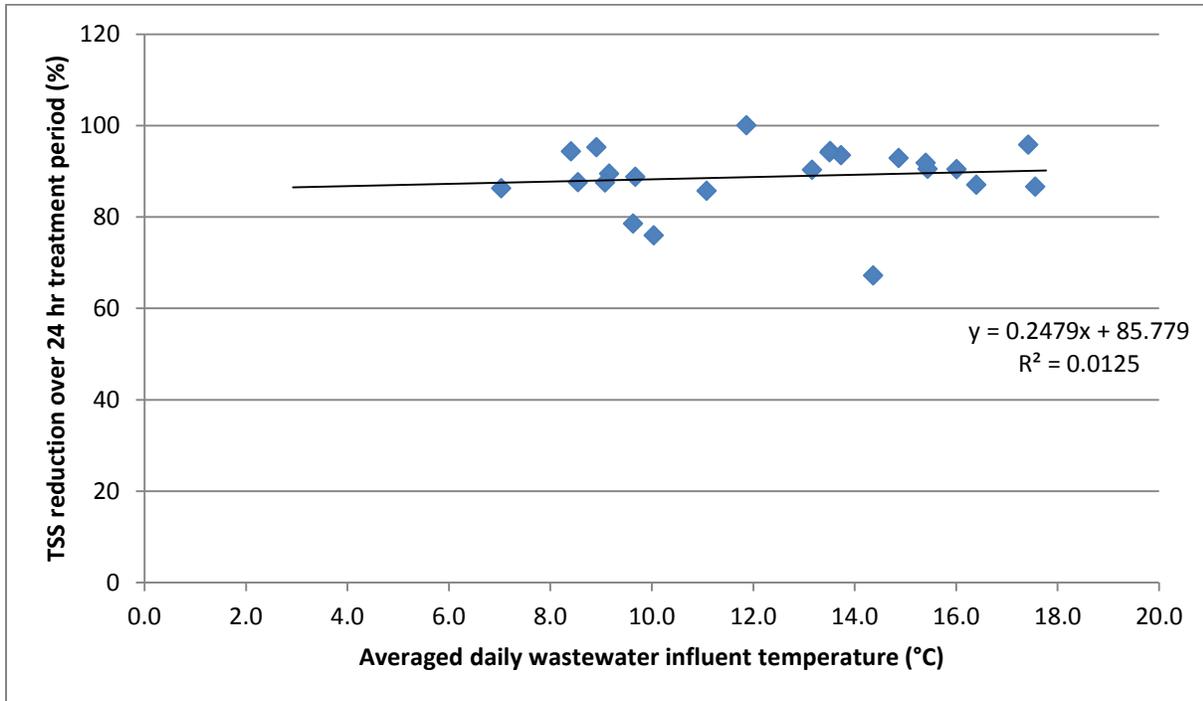


Figure 10. Relationship between TSS reduction and wastewater influent temperature. The data has a correlation value of 0.11 indicating no correlation.

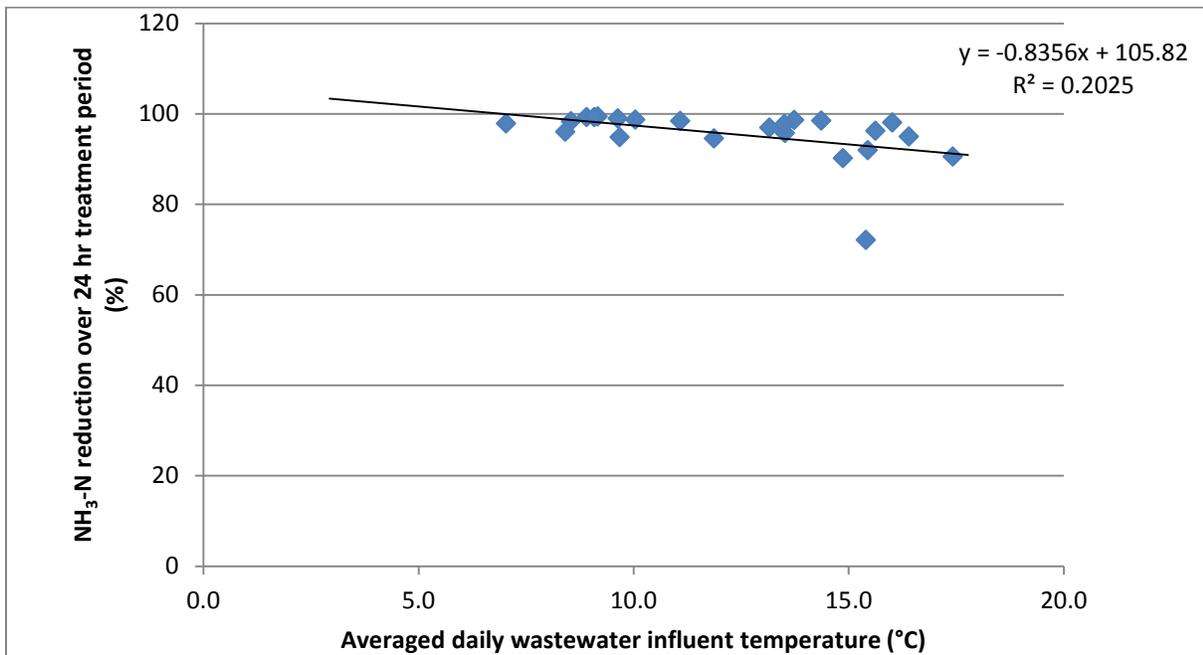


Figure 11. Relationship between NH₃-N reduction and wastewater influent temperature. The data has a correlation value of -0.45 indicating a low negative correlation.

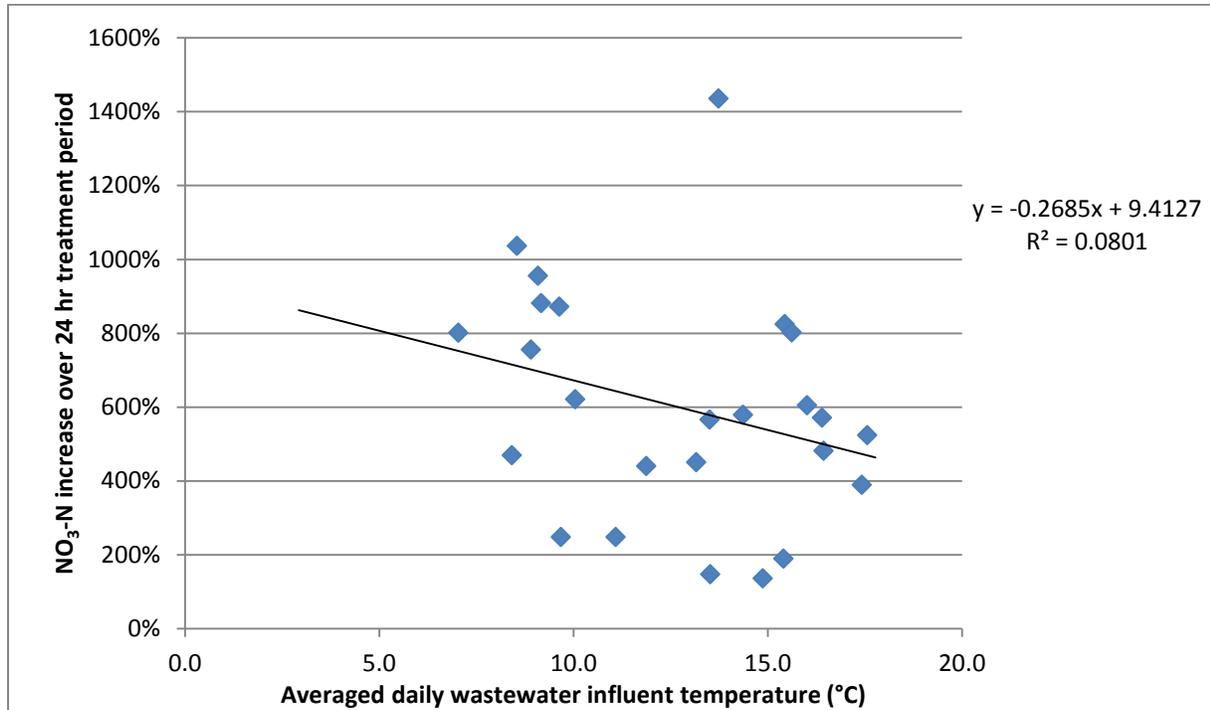


Figure 12. Relationship between NO₃-N increase and wastewater influent temperature. The data has a correlation value of -0.28 indicating a very low negative correlation.

