THE EFFECTS OF CLIMATE AND HUMIC SUBSTANCES ON DISINFECTION PERFORMANCE IN ARCTIC WASTEWATER STABILIZATION PONDS

by

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Abstract

This thesis is an investigation of disinfection in wastewater stabilization ponds (WSPs) as it relates to adequate wastewater treatment and, ultimately source water protection (SWP) in northern, particularly Indigenous, communities. WSPs are considered sustainable utilitarian wastewater treatment technologies that are cost efficient and require minimal operation and maintenance. However, their performance is highly dependent on environmental conditions and disinfection performance, specifically, can be compromised in northern climates. The research is motivated by the following question: are there any simple approaches that could improve WSP disinfection without the need for conversion to a full-scale conventional treatment plant?

The first study compared the ability of existing models in predicting cold climate disinfection performance in WSPs by comparing their predictions of mortality rates for fecal coliform bacteria with rates observed in a single-stage WSP in Pond Inlet, NU, during the 2015 treatment season. The results of this study demonstrated that existing models exhibit limitations in representing disinfection performance in Arctic WSPs.

The second study focused on the development of a sunlight-mediated disinfection model for cold climate WSPs. A $2^k$ factorial design was implemented to enable the examination of interaction effects of independent predictor variables related
to sunlight-mediated disinfection (pH, dissolved oxygen, depth-averaged irradiance) on the mortality rates of *Escherichia coli* ATCC 11229. A controlled atmosphere chamber (CAC) was designed to control these parameters. Mortality rates between $-0.8198 - 1.1057 \text{ h}^{-1}$ were observed throughout the experiments. A numerical model was presented and demonstrated a significant fit ($p < 5 \times 10^{-10}$) to the data collected in the experiment. Temperature was found to have a more complex relationship with disinfection than previously thought, likely affecting both the growth and death rates of *E. coli*.

In addition, the effect of humic substances (HS) concentrations (0 - 30 mg/L) on disinfection performance was investigated. Higher HS concentrations were found to improve disinfection performance when the conditions supporting exogenous photo-oxidation were present. Recent literature has shown that HS concentrations are positively correlated to solids retention time in membrane bioreactor systems which could indicate a similar relationship with hydraulic retention time in WSPs. Finally, suggestions are made for future WSP configurations to enhance disinfection performance.
Acknowledgments

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Chapter 1

Introduction

Canada is known as a country with rich water resources, the image of which is often of pristine conditions. This should translate into clean water supplies for residents across Canada. However, water resources and infrastructure in Canada are not as distributed nor reliable as one may think, especially in rural and remote areas. In the winter of 2015, 119 First Nation communities in Canada did not have access to clean running water. In fact, some of these communities had been on drinking water advisories (DWAs) for almost 20 years (McClearn, 2016). This includes boil water and do not drink advisories. Industrial pollution and agricultural runoff are usually identified as culprits of freshwater contamination, but a shocking statistic released by Environment and Climate Change Canada states that 150 billion litres of raw or undertreated wastewater is dumped into Canadian waterways every year (Environment and Climate Change Canada, 2016). Health Canada lists source water degradation and unacceptable microbiological quality as reasons for DWAs on Indigenous reserves (Health Canada, 2016).

Access to clean drinking water is not just a matter of treating water prior to end-user consumption, prevention of biological or chemical contaminants from reaching
the drinking source is important in preserving drinkable water resources. Source water protection (SWP) should be practiced to minimize the contaminants drinking water treatment will have to remove. Non-centralized drinking water treatment, which is used extensively in Indigenous communities, can be unreliable in providing safe drinking water (Neegan Burnside, 2011). Approximately 73% of water treatment systems on First Nations reserves in Canada were found to be medium to high risk, evaluated based on design, operation, reporting, trained personnel and source water quality (Neegan Burnside, 2011). Moreover, functioning conventional drinking water treatment can be ineffective in elimination of certain waterborne pathogens such as cysts of *Giardia lamblia*, *Endamoeba histolytica*, and oocysts of *Cryptosporidium parvum* (United States Environmental Protection Agency, 1999; von Sperling, 2007).

In practicing SWP, effective wastewater treatment should be ensured to minimize the spread of pathogens to a drinking water source. Naturalized wastewater disinfection in wastewater stabilization ponds (WSPs) has been demonstrated to be effective in elimination of pathogens, such as the resilient parasites previously mentioned (Reinoso et al., 2011; Ouali et al., 2012). This could prevent waterborne pathogens from reaching a drinking water source in the first place, as enteric viruses, such as human adenoviruses, have been reported to survive up to 120 days in freshwater, and for up to 130 days in seawater (Fong and Lipp, 2005). However, many Indigenous communities lack reliable wastewater treatment and in some cases, the community may be lacking wastewater treatment capacity completely (Neegan Burnside, 2011).

In 2012, the Canadian Council of Ministers of the Environment (CCME) released the new federal Wastewater Systems Effluent Regulations (WSER) as an amendment to the Fisheries Act. This document introduced a requirement for municipalities
1.1. THE CHALLENGES OF WASTEWATER TREATMENT IN INDIGENOUS COMMUNITIES

across Canada to achieve minimum national performance standards (NPS) for discharged wastewater effluent and provided a timely deadline of 30 years for communities to reach the discharge criteria for a range of targeted parameters. However, there are no regulations in the WSER concerning pathogen levels in wastewater effluent. Prior to the release of these regulations, there were no enforceable federal standards for wastewater quality parameters, only guidelines provided in legislation such as the Canadian Environmental Protection Act (Barlow, 2016).

However, the governments of Quebec, Nunavut, Northwest Territories, and Newfoundland and Labrador did not ratify these new regulations due to concerns associated with the challenges for remote communities in these provinces and territories to meet these regulations. Environmental and geographical constraints make it difficult for Northern communities to achieve adequately treated wastewater effluent.

1.1 The challenges of wastewater treatment in Indigenous communities

The Indigenous peoples of Canada view water as more than a resource for survival, it holds cultural and spiritual significance. Water is viewed as the giver of life that transports human consciousness from the spirit realm (Anderson, 2010). Water is also viewed as a living thing. “Living things” from the perspective of many Indigenous cultures includes abiotic factors such as water (McGregor, 2012). In fact, Anishnaabe people view their interaction with water as a relationship. Their relationship with water is so significant, that many Anishnaabe Indigenous groups refer to water as a family relative (Anderson, 2010; McGregor, 2012).

Northern Indigenous communities across Canada face a number of difficulties in
1.1. THE CHALLENGES OF WASTEWATER TREATMENT IN INDIGENOUS COMMUNITIES

providing municipal utility services to their residents due to climactic, political, economic and social challenges. Often these communities are extremely remote and isolated and the populations are often very small. In the territory of Nunavut, there are few hamlets that exceed a population of 2000 people (PricewaterhouseCoopers, 2006). The extreme climate conditions in the Canadian North present many challenges in providing water utility services. Sewage is no exception; hence both the collection and treatment of sewage in Northern Indigenous communities are limited. For example, the arctic permafrost and extremely low temperatures makes the implementation of a piped wastewater collection system impractical; therefore, communities must rely on trucks to collect wastewater from the individual sewage storage tanks at each home and deliver it to the treatment facility. Sewage hauling in northern communities can present logistical difficulties, especially in the winter, which can leave residents without service for extended periods of time (Canadian Broadcasting Company News, 2015).