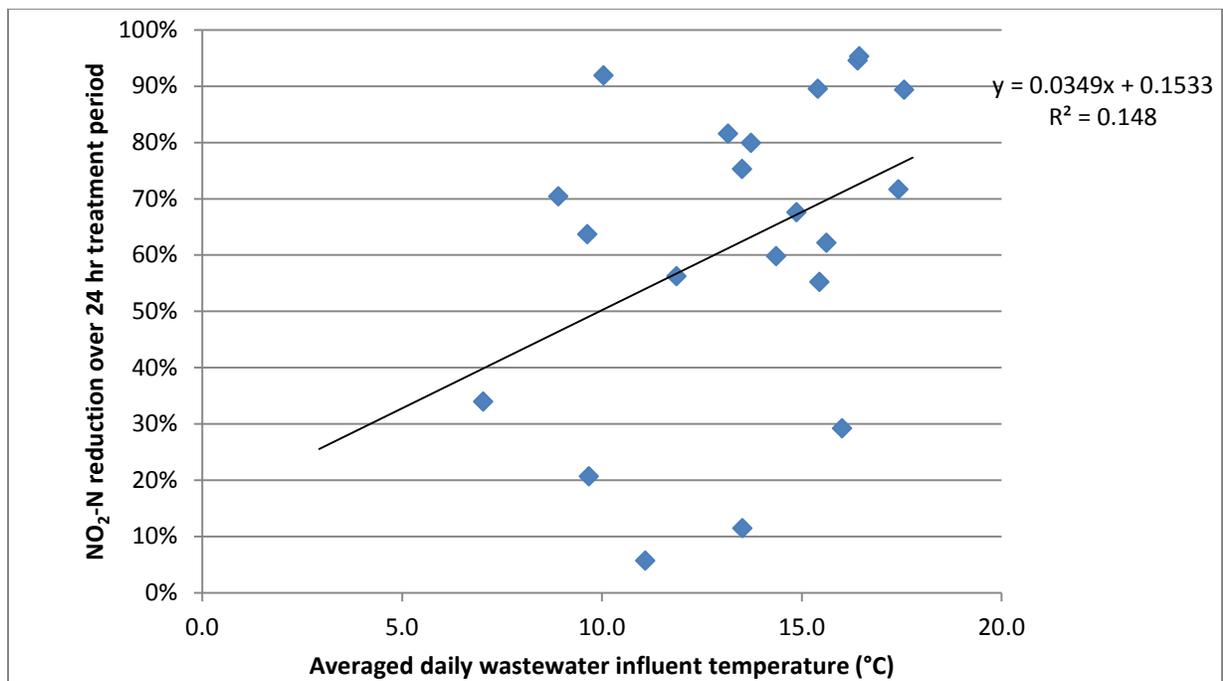


Figure 13. Relationship between NO₂-N reduction and wastewater influent temperature. The data has a correlation value of 0.39 indicating a low positive correlation.

The third hypothesis: iii) cBOD_5 concentration of influent wastewater does not negatively correlate with influent nutrient reduction rates, is illustrated in Figure 14, 15 and 16 with cBOD_5 initial influent concentrations (time 0 hr) plotted against daily $\text{NH}_3\text{-N}$ and $\text{NO}_2\text{-N}$ reduction and $\text{NO}_3\text{-N}$ increases. This shows that the null hypothesis is not disproven since as cBOD_5 influent concentrations increase, there is not a positive correlation with all nutrient reductions rates during this study period.

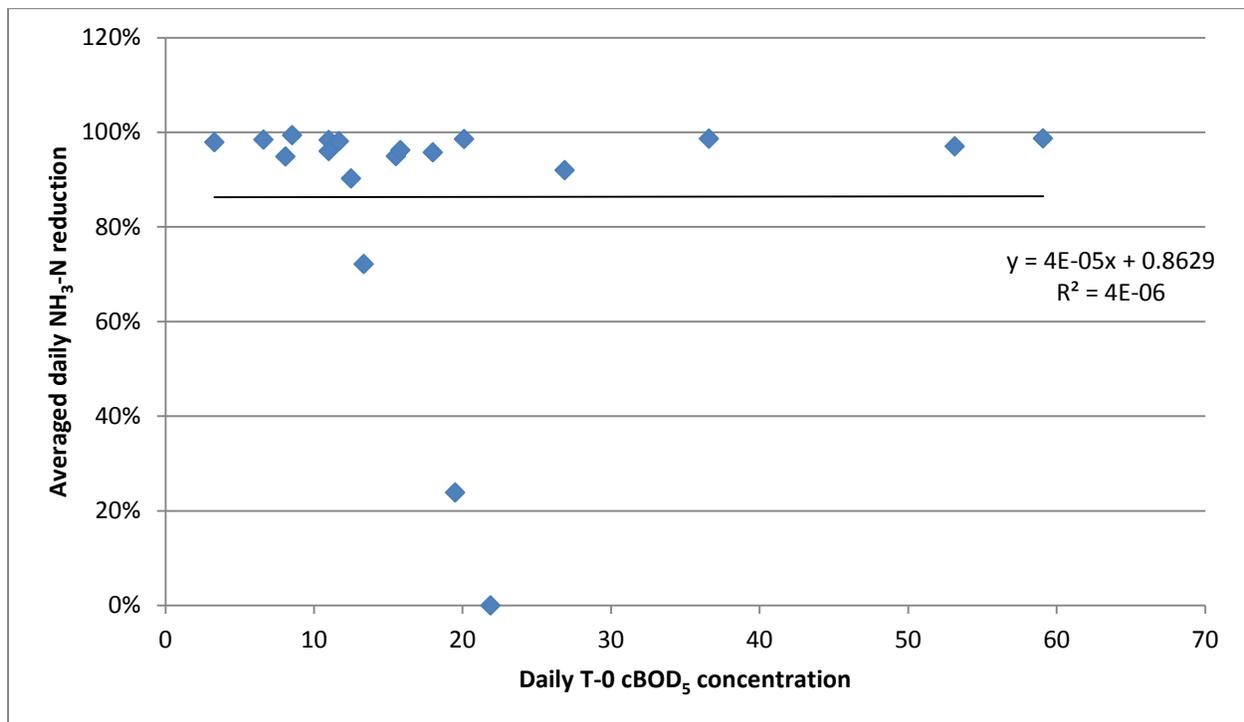


Figure 14. Relationship between T-0 cBOD_5 influent concentrations on daily $\text{NH}_3\text{-N}$ reduction rates per 24 hr batch treatment. The data has a correlation value of 0.001 indicating no correlation.