Adequate wastewater treatment in Northern Indigenous communities is another challenge on its own. In a 2005 report on drinking water challenges facing First Nations communities, the Assembly of First Nations stated that, ”...wastewater management particularly sewage, is especially problematic for First Nations. This problem is not just about how others dispose of their sewage and how this affects our lands and waters, but how inadequate our own wastewater systems are on our reserves...”. They also stated that, ”75% of the 462 wastewater treatment systems on reserves posed a medium-to-high risk to drinking water and wastewater quality” (White et al., 2012)

Inadequate wastewater treatment presents a public health hazard, and it can contribute to contaminated sources of drinking water in communities. This compounded
with limited drinking water treatment can lead to DWAs. While this is not exclusively an engineering issue, performance of existing treatment technologies implemented on reserves should be investigated and improved, while communities without wastewater treatment must be provided with practical, affordable treatment options.

1.2 Wastewater treatment

Wastewater treatment can broadly be classified into two categories: active and passive treatment. Active treatment requires energy input to promote the physical, chemical and biological processes to reduce organic compounds, nutrient and pathogen levels in wastewater. Generally, these systems are designed to increase efficiency and efficacy of natural processes by promoting optimal conditions. For example, aeration tanks with biologically activated sludge (BAS) use active aeration to maintain the optimal dissolved oxygen concentration for aerobic bacteria to consume organic compounds (Tchobanoglous and Burton, 2003). Conversely, passive treatment technologies require minimal energy input, as they rely on ambient environmental conditions to provide physical, chemical, and biological treatment processes (Mara, 2003). Examples include WSPs, constructed wetlands, and biological filters. The main advantage of these systems is the reduced cost resulting from the lower operational and maintenance requirements (Mara, 2003).

WSPs are passive centralized wastewater treatment methods that are highly effective for not only nutrient and organic compound removal, but also the removal of pathogens (Shammas et al., 2009). The lower overhead costs, previously mentioned, make WSPs an ideal treatment method for remote communities that may not have the financial resources for a full-scale wastewater treatment plant. In fact, WSPs are
the most common type of treatment system found on reserves across Canada (Neegan Burnside, 2011). However, many communities still rely on individual septic systems which require regular maintenance. In a study conducted by Neegan Burnside where 5% of the total individual septic systems on reserves were assessed, 47 percent of the septic systems exhibited operational concerns, including septage waste discharging to the ground surface (Neegan Burnside, 2011). These issues were attributed to limited maintenance, inappropriate geological settings, and the age of the systems.

The performance of WSPs can be variable as well. Biological and chemical treatment processes rely on environmental conditions for treatment, which is also true for disinfection. The mechanisms responsible for naturalized pathogen removal rely on sunlight, algae concentrations, and temperature among other factors. The disinfection mechanisms in WSPs are poorly understood, and most studies focus on empirically derived regression models (Andrianarison et al., 2010). However, regression models, such as Marais (1974) can be useful in predicting effluent bacteria concentrations, and thus can be considered for the design or to improve the performance of these WSPs. Unfortunately, regression models can be limited to the environment in which they were derived. A model developed for a WSP in Brazil may not effectively predict disinfection performance in a WSP in Northern Ontario. Currently, there have not been disinfection models developed for Arctic WSPs. A cold climate model could prove useful in improving disinfection performance and for designing future WSPs for Northern communities. Ultimately, improving disinfection performance in these treatment systems could help mitigate the DWAs in some communities by ensuring adequate treatment of wastewater treatment before it contacts drinking water sources.
1.3 Scope of studies

This thesis focuses on naturalized disinfection in cold climate WSPs, as they are extensively used as centralized wastewater treatment systems in Indigenous communities across Canada. There are 2 main objectives of this study. First, the ability of available disinfection models to predict disinfection performance in a Canadian High Arctic WSP through a comparative analysis was investigated. The second objective was to propose a disinfection model designed specifically for cold climate WSPs and examine the role of humic substances in naturalized disinfection processes.

1.3.1 Literature review

Chapter 2 of this thesis is a comprehensive literature review covering policy, the science of naturalized disinfection in WSPs, and a background on numerical modelling of naturalized disinfection. Wastewater treatment in the Canadian North, specifically Nunavut, is reviewed, presenting the regulatory framework and the challenges of delivering water and wastewater utilities. An overview of disinfection in WSPs follows, which includes a thorough report of all purported variables contributing to naturalized disinfection. There is a special focus on humic substances and their role in naturalized disinfection. Next, a summary of disinfection modelling is presented, with a special focus on sunlight-mediated disinfection models.

1.3.2 Comparative analysis of disinfection models in predicting performance in Canadian High Arctic WSPs

In Chapter 3, data collected at a WSP in Pond Inlet, Nunavut, was presented and was used to compare the ability of various disinfection models to predict fecal coliform
(FC) concentrations. Models for naturalized disinfection are often derived from data collected in tropical or temperate environments. The extreme climactic conditions in the Canadian North can lead to extrapolation of predictor variables, as the environmental parameters used to predict disinfection rates (e.g., temperature or sunlight) may be outside the range in which they were calibrated. Extrapolation of models may produce inaccurate predictions, and is not recommended (Chiang, 2003). As such, the applicability of these models to cold climate WSPs may be limited.

1.3.3 Cold-climate disinfection model and the effect of humic substances

In Chapter 4, disinfection performance in cold climate conditions was investigated. First, a disinfection model was derived using data collected from a bench-scale experiment simulating treatment conditions in a cold climate WSP. The details of the experimental setup can be found in Appendix A. Sunlight-mediated disinfection mechanisms were investigated as they are thought to be the most effective form of naturalized disinfection in WSPs. The data collected included environmental parameters that have been demonstrated to contribute to sunlight-mediated disinfection, such as depth-averaged irradiance, pH, dissolved oxygen, and temperature. The experimental design was based on $2^k$ factorial design. *E. coli* mortality rate was the measured response variable used to quantify disinfection performance. Multiple linear regression of the mortality rate of *E. coli* was performed to derive the model.

Secondly, the role of humic substances in naturalized disinfection in WSPs was investigated. Humic substances are organic acids ubiquitous in wastewater that are thought to be a crucial constituent in exogenous-photo-oxidation - a sunlight mediated disinfection mechanism in wastewater. Mortality rates of *E. coli* were measured
under varying concentrations of humic substances (0-30mg/L), previously isolated from wastewater (Curtis et al., 1992; Ouali et al., 2014). This was conducted under target depth-averaged irradiance, dissolved oxygen, temperature and pH levels. The direct influence of humic substance concentrations on disinfection with respect to exogenous photo-oxidation has yet to be investigated in detail.

1.3.4 Concluding remarks and additional materials

Studying sunlight-mediated disinfection in cold climate WSPs may provide insight to optimize controllable variables to improve disinfection performance. Also, identifying additional points of control could prove useful in improving disinfection performance in cold climate WSPs. If humic substance concentrations are identified as a variable affecting disinfection performance, future studies may investigate how to control concentrations. For example, humic substances have been shown to increase with increased solids retention times (SRT) in membrane bioreactors (Liang et al., 2007). Hence, increasing retention time in WSPs may lead to an increase in humic substance concentrations, which could potentially effect and improve disinfection performance.