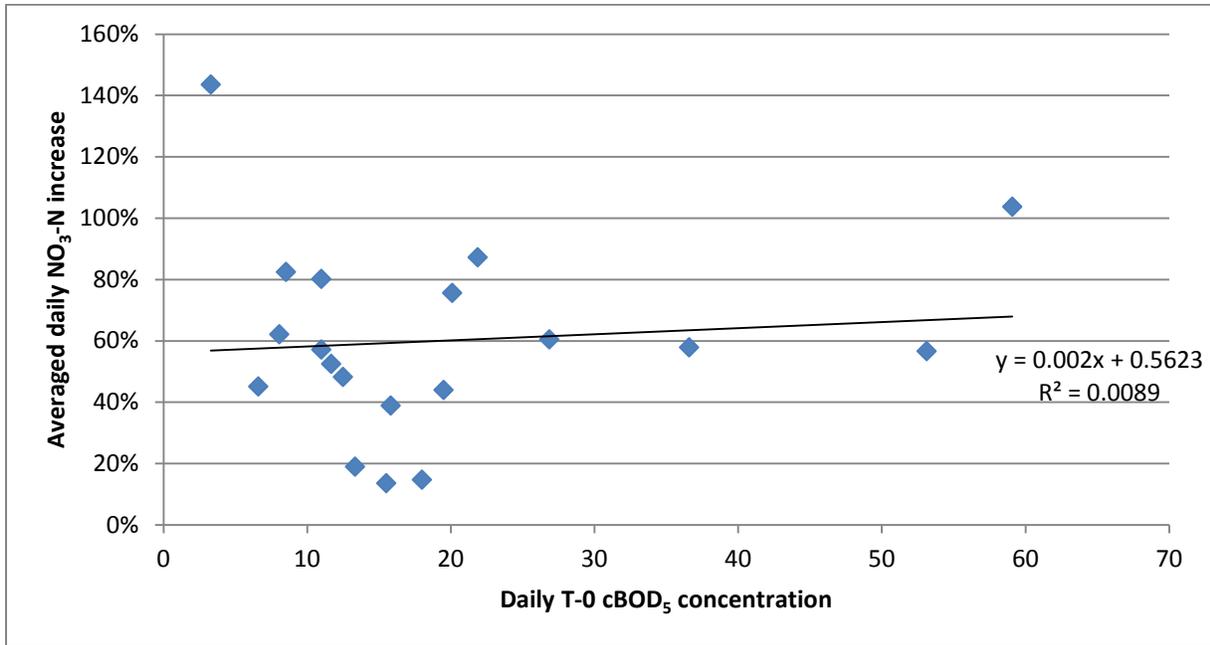


Figure 15. Effect of T-0 cBOD₅ influent concentrations on daily NO₃-N increase rates per 24 hr batch treatment. The data has a correlation value of 0.09 indicating no correlation.

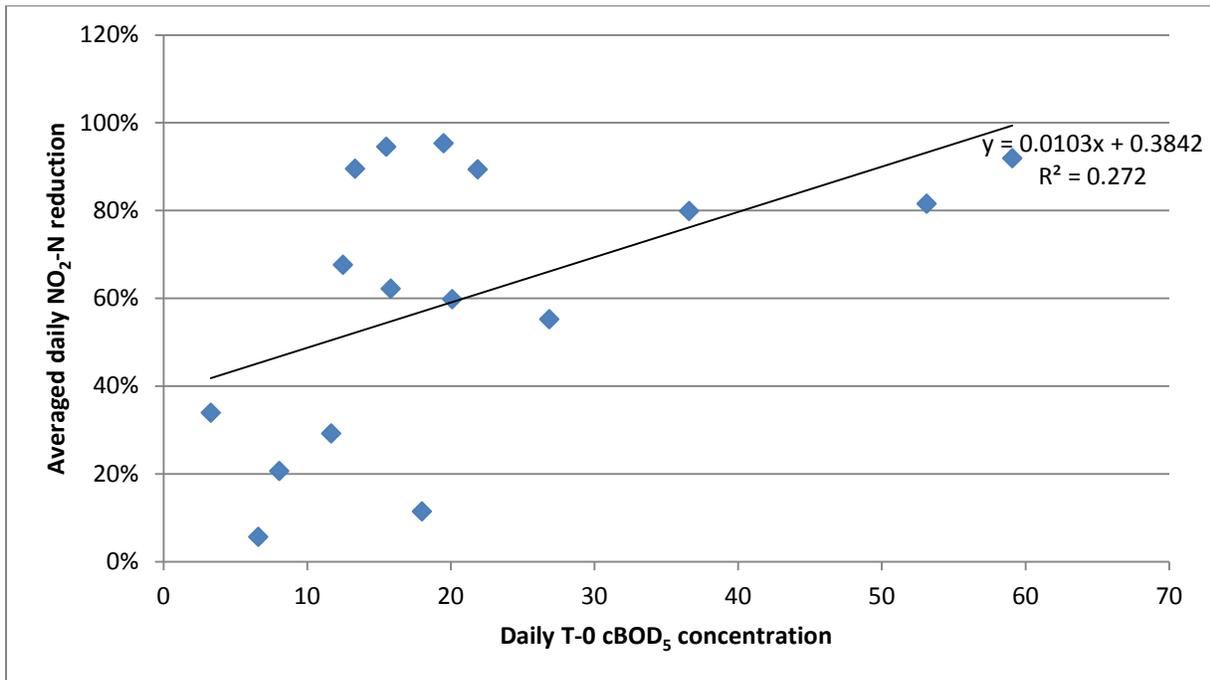


Figure 16. Effect of T-0 cBOD₅ influent concentrations on daily NO₂-N increase rates per 24 hr batch treatment. The data has a correlation value of 0.52 indicating a low positive correlation.

The fourth null hypothesis: iv) Batch treatment time (1-24 hrs) of influent wastewater recirculated over the membrane of the system does not influence nutrient reduction rates per hour has been rejected. In most the nutrient graphs, it does show a trend that reduction rates decrease as the recirculation time increases. Figures 17- 19 shows the reduction rate decline of cBOD_5 , TSS, and $\text{NH}_3\text{-N}$, over the 24 hr treatment time. Figure 20 shows the rate of increase in $\text{NO}_3\text{-N}$ concentrations over the 24 hr period. This is the expected result due to nitrification of ammonia. It does follow a similar trend as Figures 17-19 in that the concentration increase peaks around 6 hr of recirculation time and then levels off to not having much of a change. Figure 21 shows the rate of reduction for $\text{NO}_2\text{-N}$ concentrations where there appears to be an increase in $\text{NO}_2\text{-N}$ concentration at T-3 hrs (represented as -73% $\text{NO}_2\text{-N}$ reduction rate). This could be illustrating the intermediate step in nitrification where ammonia is first converted to NO_2 prior to conversion to NO_3 .

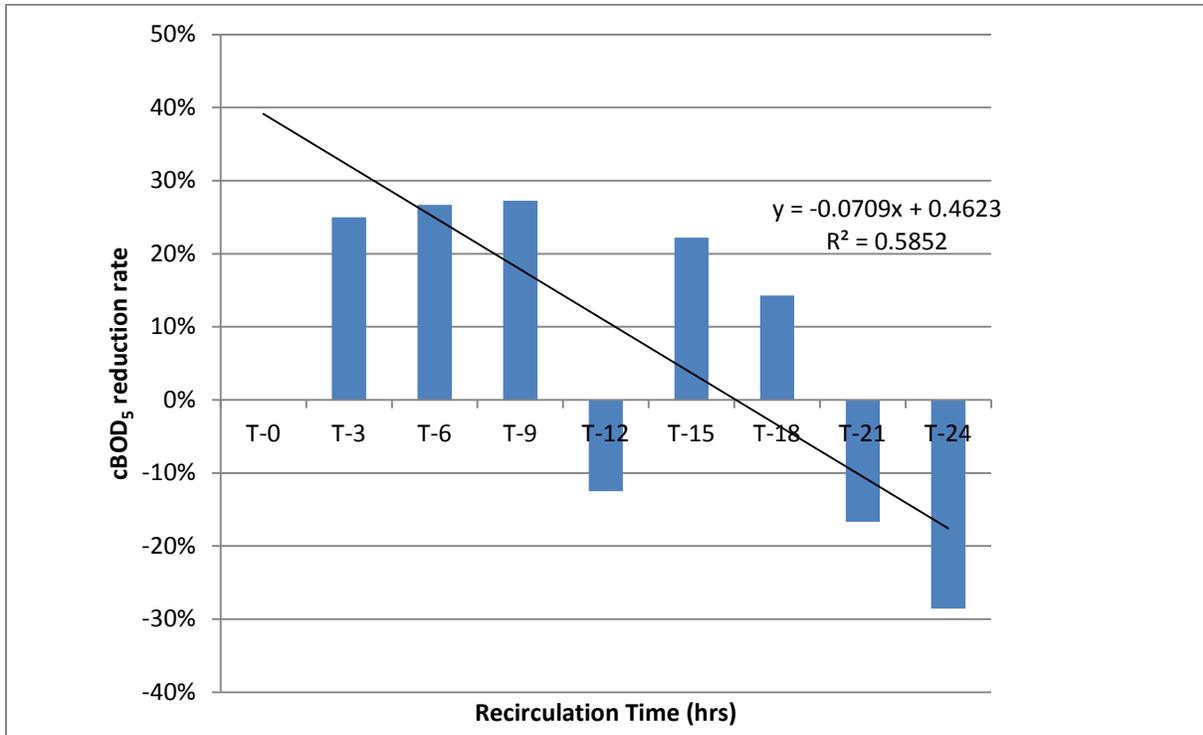


Figure 17. Averaged percent cBOD₅ concentration reductions during the sample interval times during 24 hr batch treatments.

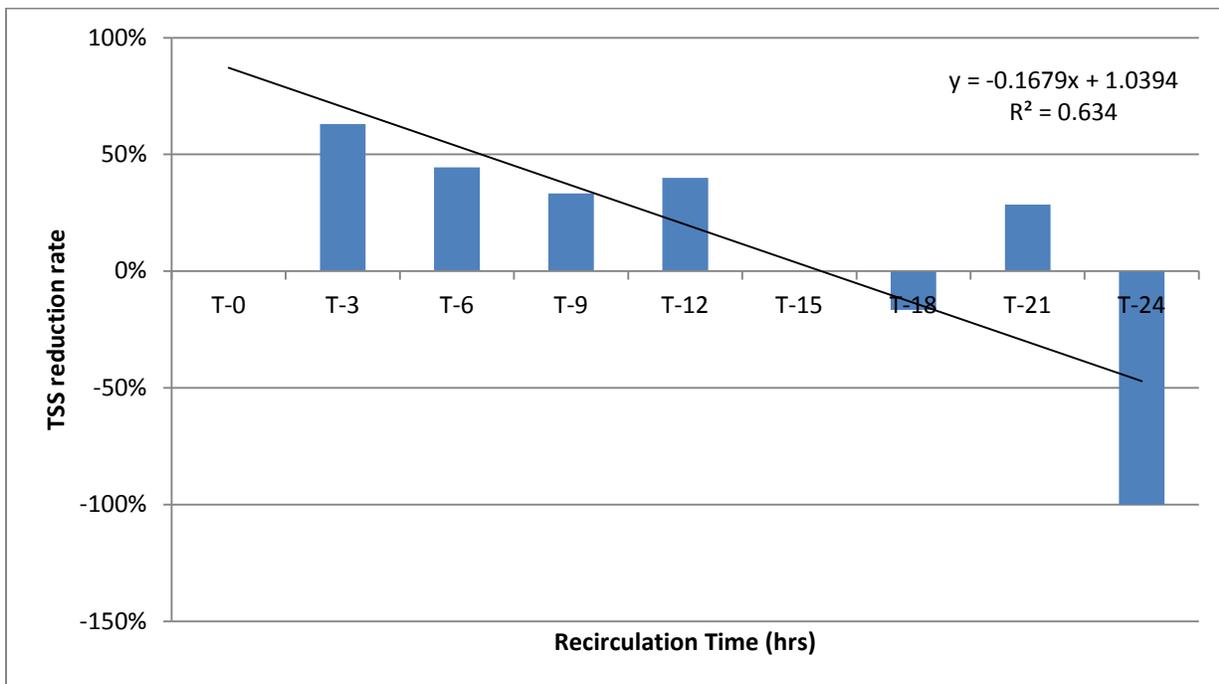


Figure 18. Averaged percent TSS concentration reductions during the sample interval times during 24 hr batch treatments.

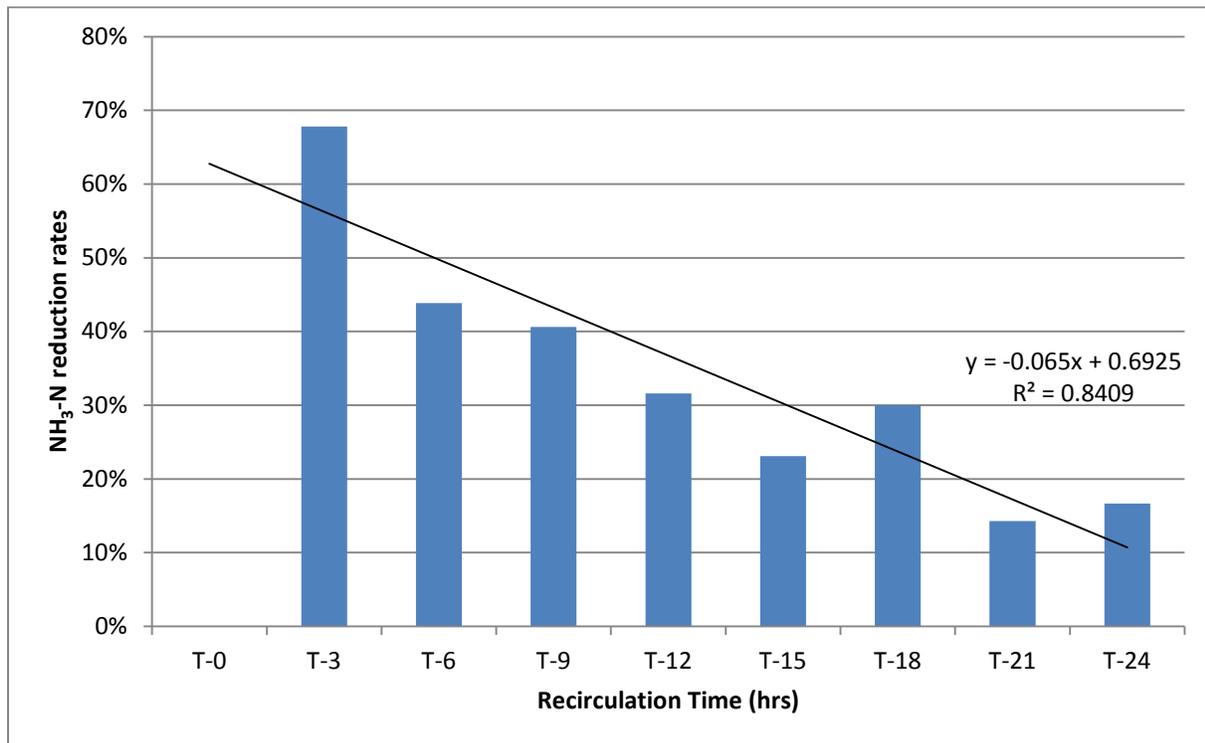


Figure 19. Averaged percent NH₃-N concentration reductions during the sample interval times during 24 hr batch treatments.