Chapter 5 summarizes the conclusions from Chapters 3 and 4 and presents engineering contributions of this thesis along with recommendations for future work.

Appendix A provides a detailed explanation of the methodology and development for the experiments described in Chapter 4.
Bibliography


United States Environmental Protection Agency (1999). Wastewater Technology Fact Sheet - Chlorine Disinfection.


Chapter 2

Literature Review

2.1 Wastewater treatment in Indigenous communities of the Canadian North

2.1.1 Policy

Currently, water and wastewater regulations on reservations in Canada’s North falls under the jurisdiction of both the territorial and federal governments. In Nunavut specifically, the Nunavut Water Board (NWB), the Nunavut Planning Commission (NPC) and the Nunavut Impact Review Board (NIRB) have jurisdiction over water resources, as stated in the Nunavut Land Claims Agreement (NLCA). While many pieces of legislation affect wastewater management in the North, the NLCA has the most significant impact. It mandates the NWB responsible for issuing licenses for water use and disposal of waste, and wastewater (including domestic) and the NWB does so using certain criteria for discharged effluent. The effluent discharge limits required are shown in Table 2.1, with Ontario’s Ministry of the Environment’s discharge limits shown for comparison. Effluent discharge requirements are imposed on a case-by-case basis in Nunavut; the discharge limits in Table 3.1 are the ranges that
Table 2.1: Various wastewater quality guidelines and regulations in Canada.

<table>
<thead>
<tr>
<th>Wastewater Quality Parameter</th>
<th>Nunavut Water Board (NWB)</th>
<th>Wastewater Systems Effluent Regulations (WSER)</th>
<th>Ontario Ministry of Environment Regulations (WSER)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cBOD (mg/L)</td>
<td>120</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>180</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Ammonia (unionized) (mg/L)</td>
<td>N/A</td>
<td>1.25</td>
<td>15-20</td>
</tr>
<tr>
<td>Escherichia coli (CFU/100mL)</td>
<td>$10^4 - 10^6$</td>
<td>N/A</td>
<td>$&lt; 200$</td>
</tr>
</tbody>
</table>

are conventionally used. It should be noted that there are 2-4 orders of magnitude difference between the guidelines in Ontario and the regulations in Nunavut. The difference is likely due to the limitations of wastewater technologies that are typically employed in the North. This will be discussed in detail in section 2.1.2. At the federal level, the Wastewater Systems Effluent Regulations (WSER), a section of the Fisheries Act introduced in 2012, provides minimal discharge criteria for the provinces with respect to wastewater quality parameters such as carbonaceous biological oxygen demand (cBOD), ammonia, and total suspended solids (TSS). The WSER discharge standards are shown in Table 3.1 as well. Presently, there are no regulated limits for pathogen indicators such as *Escherichia coli*. In addition, these guidelines were not ratified by the governments of the Northwest Territories, Quebec, Newfoundland and Labrador or Nunavut. This was due to concern that the performance standards would be difficult to achieve in communities above the 54th parallel due to environmental and geographical constraints. National performance standards for arctic facilities are being developed and will eventually be implemented. Besides the WSER, there are no enforceable federal regulations for the discharge of effluent, only non-enforceable guidelines in Acts such as the Canadian Environmental Protection Act (CEPA).

While licensing of water and waste disposal permits in Nunavut is regulated and
involves extensive review by multiple government bodies, monitoring and reporting on effluent quality from licensed treatment facilities can be lacking, especially across seasons. Regular monitoring is difficult for communities in Nunavut due to logistical issues, limited technical expertise and financial resources. In general, any compliance monitoring is conducted in the summer months (Wootton et al., 2008). The monitoring and enforcing of regulations for water use and disposal is not under the jurisdiction of the NWB despite their licensing authority, rather it is the responsibility of Indigenous and Northern Affairs Canada (INAC) (PricewaterhouseCoopers, 2006).

2.1.2 Wastewater and drinking water challenges

Providing utilities and services in the Canadian North can be challenged by physical, environmental, economical and political limitations or barriers. This is true for water and wastewater services – many communities have issues simply delivering drinking water and collecting wastewater due to the extreme climatic conditions.

Installation of above ground or buried piped distribution systems is not practical with the thick ever-present permafrost layer. Twenty-two communities in Nunavut utilize trucks to deliver utility services to each residence, however this system presents its own challenges (Wootton et al., 2008). In the winter of 2015, the hamlet of Pond Inlet had to declare a state of emergency over a sewage crisis. Year-round, the hamlet used three sewage haulers for the collection of wastewater from holding tanks at each residence and delivery to the hamlet’s single sewage lagoon. In winter temperatures of -40 °C, two of the sewage haulers broke down, and the hamlet utility services were simply overwhelmed trying to meet the needs of the community. Meeting the
requirements of the community is difficult with 3 haulers, near impossible with a single hauler. Across Pond Inlet, residences had spilled sewage resulting from the operation of storage tanks over their capacity limits (Canadian Broadcasting Company News, 2015).

Infrastructure Canada prepared a report in 2012, describing the difficulties experienced by communities north of the 54th parallel. Many of these communities are extremely remote, and for much of the year, the only access is via airplane. In Nunavut, sealift is the only means of transporting large cargo, and is only in operation during the months without significant sea ice, from July to October. (Wootton et al., 2008). Transfer of goods and labour between communities over a large area becomes very difficult, creating high costs and inflexible timelines.

In Nunavut, there are few communities that exceed a total population of 1500 people. Within these communities, the unemployment rates are very high compared to the national average, and the average income is well below the federal poverty line (Wootton et al., 2008). Water and sewage services are required to be self-funding. Sewer operation and maintenance costs are ten times greater than average costs in southern Canada due to high fuel prices, and infrastructure degradation due to climate, among other factors. In fact, as of 2009, 15 communities were in need of major repairs to existing wastewater treatment facilities, many of the communities requiring these repairs for over 5 years.

Neegan Burnside conducted a National Assessment on current deficiencies and operational needs of water and wastewater systems in 97 percent of the First Nation communities across Canada, identifying long-term needs of communities, and proposed a ten-year plan for infrastructure development (Neegan Burnside, 2011).
Although this report did not exclusively target Northern communities, the results illustrate the infrastructure gap between Indigenous and Canadian communities in general. The results from the assessment, completed according to the Indigenous and Northern Affairs Canada (INAC) Risk Level Evaluation Guidelines, included the following highlights:

- Out of 807 water systems inspected, 73 percent of the systems were considered medium to high risk

- Out of 532 wastewater systems inspected, 65 percent were considered medium to high risk

Neegan Burnside estimated the total construction cost to meet INAC’s Protocols, as well as federal and provincial guidelines, standards and regulations would be $1.08 billion. The non-construction costs were estimated to be $79.8 million. These included costs for increasing the capacity of communities to maintain their systems and preserve their source water.