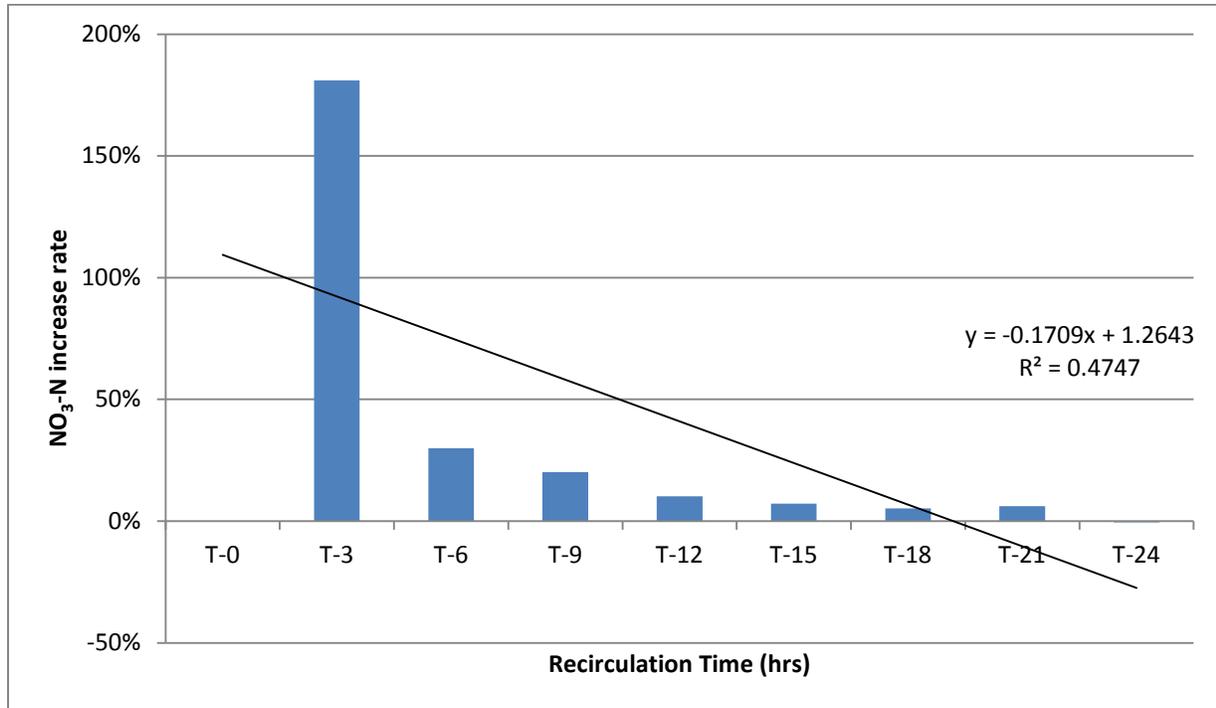


Figure 20. Averaged percent NO₃-N concentration increase rates during the sample interval times during 24 hr batch treatments. The NO₃-N concentration increases substantially during the first 3 hrs of treatment, where all other treatment times have only a minimal increase in NO₃-N concentration.

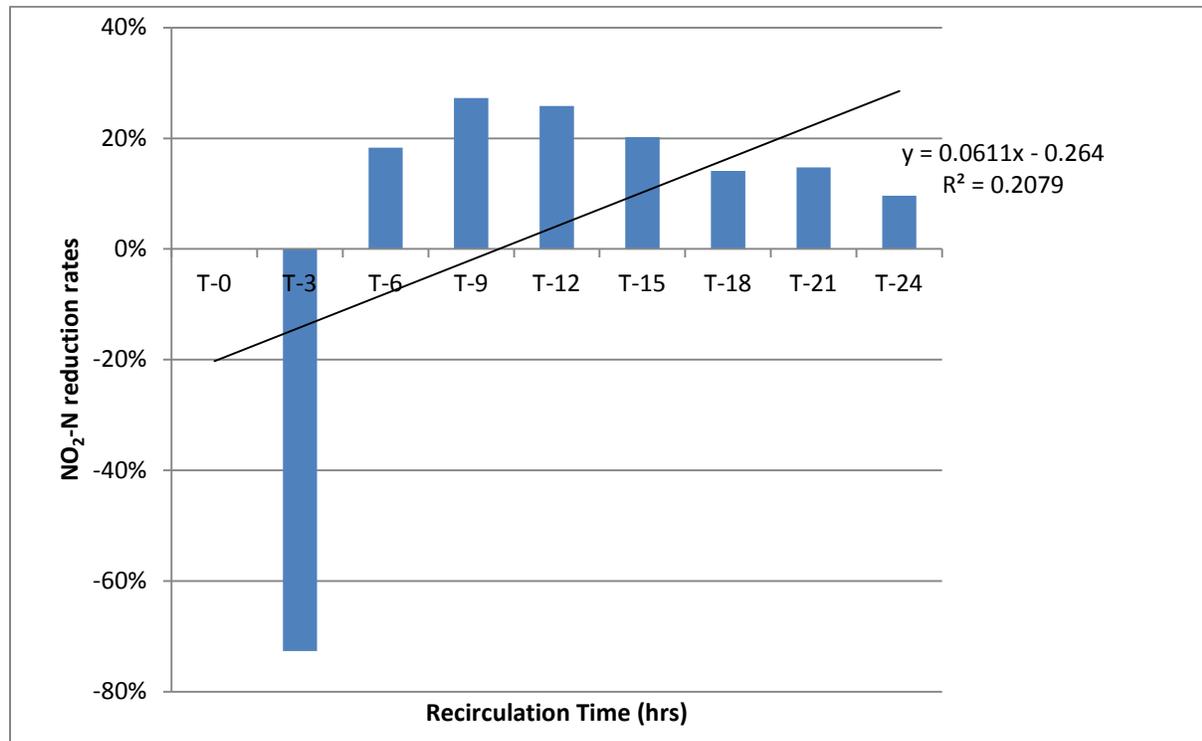


Figure 21. Averaged percent NO₂-N concentration reductions during the sample interval times during 24 hr batch treatments.

Discussion

These data were collected to answer the research questions and objectives set out above. The results provided a good illustration of the bioreactor technology's capabilities under a typical Southern Ontario aquaculture facility.

Overall this study's data show that under a 24 hr batch treatment of wastewater effluent from a rainbow trout aquaculture facility (Coldwater Fisheries Ltd), the bioreactor can effectively reduce (or maintain) wastewater parameters to levels acceptable for recirculation of hatchery waters for that species. The one exception to this is nitrate which actually increased during the 24 hr treatment period. This is expected though because nitrification occurs on the biofilm growth on the bioreactor membrane in the system. This biofilm is comprised of bacteria including autotrophic nitrifiers (Saidu, 2009). Since the design of the bioreactor is an

unsubmerged, trickling biofilter, the environment has plenty of oxygen to facilitate the nitrification process (Biogill®, 2014). In aquaculture effluents, total ammonia can be quite high predominantly due to fish excretion, and it is very toxic to fish. The nitrification process converts ammonia nitrogen to nitrite and ultimately nitrate, a less toxic nitrogen compound (Saidu, 2009). As shown by the above data, there is a reduction of ammonia and nitrite during the treatment batches, with an overall increase of nitrate, showing a fairly effective nitrification process. However, even with the increase in nitrate concentration over time, the values were still below that required for a RAS. For a complete removal of nitrogen, the technology would have required an anaerobic biomass to facilitate growth of heterotrophic facultative anaerobic bacteria to convert nitrate to elemental nitrogen which would be released as a gas (Saidu, 2009). Von Sperling (2007) states that there is a layer of anaerobic biofilm that can exist between the layers of biofilm growth; however this was not illustrated in the results of this study. Nitrification can also be inhibited especially in wastewaters with high biodegradable organic matter content (Saidu, 2009). The presence of this organic matter can cause the rapid growth of heterotrophic bacteria on the biofilm which can out compete autotrophic bacteria for oxygen and space (Saidu, 2009). Since the cBOD₅ levels in this study were not high, there appears not to have been this bacterial competition. Overall, this is an indication of requiring a good understanding of wastewater compositions in order to maximize the effectiveness of the unsubmerged bioreactor technology.

The graphs above do not show a clear correlation between temperature and rate of reduction of wastewater parameter concentrations. While this study only took place over September and October, it is hard to determine from this study's results if the lower temperatures during the study's duration would have reduced the rate of reduction. Research such as Saidu's

dissertation paper (2009) found that the optimal ammonia removal from wastewaters occurred at 30°C, indicating that this may be an ideal growth temperature for nitrifying bacteria. The removal rate was significantly higher than those at 13° and 20° C (Saidu, 2009). With a biological based system such as the bioreactor, this factor could cause issues with effective treatment capability in locations with a fluctuation of temperatures, such as an outdoor location or a facility for cold-water fish species.

cBOD₅ influences on wastewater parameter reduction rates would be expected to be apparent due to the growth of the biofilm. Biofilm growth will only be substantial given a fairly nutrient rich influent, therefore it would be expected that low cBOD₅ wastewaters would not grow sufficient biofilm, which could result in lower reduction rates of wastewater parameters. However, as this study used real aquaculture waste, the cBOD₅ values of the influent waters varied throughout the course of the study. If the waters were more nutrient rich at the beginning, the biofilm could grow sufficiently and if days of lower cBOD₅ influent concentrations were apparent, the biofilm would not automatically decrease due to that one day. Therefore it would have some robustness in order to handle low or high concentration waste days. In that same line of thinking, too many low concentration days would result in a lesser biofilm and less removal capacity, and too many high loading days could result in an overgrowth of the biofilm, potentially suffocating the microbial population and killing off the biofilm all together, resulting in no treatment capacity. Overall, this unsubmerged bioreactor would have an optimal value range of wastewater strength required to effectively grow and maintain the system coupled with temperature and HRT. This again illustrates the important need to understand fully the operations of the facility to utilize the treatment. The data above illustrate a clear relationship but the range

of cBOD₅ concentrations, from 0 to 60 mg/L all are considered on the low side for what the unsubmerged bioreactor is designed to handle so it would be difficult for any trends to stand out.

The above graphs also illustrate that the 24 hrs of treatment is more than adequate for reduction of wastewater parameters. Most wastewater parameters were reduced (or maintained) to satisfactory levels by the 9th hour of treatment. After the 9 hrs the treatment was minimal. It should be noted that some nutrient concentrations were already below target concentrations at time 0 hrs of treatment. This would be due to the large volumes of water being used in the facility which would dilute wastewater concentrations and also the fact that a drum filter in the facility is utilized for all wastewater prior to the waters leaving the facility. This physical filtration removes a great deal of the solids which would otherwise increase nutrient levels.

This information could be used for further investigation into best practices for operation when the unsubmerged bioreactor system is in use. Again each site would be unique in terms of influent quality and effluent goals required, so each site would need to have its own evaluations. Our study was supplied with an available Biogill® technology unit as the unsubmerged bioreactor that was likely oversized for the nutrient concentrations of the influent wastewater. Typically the bioreactor would be sized according to BOD₅ concentrations and these wastewaters were quite low in cBOD₅ with average values of 20 mg/L in the influent. Therefore the time deciphered here for most efficient in terms of nutrient removal, can only be used as an illustration for this specific wastewater stream.

Technology Comparisons

Overall the bioreactor technology does show some promising results, however it is dependent on the fish species being reared, and temperatures of waters and the ambient environment. This study used relatively low concentrations of wastewater tested over a short

period of time. It wasn't long enough to determine if operational issues such as fungal growth or biomass dieback would be a problem for this technology, but it did give an indication that the technology could possibly be beneficial to the aquaculture industry. It would be expected however, that the bioreactor technology could not grow an adequate biomass if situated outdoors in Ontario during the fall and winter months with below freezing temperatures and therefore could not have an effective treatment of wastewaters. With it also being a biological treatment, there is concern that any chemical treatments could impact the biofilm, which could have the potential to cause it to degrade or die off and affect treatment capacity. While this specific technology would require further site specific testing, some technologies have been tested and shown to be appropriate for RAS.

The overall goal of a RAS is to have high production and be environmentally sustainable; therefore it needs a carefully designed and carefully managed waste management strategy (van Rijn, 2013). This includes developing an efficient feed utilization strategy to create low waste production and the proper incorporation of appropriate treatment technologies into the recirculation loop of the facility (van Rijn, 2013). Appropriate treatments should be chosen in order to re-use as much of the water as possible, which results in a concentrated discharge waste with the ability to consider re-use options for this waste (such as being used as a fertilizer or as compost) (Martins et al., 2010; van Rijn, 2013). Treatment technologies that can be applied to RAS are typically broken down into these categories:

1. Physical filtration such as drum filters and membranes
2. Biological systems including trickle filters and membrane bioreactors
3. Chemical addition for disinfection

The most effective RAS wastewater treatment system would actually be a combination of all the categories above, allowing the system to re-use most of its waters in recirculation.

Facilities with a RAS will have some means of a mechanical treatment for solids removal, such as a drum filter, as the removal of these solids greatly reduces the nutrient content of the effluent waters, making it easier to treat. Most land-based flow-through aquaculture facilities would also, at a very minimum, have a drum filter to remove these solids to allow for discharge of wastewaters to the environment under an ECA. The facility would then require some means of disposing the solids wastes as well, where options of reuse as fertilizer could come into play under appropriate circumstances (van Rijn, 2013).

Trickle filters and membrane bioreactors work on the same biological principles as the unsubmerged bioreactor technology treatment. The technology involves the creation of a microbial population in an aerobic environment that encourages the nitrification of ammonia into nitrite and ultimately nitrate, which is much less toxic to fish (UNEP, 2004). Trickle biofilters are much like the unsubmerged bioreactor as it is comprised of a fixed bed of media in a tank or housing, in which the wastewater, pre-treated with some initial solids removal, is sprayed or trickled over the media to create a biofilm layer in which the wastewater would be treated. They are predominantly used for BOD₅ removal, but will have some nitrification depending on the organic loading, temperature, and oxygen available (UNEP, 2004). Membrane bioreactors are somewhat of a two-stage process in where the membranes, either microfiltration or ultrafiltration, separate the activated sludge from the effluent (Melin et al., 2006). The bioreactor utilizes the high sludge content for efficient treatment of wastewaters, much in the way as a conventional activated sludge process at a municipal wastewater treatment plant (Melin et al., 2006). The technology has been demonstrated to be highly effective in municipal treatment and

is considered a key element of advanced wastewater reclamation and reuse, making it a fit for RAS treatment systems (Melin et al., 2006), although it does come with a high initial capital cost along with required expertise to operate it.

Chemical additions, such as formalin are sometimes added as a final disinfection treatment step for pathogens, fungus or parasites within the RAS (Moller, Arvin, & Pedersen, 2010). While the additions of chemical treatments are widely used, in the case of formalin, there is some concern that excess formaldehyde could be harmful to the receiving water bodies (Moller et al., 2010). Any additions of chemicals into the system would require in depth testing of the treated waters. It would be extremely detrimental to fish health and the aquaculture business if a RAS were to spread pathogens throughout the facility, so this aspect would be closely monitored.

In addition to the above treatments, RAS engineers see more room for improvement. While the above technologies can reduce water and wastewater parameters, further biological treatments and forward thinking waste management strategies can make RAS meet and exceed environmental requirements (van Rijn, 2013). Waste management can be viewed in two different ways: capture of waste and the conversion of NH_3 or; waste reduction with denitrification and sludge removal (van Rijn, 2013). Some advanced treatment options that can be considered for RAS are denitrification reactors, sludge thickening technologies, and ozone and UV irradiation treatments (Martins et al., 2010).