**Disinfection in Northern WSPs**

As previously mentioned, monitoring of wastewater treatment facilities in northern communities is not performed on a consistent basis. Therefore, disinfection performance data is rather limited. In 2012, 2013, a collaborative research effort between Dalhousie University and Queen’s University culminated in a wide range of data to evaluate the performance of Northern wastewater stabilization ponds. For disinfection performance, monitoring was conducted at WSPs in Pond Inlet and Clyde River, Nunavut. Information on disinfection performance was among the data collected (Huang et al., 2014; Krumhansl et al., 2015).
The results from this study showed that the WSPs inconsistently met NWB standards. Moreover, when these ponds were in compliance of the criteria of $10^4 - 10^6$ CFU/100mL in terms of indicator coliform bacteria, pathogenic bacteria such as *Salmonella*, *L.monocytogenes* and *Campylobacter spp.* were present in the pond water prior to decant in September (Huang et al., 2014). This tends to suggest the compliance standards may not be adequate to ensure safely treated wastewater. Although these communities decant their wastewater directly into the ocean rather than a source water body, pathogens such as enteric viruses have been reported to survive and remain infective for up to 130 days in seawater and for up to 120 days in freshwater and sewage (Fong and Lipp, 2005). In ecosystems, waterborne diseases transmitted by effluent can pose a risk to biodiversity and ecosystem health. For example, outbreaks of botulism have caused substantial mortalities in waterfowl in locations across Canada (Environment Canada, 2001). In addition, pathogens can survive and be transported in groundwater, so freshwater sources for communities are at risk of contamination even if the inadequately treated wastewater effluent is not directly dumped into the freshwater body (Fong and Lipp, 2005).

### 2.2 Waste stabilization ponds

As both urban and rural communities continue to develop and grow across the globe, so has the need for treatment and management of septic waste. The disposal of septage and the effective treatment of wastewater is a crucial aspect in the prevention of disease and water borne illnesses (Ashbolt, 2004). Wastewater stabilization ponds (WSPs) are an effective, cost-efficient way to exploit the natural ability of
lagoon systems to improve the quality of water. As the importance of sustainability and long-term efficiency have continued to grow and become a more significant focus to the public, WSPs have become an increasingly favoured - albeit considered non-conventional - method for natural, centralized, eco-engineered wastewater treatment. In particular, minimal maintenance requirements and low operational costs have made WSPs an attractive option in developing and third-world countries, as well as small, rural and remote communities, such as those Indigenous communities in the Canadian North. These are all areas where conventional treatment methods such as electromechanical treatment plants or even septic tanks would be too costly and difficult to implement and maintain (Mara, 2003). The tropical or sub-tropical climactic environments typical of developing countries also provide ideal conditions for optimal efficiency and operation of stabilization ponds, however, polar climate conditions can be problematic for reliable and predictable WSP performance (Tilsworth and Smith, 1984).

WSPs are shallow lagoons designed to take in a flow of domestic or industrial waste. The naturally occurring microorganisms present in lagoon environments use a number of metabolic processes in order to break down organic compounds and remove nutrients from the wastewater effluent. The incoming solids and particulates flocculate and settle to the bottom of the WSPs as sludge, which is subsequently removed on a periodic basis (approximately every five years) (Mara, 1997). This provides the system with a physical method of separation and treatment.
2.2. WASTE STABILIZATION PONDS

There are three kinds of stabilization ponds: anaerobic, facultative, and maturation ponds. It is typical for all three types of ponds to be present in a complete wastewater treatment system, with anaerobic ponds being the primary treatment lagoon mainly for physical separation of solids from the liquid stream, followed by one or more facultative ponds, and ending with a maturation pond for effluent polishing. Ideally, a treatment train of WSPs will have one or more maturation ponds for tertiary treatment of wastewater. These ponds are designed for pathogen removal and can achieve coliform removal efficiencies of 99.99 percent and the usually reach 100 percent removal of protozoan cysts and helminth eggs (von Sperling, 2007). Their shallow depth and tertiary low-turbidity wastewater ensures that the pond volume is effectively contributing to disinfection by penetration of solar radiation, pH and dissolved oxygen as a result of photosynthetic activity.

As opposed to natural disinfection in WSPs, chemical disinfection with chlorine is the most commonly used method for municipal wastewater, as chlorine is an effective oxidizer of cellular material (United States Environmental Protection Agency, 1999). It is one of the more cost efficient methods as well, although it is not without its

Figure 2.1: A panoramic view of a single-celled WSP in Pond Inlet, Nunavut in summer 2015.
2.3 DISINFECTION IN WSPS

2.3.1 Indicator organisms

The disinfection performance of a treatment system can be evaluated by its ability to reduce influent bacteria, virus, helminths and protozoa populations. However, regulatory authorities often use indicator organisms, such as \textit{E. coli}, as a proxy for the presence of harmful microorganisms. \textit{Enterococcus}, \textit{fecal coliform} and \textit{total coliform} are also commonly used. By using a single species or class of bacteria, one can establish a standard by which all treatment systems can be compared and target concentrations can be regulated (Hach Company, 2000). An indicator organism’s reliability can be evaluated based on the following criteria (Hach Company, 2000):
2.3. DISINFECTION IN WSPS

1. It must have a greater population than the associated pathogen.

2. The organism must be strictly of fecal origin and ubiquitous in new fecal waste.

3. It must be resilient to environmental conditions and have a longer survival time than the pathogen.

4. It must not greatly proliferate in the environment outside of the source.

5. Detection, enumeration and identification of the indicator must be simple, reliable and inexpensive.

Despite being the gold standard indicator organism, *E. coli* does not meet all of the criteria. For example, *E. coli* may accurately indicate the presence of pathogenic bacteria, such as *Salmonellae* and *Shigellae*, but because their biology is much different than enteric viruses, *E. coli* may not be an accurate indicator for viruses such as *Vibrio cholerae* (Curtis, 1990; Li et al., 2014). However, for the intents and purposes of this thesis, *E. coli* and *fecal coliform* will be used and assumed to accurately represent overall pathogen concentrations in WSPs.

2.3.2 Viable but non-culturable

The membrane filtration technique measures culturability of microorganisms. An underlying assumption of this technique is that culturability and cell viability are one in the same, therefore, when measuring disinfection rates, techniques such as membrane filtration can be used to measure the living cells in a sample. However, studies have shown that cells that become unculturable do not always indicate the cells are no longer living or viable (Li et al., 2014). Pathogen and indicator organism
cells can become viable but non-culturable (VBNC), and therefore undetectable by membrane filtration. This includes \textit{E. coli}. Because public and wildlife health is the main concern of studies such as this, the real question is whether VBNC cells remain infectious. The answer appears to be on a species-to-species basis.

Li \textit{et al.} (2014) stated that new technology is needed to both accurately and cost efficiently measure viable pathogen cells. For the purposes of this study, the current standard for quantification, membrane filtration, will be used to measure \textit{coliform} concentrations in samples.

\subsection*{2.3.3 Overview of disinfection mechanisms}

Disinfection mechanisms in WSPs are not well understood; most of our understanding is derived from blackbox models, correlating different environmental variables to the mortality rate of indicator organisms, such as \textit{E. coli}. Modelling the mortality rate will be discussed in detail in a later section. First, the different factors that have been attributed to pathogen removal in WSPs will be explored.