Nitrifying reactors such as trickle filters and membrane bioreactors create concentrations of nitrate (NO_3) that will determine the requirement for external water exchange rates (Martins et al., 2010). If too high, additional water will need to be introduced into the RAS in order to dilute the concentration, therefore reducing the reusability of the RAS waters. In order to keep a RAS

system as “closed” as possible, a denitrification reactor can be added to the treatment train in the RAS. Martins et al., (2010) describes one denitrification reactor design called an up flow sludge blanket denitrification reactor (USF-denitrification reactor). This design consists of a cylindrical anoxic reactor tank, fed with the waste captured by the solids removal technology at the start of the RAS treatment. The waste enters through the bottom of the tank at a velocity slower than the settling ability of the solids in order to create a sludge bed on the bottom of the tank where denitrifying bacteria can digest the solid waste (carbon source) and reduce nitrate to nitrogen gas. The final treated waters exit through the top of the tank. Having this system included in a RAS can reduce the amount of external water required to create the exchange water for the RAS by reducing the nitrate concentrations. The denitrification process requires a carbon source to feed the bacteria and at times an external source such as methanol is used. But by using the solids removed at the start of treatment, the RAS will also be reducing a waste product that would otherwise need to be disposed of. Economically the cost of installing and running a denitrification unit is superior to the costs associated with increased water supply need, dumping fee of solid wastes, and heating costs (Martins et al., 2010). However with all these advantages there is still limited utilization due to higher initial investments and the requirement of a skilled and experienced personnel to operate the systems.

Sludge thickening technologies are also being investigated as a potential new development for RAS. This technology includes the use of belt filter systems or geotextile bags or tubes (Martins et al., 2010). These systems account for dewatering of the RAS accumulated sludge so that there can be a lower overall total volume of sludge; this can lead to a decrease in cost of storage, transportation, labour and disposal of the sludge discharge (Martins et al., 2010). With the addition of alum or other flocculants, the facility can potentially achieve high removal

rates of TSS, PO₄, TP, BOD and COD, while N removal rates can be moderate (Martins et al., 2010).

Ozone and ultraviolet irradiation are being used as a final stage treatment method to disinfect recirculation waters by controlling pathogens and oxidizing NO₂, NO₃, organic matter, TAN, and fine suspended particles (Martins et al., 2010). While the combined technology has shown promise in studies demonstrating nearly complete inactivation of bacteria in freshwater RAS, ozonation by-products can be produced and can potentially be harmful (Martins et al., 2010).

Other technologies that have been investigated are being viewed as components that would add to an integrated treatment system since, to date, one technology has not shown to be a complete system for treatment. These technologies would include: wetlands, algae controlled systems, and micro-algae based water treatment (Martins et al., 2010).

There are also some downsides to incorporating a highly effective treatment technology into a RAS facility that is so efficient that it requires only minimal uptake of new water due to the recirculation water being treated so effectively. When there is a small exchange rate of new water into a facility, there could be an accumulation of growth inhibiting factors such as cortisol from fish, metabolites from waste, and metals from feed (Martins et al., 2010). Depending on the type of facility, this could have detrimental effects on embryonic and larval development (Martins et al., 2010). Overall in the European Union, the economic feasibility of establishing a denitrification system still has not been shown. The higher initial investments, required ongoing technology expertise, and accumulation of TDS have so far outweighed the benefits of such a technology (Martins et al., 2010).

In their 2010 paper, Martins et al., stated that the major areas of future research for RAS are in efficiency of waste removal for solids, nitrogen and phosphorus, as well as research into more technologies that reduce the water content in backwash water. They identified these areas of research as bring a priority to establish the ecological sustainability of RAS.

Economic reality

Overall, wastewater treatment technologies for RAS need substantial investigation into its appropriate usage for each individual site before it can be determined to be both economically and ecologically sustainable. The bioreactor technology is no exception. The data shows that the technology can effectively remove some contaminants for a freshwater RAS facility; however it is not a complete treatment technology to allow for a successful RAS independently. Based on what I have learned from this study, I believe an integrated approach to water treatment at a RAS using the bioreactor and perhaps some early solids removal and end of line disinfection, could prove to be an all-encompassing treatment setup for a freshwater RAS. The bioreactor does have the benefit of having simple set up and operation, relying mostly on the biological processes of the biofilm to be effective. This could result in a facility not requiring specialized operators to ensure its day to day operations. It is also fairly low cost and can be scalable to the size of the operation. However, economically this may not to be feasible in Ontario unless stricter regulations are put on the land-based aquaculture industry to encourage the adoption of more advanced environmentally conscious treatment systems to encourage RAS. Currently many of the aquaculture facilities in Ontario are located outside of populated areas on abundant land and near large water supplies, which in Canada are not currently seen as scarce (Moccia & Bevan, 2005). While there are some waste effluent parameters to meet, they typically are reachable with just a drum filter removing solids and it doesn't necessarily take into account mass loading of the

low levels of nutrients; again because of the availability of water supplies to dilute the effluent. Until there are stricter regulations on water usage and effluent disposal, aquaculture facilities will likely have little incentive to proactively improve facilities unless there was a driving business case to do so (Moccia & Bevan, 2005).

Conclusions and Recommendations

Generally speaking, the unsubmerged bioreactor did prove to be relatively effective for wastewater parameter removal from the Coldwater Fisheries study site. The information collected from this study could be used to help improve operation guidelines for the bioreactor installations since there does appear to be a shorter recirculation time required for effective treatment. This information will be relative to the specific site, but is a good indicator that a more efficient option is likely available. The data collected should help the suppliers of the unsubmerged bioreactor technology such as the Biogill® company gain a better understanding of the operation and constraints of the technology and could help the company in determining what wastewater markets should be pursued and which ones may not be the best suited for the technology. From the point of view of aquaculture facilities, this information could be used to understand the best use of low-tech wastewater treatment options that are available and how best to efficiently and economically include them in a RAS. In terms of environmental management, having a low cost treatment solution could entice aquaculture facilities to adopt the technology early and over other technologies that have a high initial investment and require experienced staff to operate it.

References

- Badiola, M., Mendiola, D., & Bostock, J. (2012). Recirculating Aquaculture Systems (RAS) analysis: main issues on management and future challenges. *Aquacultural Engineering*, 51, 26-35.
- Biofilm. (n.d.). In *Merriam-Webster Online*. Retrieved June 29, 2015, from <https://www.merriam-webster.com/dictionary/biofilm>
- Biogill®, (2014). *Technical and System Specification Guide*. Retrieved from: <http://www.Biogill.com/downloads>
- Camargo, J.A. (1994). The importance of biological monitoring for the ecological risk assessment of freshwater pollution: a case study. *Environment International*, 20(2), 229-238.
- Chang, C-Y., & Cajucom, S. S. (2011). Feasibility study of fish farm effluent treatment by sequencing batch membrane bioreactor. *Journal of Water Sustainability*, 1(1), 103–112.
- Charlton, T. (2013). *Assessment of a BioGill® water treatment unit at Port Stephens Fisheries Institute*. Retrieved from Biogill website: http://www.biogill.com/uploads/74946/ufiles/BioGills_at_PSFI_Hatchery_-_AFS_Report_24_June_2013_FIN.pdf
- Davidson, J., Helwig, N., & Summerfelt, S.T. (2008). Fluidized sand biofilters used to remove ammonia, biochemical oxygen demand, coliform bacteria, and suspended solids from an intensive aquaculture effluent. *Aquacultural Engineering*, 39, 6- 15.

Eutrophication. (n.d.). In *Merriam-Webster Online*. Retrieved June 23, 2015, from

<https://www.merriam-webster.com/dictionary/eutrophication>

Food and Agricultural Organization of the United Nations, FAO Fisheries and Aquaculture

Department. (2012). *The State of World Fisheries and Aquaculture 2012*. Retrieved from the Food and Agricultural Organization of the United Nations website:

<http://www.fao.org/>

Food and Agricultural Organization of the United Nations, FAO Fisheries and Aquaculture

Department. (2014). *The State of World Fisheries and Aquaculture 2014*. Retrieved from the Food and Agricultural Organization of the United Nations website:

<http://www.fao.org/>

Guerdat, T.C., Losordo, T.M., Classen, J.J., Osborne, J.A., & DeLong, D. (2011). Evaluating the effects of organic carbon on biological filtration performance in a large scale recirculating aquaculture system. *Aquacultural Engineering*, 44, 10-18.

Health and Ecological Effects.(n.d.). In *Nutrient Policy and Data, United States Environmental Protection Agency*. Retrieved December 1, 2016, from <https://www.epa.gov/nutrient-policy-data/health-and-ecological-effects>

Le-Clech, P., Chen, V., & Fane, A.G. (2006). Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science*, 284, 17-53.

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., Roque d'Orbcastel, E., & Verreth, J.A.J. (2010). New developments in recirculating

aquaculture systems in Europe: A perspective on environmental sustainability.

Aquacultural Engineering, 43, 83- 93.

Masser, M.P., Rakocy, J., & Losordo, T.M. (1999). *Recirculating Aquaculture Tank Production Systems: Management of Recirculating Systems* (SRAC Report 452). Retrieved from University of California, Davis, California Aquaculture website
<http://aqua.ucdavis.edu/DatabaseRoot/pdf/452RFS.PDF>

Melin, T., Jefferson, B., Bixio, D., Thoeye, C., De Wilde, W., De Koning, J., Van der Graaf, J., & Wintgens, T. (2006). Membrane bioreactor technology for wastewater treatment and reuse. *Desalination*, 187, 271 – 282.

Moccia, R.D., & Bevan, D.J. (2000). *Aquaculture legislation in Ontario*. Retrieved from the University of Guelph Aquaculture website:
<http://www.aps.uoguelph.ca/aquacentre/files/about-ontario-aquaculture/Aquaculture%20Legislation%20in%20Ontario.pdf>

Moccia, R.D., & Bevan, D.J. (2005). *Environmental issues concerning water use and wastewater impacts of land-based aquaculture facilities in Ontario*. Retrieved from the University of Guelph Aquaculture Centre website:
[http://www.aps.uoguelph.ca/aquacentre/files/research-publications/OSAWG%20Report%201%20Land-Based%20Issues%20\(Sep2005\).pdf](http://www.aps.uoguelph.ca/aquacentre/files/research-publications/OSAWG%20Report%201%20Land-Based%20Issues%20(Sep2005).pdf)

Moccia, R.D., Naylor, S., & Reid, G. (1997). *An overview of aquaculture in Ontario*. Retrieved from the University of Guelph Aquaculture website:
<http://www.aps.uoguelph.ca/aquacentre/files/about-ontario-aquaculture/An%20Overview%20of%20Ontario%20Aquaculture.PDF>

- Molleda, M.I. (2007). *Water quality in recirculating aquaculture systems for Arctic charr (Salvelinus alpinus L.) culture*. Retrieved from United Nations University Fisheries Training Programme website:
<http://www.unuftp.is/static/fellows/document/mercedes07prf.pdf>
- Moller, M.S., Arvin, E., & Pedersen, L.F. (2010). Degradation and effect of hydrogen peroxide in small-scale recirculation aquaculture system biofilters. *Aquaculture Research*, 41, 1113-1122.
- Northern Ontario Aquaculture Association (NOAA). (2006). *Best management practices for sustainable aquaculture in Ontario*. Retrieved from NOAA website (no longer available).
- Ontario Animal Research and Services Committee (OARSC). (2005). *Ontario Aquaculture Research and Services Committee: 2005 Strategic Report (For the Period 2005-2009)*. Retrieved from the University of Guelph Aquaculture website:
<http://www.aps.uoguelph.ca/aquacentre/files/research-bulletins/Annual%20Report%20Ontario%20Aquaculture%202005.pdf>
- Pulefou, T., Jegatheesan, V., Steicke, C., & Kim, S-H. (2008). Application of submerged membrane bioreactor for aquaculture effluent reuse. *Desalination*, 221, 534–542.
- Rice, E.W., Baird, R.B., Eaton, A.D., & Clesceri, L.S. (Ed.).(2012). *Standard Methods for the Examination of Water and Wastewater, 22nd Edition*. American Public Health Association, American Water Works Association, Water Environment Federation.
- Saidu, M. MG. (2009). *Temperature impact on nitrification and bacterial growth kinetics in acclimating recirculating aquaculture systems biofilters* (Doctoral dissertation).

Retrieved from http://etd.lsu.edu/docs/available/etd-07092009-141241/unrestricted/Dissertation_CombinedFinalF.pdf

- Schneider, O., Schram, E., Poelman, M., Rothuis, A., van Duijn, A., & van der Mheen, H. (2010). *Practices in managing finfish aquaculture using ras technologies, the dutch example*. OECD Workshop on Advancing the Aquaculture Agenda. OECD, Paris, France.
- Schreier, H.J., Mirzoyan, N., & Saito, K. (2010). Microbial diversity of biological filters in recirculating aquaculture systems. *Current Opinion in Biotechnology*, 21, 318-325.
- Snow, A., Anderson, B., & Wootton, B. (2012). Flow-through land-based aquaculture wastewater and its treatment in subsurface flow constructed wetlands. *Environmental Reviews*, 20, 54- 69.
- Summerfelt, S.T., Davidson, J.W., Waldrop, T.B., Tsukuda, S.M., & Bebak-Williams, J. (2004). A partial-reuse system for coldwater aquaculture. *Aquacultural Engineering*, 31, 157-181.
- Summerfelt, S.T., Wilton, G., Roberts, D., Rimmer, T., & Fonkalsrud, K. (2004). Developments in recirculating systems for Arctic char culture in North America. *Aquacultural Engineering*, 30, 31- 71.
- Tal, Y., Schreier, H.J., Sowers, K.R., Stubblefield, J.D., Place, A.R., & Zohar, Y. (2009). Environmentally sustainable land-based marine aquaculture. *Aquaculture*, 286, 28-35.

Taylor, T. (2013). *Resort wastewater treatment system using Biogill technology*. Retrieved from

Biogill website:

http://www.biogill.com/uploads/74946/ufiles/Resort_Sewage_Treatment_by_BioGills.pdf

U.S. Environmental Protection Agency, Office of Science and Technology. (2004). *Economic and environmental benefits analysis of the final effluent limitations guidelines and new source performance standards for the concentrated aquatic animal production industry point source category*. (EPA -821-R-04-013). Retrieved from

https://www.epa.gov/sites/production/files/2015-11/documents/caap-aquaculture_eeba_2004.pdf

UNEP (2004). Wastewater Technologies. In UNEP (Ed.), *A Directory of Environmentally Sound Technologies for the Integrated Management of Solid, Liquid and Hazardous Waste for SIDS in the Caribbean Region* (pp.63-125). Nairobi.

United Nations Environment Programme, Division of Technology, Industry and Economics.

(n.d.). *Water quality: The impact of eutrophication* (Lakes and Reservoirs vol. 3).

Retrieved from http://www.unep.or.jp/ietc/publications/short_series/lakereservoirs-3/index.asp

Urban Agriculture Business Information Bundle. (2016). Retrieved October 3, 2016, from

Ontario Ministry of Agriculture, Food and Rural Affairs website:

<http://www.omafra.gov.on.ca/english/livestock/urbanagbib/fish.htm>

van Rijn, J. (2013). Waste treatment in recirculating aquaculture systems. *Aquacultural Engineering*, 53, 49- 56.

What is Aquaculture? (n.d). Retrieved September 23, 2016, from National Oceanic and

Atmospheric Administration website:

http://www.nmfs.noaa.gov/aquaculture/what_is_aquaculture.html

Zhang, S.Y., Li, G., Wu, H.B., Liu, X.G., Yao, Y.H., Tao, L., & Liu, H. (2011). An integrated recirculating aquaculture system (RAS) for land-based fish farming: The effects on water quality and fish production. *Aquacultural Engineering*, 45, 93- 102.

Zohar, Y., Tal, Y., Schreier, H.J., Steven, C., Stubblefield, J., & Place, A. (2005). Commercially feasible urban recirculated aquaculture: addressing the marine sector. In B. Costa-Pierce (Ed.), *Urban Aquaculture* (pp. 159-171). Cambridge, MA:CABI Publishing.