\textbf{Temperature}

Previous to studies concerning sunlight mediated disinfection mechanisms, temperature was thought to be the main factor to predict disinfection in WSPs. In fact, the first disinfection model for WSPs included only temperature as the sole factor for predicting disinfection (Marais, 1974). Many studies since have reinforced the relationship between temperature and disinfection (Klock, 1971; Flint, 1987; Mancini, 1978; Pearson \textit{et al.}, 1987; Mayo, 1995; Ouali \textit{et al.}, 2014). However, there has been some contention over whether temperature is actually an important parameter, or if
it is confounding with the effect of sunlight on disinfection rates or depletion of nutrients. Relationships between temperature and pathogen die-off are highly variable (Klock, 1971; Flint, 1987; Mancini, 1978; Pearson et al., 1987; Ouali et al., 2014). A summary of these studies can be found in Table 2.2. Figure 2.2 shows the variability in the relationship between temperature and disinfection rate for 5 independent studies of the same sample source in Onondaga Lake. Some studies have found no significant relationship between temperature and E. coli death (Auer and Niehaus, 1993; Curtis, 1990). However, studies such as Ouali (2014) found a clear increase in disinfection rates from a temperature of 13 to 28°C. The high variability between these studies suggests the role of temperature is more complex, and is likely interacting with other variables to affect disinfection performance.

Table 2.2: Relationship between temperature and mortality rate from past studies. The range of other variables affecting disinfection performance are included if reported in the study.

<table>
<thead>
<tr>
<th>Author</th>
<th>Temperature (°C)</th>
<th>Effect on mortality rate</th>
<th>DO (mg/L)</th>
<th>pH</th>
<th>Irradiance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mancini (1978)</td>
<td>3-40</td>
<td>Positive</td>
<td>-</td>
<td>-</td>
<td>5-20 lys/hr</td>
</tr>
<tr>
<td>Flint (1987)</td>
<td>4-37</td>
<td>No &lt;25; Positive &gt;25</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Auer et al. (1993)</td>
<td>10-35</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>0-230 cal/cm/day</td>
</tr>
<tr>
<td>Curtis et al. (1992)</td>
<td>30-40</td>
<td>No</td>
<td>0.8-7.5</td>
<td>7-9</td>
<td>429-1096 W/m²</td>
</tr>
<tr>
<td>Mayo (1995)</td>
<td>26-31</td>
<td>Positive</td>
<td>-</td>
<td>7-9</td>
<td>188-528 cal/cm²/d</td>
</tr>
<tr>
<td>Ouali et al. (2014)</td>
<td>13-28</td>
<td>Positive</td>
<td>1.2-8.9</td>
<td>5.16-12.17</td>
<td>0-25 W/m²</td>
</tr>
</tbody>
</table>
In terms of bacterial population growth, an optimal temperature is crucial. The growth rate of bacteria will double with temperature increases of 10 °C until the optimal growth temperature is reached (Tchobanoglous and Burton, 2003). Beyond the optimal temperature bacteria growth declines. In an environment outside the host where resources for survival may be limited, like a maturation pond, an increased
bacterial growth rate and higher population could lead to depletion of food and nu-
trient sources. This could lead to an increased death rate. This effect could help
explain the high variability in relationships between temperature and bacteria death.

**Attachment and sedimentation**

The physical separation of solids by settling from wastewater liquid streams is crucial
for reducing total suspended solids (TSS) and biological oxygen demand (BOD),
and it can play an important role in removing pathogens as well. Before pathogen
removal via sedimentation can occur, pathogens must attach to suspended particles.
Most bacteria, viruses and parasites attach to a host tissue for survival, and have
developed various methods for doing so. Two structures are required for attachment.
An adhesin, which is a structure or macromolecule on the surface of the pathogen
which can bind to a receptor on the targeted host cell. These two complementary
structures will bind in a lock-and-key fashion. For example, *E. coli*, which uses the
human intestinal tract as a habitat, will bind with receptors on epithelial cells via the
adhesins on the small hair-like appendages, called pili, on the surface of the bacterial
cell (Awuah, 2006).

In wastewater, pathogens use adhesins to attach to nutrient-dense suspended solids
to facilitate survival. This causes a change in shape and size of the suspended particle
and can ultimately lead to the particle settling out of the liquid stream, thus disin-
fecting the wastewater (Auer and Niehaus, 1993; Awuah, 2006). In scenarios where
other disinfection mechanisms are limited, sedimentation can be responsible for close
to all the disinfection in WSPs, and may be crucial for disinfection in primary ponds
(Mayo, 1995; Maynard et al., 1999).
2.3. DISINFECTION IN WSPS

Sunlight

Past studies have suggested sunlight as one of the most important factor in passive disinfection (Mayo, 1990; Curtis et al., 1992a; Davies-Colley et al., 1997, 1999; Craggs et al., 2004; Maïga et al., 2009; Ouali et al., 2014; Kohn et al., 2016; Young et al., 2016). Sunlight disinfects wastewater through multiple pathways; characterizing these pathways could yield a more mechanistic understanding of disinfection.

Terrestrial sunlight can be categorized as follows:

- UV-B 290-320 nm
- UV-A 320-400 nm
- Visible 400-700 nm
- Infrared >700 nm

UV-C (200-290 nm), despite being germicidal, is attenuated by the upper atmosphere, and therefore does not contribute to naturalized pathogen removal. UV-B light has been shown to reduce faecal coliforms by 99 percent in WSPs (Davies-Colley et al., 1999). However, some studies have suggested that the UV-A and visible light spectrum is more active in disinfection (Kadir and Nelson, 2014). Two studies on sunlight-mediated disinfection yielded two different results. Curtis et al. (1992) found that a greater portion of fecal coliform bacteria were inactivated by visible light than UV. Davies-Colley et al. 1999 found that *E. coli* death by visible light only occurred at pH levels above 9.0, below this pH, *E. coli* removal related to UV-A and B wavelengths predominated. They concluded that visible light can only produce high disinfection when certain conditions are met; a pH over 9.0 and moderate to high
concentrations of dissolved oxygen. Davies-Colley et al. (1999) suggested that the difference was likely because Curtis et al. (1992) did not employ pH control. The presence of sunlight could lead to a pH rise due to an increase in algal activity enhanced by extended exposure. However, Curtis et al. (1992) did use a control; CHES buffer was used to maintain a constant pH, along with an algal growth inhibitor.

Despite the findings of previous studies, UV light is attenuated within the first few centimeters of the pond due to its short wavelengths in WSPs, and therefore, its contribution to disinfection could be limited, especially if the WSP is particularly turbid. In each study mentioned, light attenuation was not considered in their experiments, and only surface irradiance was measured with no mention of light attenuation coefficients. Considering light attenuation is crucial in predicting disinfection performance, it should be included in disinfection models (Maiga et al., 2009).

What is becoming increasingly evident in the research over the past 20 years, is that light-mediated disinfection is highly synergistic, and sunlight should not be considered independently in pathogen removal. Interaction between pH, DO and photo-sensitizers, either within pathogens or in the wastewater environment, create the conditions for disinfection (Curtis et al., 1992b; Mayo, 1995; Davies-Colley et al., 1999; Craggs et al., 2004; Ouali et al., 2014; Kohn et al., 2016). Studies by Curtis et al. (1992) and Davies-Colley et al. (1999) concluded multiple mechanisms for sunlight-mediated disinfection. After the experiments with *E.coli*, *enterococci* and *F*-DNA, and *F*-RNA viruses, Davies-Colley et al. (1999) postulated three mechanisms for disinfection:

1. Oxygen-independent UV disinfection
2. Endogenous photo-oxidation
3. Exogenous photo-oxidation

Oxygen-independent UV disinfection, mainly attributed to UV-A and UV-B radiation, directly damages DNA material in pathogen cells, as shown in figure 2.3 (Kadir and Nelson, 2014). When UV light is absorbed by DNA, molecular lesions on the double helix structure can form, thereby inhibiting replication transcription and translation. Ultimately, this results in cell death. This is the only pathway that involves direct interaction between sunlight and DNA.

Figure 2.3: A graphical representation of mechanism 1; an \textit{E. coli} organism is directly killed by UV-A and UV-B radiation.

The next mechanism is endogenous photo-oxidation, where UV-A and UV-B light indirectly kill pathogens. UV-A and UV-B light are absorbed by an endogenous photosensitizer in the pathogen cell. A photosensitizer is a molecule that can absorb light and change the chemical or physical properties of another chemical (Curtis et al., 1992b; Curtis, 1990; Davies-Colley et al., 1999; Muela et al., 2002; Benchokroun et al., 2003; Ouali et al., 2014; Kadir and Nelson, 2014; Kohn et al., 2016). Subsequently, reactive oxygen species (ROS) are produced by the photo-excited photosensitizer
transferring its energy to a dissolved oxygen molecule. This process is shown in Figure 2.4. Examples of ROS are singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radicals (Curtis et al., 1992b). Many cell constituents could be damaged by ROS, including DNA (Davies-Colley et al., 1999). Endogenous sensitization was first discovered by Hollaender in 1943, where there was a marked difference between the absorption spectra of DNA in *E. coli* and the action spectra for killing (Hollaender, 1943). Subsequent studies showed that DNA light absorbance decreases at 320nm, but aerobic inactivation peaks at 340, 365, 410 and 500nm (Webb and Brown, 1979). The chlorophyll pigment in plant cells is an example of a photosensitizer. Porphyrins and riboflavins have been shown to be related to endogenous photo-oxidation, and are thought to be common endogenous photosensitizers found in a wide variety of bacterial pathogens (Sammartano and Tuveson, 1987; Curtis, 1990)

![Figure 2.4](image)

Figure 2.4: A graphical representation of mechanism 2; an *E. coli* organism is indirectly killed by UV A or B radiation by first being absorbed by an *endogenous* photosensitizer, catalyzing the production of ROS which will damage cell constituents.

The third mechanism is called exogenous photo-oxidation, and it occurs primarily outside of the cell. The process is very similar to endogenous photo-oxidation
2.3. DISINFECTION IN WSPS

but there are a few important distinctions. UV-A, UV-B and visible radiation (390-700nm) are absorbed by an exogenous photosensitizer. In wastewater, this photosensitizer includes a class of organic matter called humic substances. Porphyrins, flavins and similar molecules are likely present in the water, but at much lower concentrations than humic substances. In addition, the insolubility of chlorophyll in water may limit its influence as a photosensitizer (Curtis et al., 1992b). ROS production is catalyzed by the photosensitizer, which will damage the external structures, such as the cellular membrane, of a pathogen. This renders the cell more sensitive to external factors that could impact survivability, such as pH and salinity. The general process can be seen in Figure 2.5. Dissolved oxygen (DO) and pH effects in photo-oxidation will be discussed further. Exogenous photo-oxidation was first reported by Raab (1900), who observed the removal of Paramecium in solution with acridine orange, a cationic dye used for cell-cycle determination.

Figure 2.5: A graphical representation of mechanism 3; an E. coli organism is indirectly killed by UV A and UV-B or visible light radiation being absorbed by an exogenous photosensitizer, catalyzing the production of ROS which will damage the cellular membrane of organisms.
Both mechanism 1 and 2 are dependent on UV-A and UV-B light as the main energy source for disinfection. These methods could be limited by the low penetration of UV light. Humic substances are able to absorb a wide range of light, from 200nm to 700nm, accessing potential energy from both the UV and visible spectra (Curtis et al., 1992b). Visible light inherently has a longer wavelength, meaning that it is more resistant to attenuation, and can reach lower depths in the WSP than UV. This effectively increases the proportion of the pond that produces ROS, and thereby increasing disinfection rates. Therefore exogenous photo-oxidation could very well be the most important sunlight-mediated disinfection mechanism in WSPs.

After a pathogen is exposed to sunlight and damaged through the various sunlight-mediated mechanisms, the pathogen may become unculturable. However, this does not necessarily indicate death of the microorganism, as discussed previously. Pathogens and indicator organisms, such as E. coli can undergo dark repair of damage from sunlight at night (Liltved and Landfald, 2000). This can result in an overestimation of the effect of sunlight on pathogen removal. When evaluating the performance of a WSP, sampling of the effluent should be performed in the morning after organisms have undergone repair to get a more accurate estimate of disinfection.

Retention time

There have been conflicting reports on the effect of hydraulic retention time on disinfection rates. Logically, increasing hydraulic retention time should increase the time pathogens are exposed to environmental factors that cause disinfection, therefore increasing pathogen removal. Increasing retention time also allows for more attachment, flocculation and settling (Tchobanoglous and Burton, 2003). A longer exposure time
would lead to a lower concentration, as decay rate is time dependent.

However, Mayo (1989) found no relationship between hydraulic retention time and disinfection. This comes down to an issue of geometry. Depth has an inverse relationship with disinfection rates; increasing depth would decrease disinfection rates in the WSP and vice versa. This is related to attenuation of sunlight in the pond. The depth to which sunlight can penetrate to is fixed for a particular wastewater. Ultimately, as the depth of the pond increases the portion of the pond protected from sunlight increases as well. This decreases the proportion of the pond contributing to higher disinfection rates via sunlight-mediated mechanisms, decreasing the overall rate. Therefore, increasing retention time by increasing depth will have a negative effect on the disinfection rate. Increasing the surface area of the pond to increase retention time, however, could conceivably have a positive effect on disinfection by increasing sunlight exposure time in the pond, while maintaining the volume ratio in the pond contributing to disinfection. Light attenuation will be explained in greater detail in the modelling section.

**pH**

The number of hydrogen ions in solution (pH) has a noticeable effect on disinfection in both light and dark reactions (Curtis et al., 1992b; Mayo, 1995; Davies-Colley et al., 1999; Ouali et al., 2014). As mentioned previously, pH plays an important role in exogenous disinfection as the cause of cell mortality after the cell membrane is damaged by the ROS produced in the reaction.

Ouali *et al.* 2014 and Davies-Colley *et al.* 1999 both found that there was a significant increase in pathogen removal when pH reached 8.5-9.0, and they also noticed
a synergistic effect between increasing pH and DO concentrations. They hypothesize that under moderate pHs (under 8.5), *E. coli* was inactivated primarily by pH-independent endogenous photo-oxidation, while at higher pHs, disinfection was more rapid because of the exogenous photo-oxidation effect. Curtis *et al.* 1992a argued that increasing pH could have 2 different effects on exogenous photo-oxidation. The elevated pH could provide a higher concentration of hydroxyl ions which might raise the internal pH of the microorganism making it more susceptible to the exogenous photo-oxidation effect. The second possibility, is that the higher pH induces more production of ROS in wastewater. A higher ROS production could happen 2 different ways. Studies on the photochemistry of natural waters noted an increase in light absorbance at higher pH levels (presumably by humic substances), which could theoretically lead to a higher production of ROS. However, there was little to no increase in ROS production noted in these studies (Zepp *et al.*, 1981; Haag and Hoigne, 1986). There may also be changes in the ionization or speciation of the many chemical constituents involved with this complex process that could increase success of ROS production (Curtis *et al.*, 1992b).

pH has been shown to have an effect in the absence of light, however, the overall dark disinfection rate, the disinfection rate without any light, has been shown to be orders of magnitude lower than that of sunlight-mediated disinfection further cementing the synergy between pH, DO, and sunlight (Curtis, 1990; Maïga *et al.*, 2009; Bolton *et al.*, 2010; Ouali *et al.*, 2014).
Dissolved oxygen (DO)

Fecal bacteria are facultative anaerobes, so they are able to survive under both aerobic and anaerobic conditions. However, Klock (1971) found that survivability of fecal coliform bacteria was lower in aerobic than anaerobic environments. In the absence of light, DO concentration was found to have no effect on fecal coliform bacteria (Curtis, 1990). Concentrations below 0.5 mg/L have been shown to have no significant effect on the mortality rate of fecal coliform bacteria (Van Buuren and Hobma, 1991). In endogenous and exogenous photo-oxidation, DO is crucial in production of ROS, such as superoxide or hydrogen peroxide, that are responsible for the physical damage of pathogen cells leading to cell death (Curtis et al., 1992b; Mayo, 1995; Davies-Colley et al., 1999; Ouali et al., 2014; Kohn et al., 2016).

In WSPs, DO is used by aerobic and facultative anaerobic bacteria in cellular respiration to oxidize organic matter. DO concentrations should therefore be relatively low, as surface reaeration of oxygen alone can not out compete the biological activity. However, the presence of algae in most ponds counteracts this effect and replenishes the supply of DO as a by-product of photosynthesis. Some ponds can even reach DO concentrations above saturation in the presence of algae.

Effect of algae

Algae can affect disinfection rates by several different means. Firstly, an uptake of carbon dioxide in photosynthesis (Park and Craggs, 2010) causes a domino effect in the bicarbonate buffer system. When carbon dioxide is removed from the water, the equilibrium system shifts to replace the $CO_2$ by increasing concentrations of carbonic acid [$H_2CO_3$] and bicarbonate [$HCO_3^-$] according to Le Chatelier’s principle.
In doing so, hydrogen ions $[H^+]$ in the system are consumed, increasing hydroxide concentration, effectively raising the pH. This is shown in Figure 2.6:

![Figure 2.6: The bicarbonate buffer system present in WSPs, and algae’s effect on the equilibrium reactions.](image)

Consequently, high pH values varying diurnally between 7-9.4 are often observed in WSPs (Curtis, 1990; Sweeney et al., 2007). As mentioned previously, increasing pH has a positive influence on disinfection rates. A by-product of photosynthesis is DO, where increasing algae concentrations and photosynthetic productivity will lead to higher concentrations of DO. This can improve the endogenous and exogenous photo-oxidation rate as mentioned previously (Curtis, 1990).

Algae can have other effects on pathogenic bacteria, either improving survivability or contributing to disinfection. The specific effect depends on the algae and the pathogen species in question. Some macrophytes are believed to produce chemical substances that affect survivability. For example, hot water extracts of the leaves
of cypress, *Cupressus macrocarpa*, inhibited the growth of bacteria such as *E. coli*, *Citrobacter freundii*, *Enterobacter cloacae*, and *Salmonella derbyi*. Microphytes may also have an inhibitory or growth effect on pathogens. For example, in the presence of cyanobacteria, a higher removal of *E.coli* than *V.cholerae* has been observed but in the presence of *Chlorella* the opposite was found. In darkness, Chlorella releases a toxin that inhibits fecal coliform growth. *Chlorella vulgaris* has been shown to produce toxins when under high pH stress conditions (Robertson Pratt, 1940). This could help explain the high dark disinfection rate found at high pH levels. In addition, algae may compete with heterotrophic bacteria for food or nutrients, such as glucose, which could also contribute to reductions in pathogenic populations (Mayo and Noike, 1994).

**Competition, starvation and predation**

Protozoans have been shown to prey on fecal coliform bacteria and other pathogens in wastewater, as well as algae. In fact, some studies have shown that increasing protozoan concentrations in WSPs can increase the wastewater treatment disinfection performance (Atlas and Bartha 1981; Sinclair and Alexander 1989; WPCF 1990). Nutrient availability in WSPs can vary depending on the prior treatment stages. In systems where nutrients may be limited, bacteria and pathogens must compete for resources. Fecal coliform bacteria die-off rates have been suggested to be dependent on nutrient supply and the presence of other bacteria populations in the environment. Species that are adapted to the WSP environment may out-compete intestinal bacteria for nutrients, contributing to their removal (Legendre et al., 1984; Wanjugi and Harwood, 2013). Starvation occurs when organisms no longer have access to their
required nutrients, and rely on intracellular constituents such as glycogen, protein and ribosomal RNA as sources of energy (Curtis, 1990). This reliance on internal constituents will continue until the organism metabolizes all of its ribosomal RNA or it can no longer maintain its internal pH. In disinfection models, competition, starvation and predation are represented by dark reaction rates. Many studies have shown significant differences between disinfection rates in samples exposed to sunlight versus those dark reaction rates, suggesting that predation and competition contributes less to disinfection than sunlight-mediated mechanisms (Curtis et al., 1992b; Mayo, 1995; Davies-Colley et al., 1999; Ouali et al., 2014).

However, there have been some conflicting reports in literature. Some studies have shown no significant difference between sunlight and dark disinfection rates, but an increase in disinfection rates of *E. coli* only when predators and competitors are present versus absent in water and sediment from freshwater and saline environments (Korajkic, 2010; Staley et al., 2011). The reason for this discrepancy is unknown, but the freshwater and saline mesocosm samples used in these experiments would likely have limited humic substance concentrations, and therefore limited exogenous photo-oxidation, reducing the overall sunlight-mediated disinfection rate. Concentrations of humic substances in freshwater have generally been found to be very low, between 0.1-6mg/L (Visser, 1984). Concentrations in wastewater are likely much higher considering the deeper yellow colour indicative of humic substances (Curtis et al., 1992b). In addition, humic substances concentrations have been shown to increase with increased solids retention time (SRT) in wastewater effluents from membrane bioreactors (MBR) (Liang et al., 2007).
2.3.4 Humic substances and exogenous photo-oxidation

Exogenous photo-oxidation is thought to be the predominant disinfection mechanism in a functional maturation WSP (Curtis et al., 1992b; Davies-Colley et al., 1999). Humic substances are essential constituents necessary for exogenous photo-oxidation, and the entire process is believed to hinge on the presence of humic substances in wastewater. They absorb sunlight and act as an electron shuttle, providing energy for the entire disinfection process. In fact, Curtis et al. (1992b) stated that the identity, location and concentration of the sensitizer are important variables in the photo-oxidation process. To date, there have not been any studies that have directly observed the effect of these variables on the photo-oxidation process.

The nature and origin of humic substances involved in exogenous photo-oxidation are questions that must be answered and then a more detailed explanation of their role in photo-oxidation must be examined.

Origin and composition

Humic substances (HS) are the yellowish-brown substance that can give natural waters and wastewater a yellowish hue. HS are ultimately by-products of the microbial breaking down of organic matter for energy and nutrients. They can be found in a wide variety of environments, where decomposition via microbial activity can occur. HS can be found in soils, freshwater, saltwater, peat bogs, compost heaps, carbonaceous shales, and lignites (Nova Scotia Environment, 2008; Lykins and Clark, 1988).

There are a few different theories that describe the formation of humic substances, but they all describe a polymerization reaction involving the resynthesis of organic
compounds such as complex carbohydrates, nitrogenous substances and phenolic compounds (Pettit, 2014). HS can form with many different precursors and in many different environments, leading to variability in the molecular features of HS (Pettit, 2014). Despite the vast difference in sources, the properties of humic substances are largely similar.

Generally, they can be classified into three different categories based on their solubility at different pH levels, colour and their relative molecular size. These classifications are displayed in Figure 2.9. Humin, the first class, is insoluble in both high and low pHs, and its molecular size is considered very large. Typically, humin is darker in colour than the other categories of HS. They are present in soils and are the most resistant to breakdown out of all HS.

Humic acids (HA) (Figure 2.7), are comprised of aliphatic and aromatic organic acids which are only soluble at pH conditions above 2.0. They are typically brown or dark yellow in colour. Fulvic acids (FA)(Figure 2.8) consist of the the smallest humic molecules, soluble under all pH conditions, and are typically lighter yellow in colour (Pettit, 2014). They are generally more reactive than humin and
2.3. DISINFECTION IN WSPS

HA because of a higher number of carboxyl and hydroxyl groups in their structure.

Figure 2.9: Classification of humic substances (HS) based on solubility in varying pHs, colour and relative molecular size (Stevenson 1982).

HS terrestrial sources are commonly associated with the decomposition of plant matter; lignin or polysaccharides (Lykins and Clark, 1988). Lignin is a structural polymer found in vascular tissue in plants and some algae. Humus, the active layer of soil that is consistently being renewed, contains large amounts of humin, humic and fulvic acids. Aquatic humic substances (AHS), have two main sources: allochthonous substances from terrestrial plants and soil, and authochtonous substances from the by-products of microbial activity within the body of water (Lykins and Clark, 1988).

In wastewater, the authochtonous source of HS is present in soluble microbial products (SMP), which can be defined as a mixture soluble organic matter produced by the bacterial population in wastewater treatment. SMP can be produced from two sources: as the by-products of the growth and metabolism of microbes, or from the decomposition of biomass. HS has been shown to be the most abundant fraction of SMP, and the humic and fulvic content have been shown to increase with a lengthened
SRT (Liang et al., 2007). Liang et al. (2007) found that increasing the SRT of sewage increase HS concentration in both the sludge and the water column. This is demonstrated in figure Figure 2.10.

**Role in exogenous photo-oxidation**

In order for light to fatally damage pathogens, it must pass its energy onto and destroy cellular components, like organelles, DNA or the cellular membrane. Light does not have to come in direct contact with the substrate being damaged. Light, especially at wavelengths less than 313 nm is known to have a synergistic effect with DO leading
to disinfection (Curtis, 1990). Oxygen can act as the transporter for the sunlight energy. But this is only part of the whole picture. The energy has to be captured and converted to make it usable and can be absorbed by DO. The process is called photo-oxidation, and can occur inside (endogenously) or outside (exogenously) the pathogen. To reiterate, exogenous photo-oxidation is a sunlight-mediated disinfection process where UV-A, UV-B and visible light indirectly damage pathogen cells by production of toxic reactive oxygen species that damage the cytoplasmic membrane. The cytoplasm becomes exposed to external conditions, including pH and salinity, which can lead to cell death. In both endogenous and exogenous photo-oxidation, the toxic ROS production is catalyzed by what is called a photosensitizer capable of absorbing UV light. Some photosensitizers can absorb light from the visible spectrum as well. This is advantageous because red light of 700nm has been shown to have enough energy to produce ROS. Conceivably, a large portion of the light spectrum can contribute to disinfection, assuming the photosensitizer is capable of absorbing wavelengths of light up to 700nm. In wastewater this photosensitizer is HS (Zepp et al., 1985).

In both exogenous and endogenous photo-oxidation a photosensitizer can produce ROS by two pathways: Type I or Type II photosensitization. The type of reaction pathway that occurs will depend on the sensitizer, the substrate and the reaction conditions. Reactions are not necessarily restricted to one pathway; each pathway can occur with the right photosensitizer and substrate and may compete for sunlight energy with each other. In either pathway, a successful reaction requires that the photosensitizer remains in its excited state for an extended period of time. An excited photosensitizer can have two different electron configurations, either singlet state, $^1S$,汉语
or triplet state, $^3S$. Singlet state can spontaneously convert to triplet state, as shown in Equation 2.2. The singlet excited state typically lasts between $10^{-9}$ to $10^{-6}$ seconds, while the triplet state can last up to 10 seconds. (Smith, 2013). Singlet state and triplet states can be produced by the following reactions:

\[ S_0 + hv \rightarrow ^1S \]  
\[ ^1S \rightarrow ^3S \]

Where $hv$ is a photon, $S_0$ is the ground state photosensitizer, and superscripts 1 and 3 represent triplet states 1 and 3 respectively. Because the photosensitizer must pass its energy on in order for photo-oxidation to proceed, the triplet state is more successful in transferring energy than singlet state, since the longer lifespan will increase the probability of the sensitizer coming in contact with another molecule. The photosensitizer can lose the energy gained through fluorescence or heat loss if the energy is not transferred within the lifespan of the singlet or triplet state. The sensitizer can also be converted to ground state by certain compounds called quenchers (Smith, 2013). A substrate can be defined as the target of the reaction. In disinfection, the targets could be cellular membranes, organelles, DNA or RNA of a pathogen.

The Type I photosensitization pathway is essentially a redox reaction, where an electron is transferred between the photosensitizer and the substrate. Most often, the photosensitizer is reduced and the substrate is oxidized.

\[ ^3S + A(substrate) \rightarrow S^- + A^+ \]  
\[ A^+ + O_2 \rightarrow A_{ox} \]
Two things can subsequently happen to the reduced photosensitizer:

1. If oxygen is present, the sensitizer can form oxygenated products. In this scenario, the photosensitizer is lost and can no longer participate in photo-oxidation.

\[
S^- + O_2 \rightarrow S_{ox} \tag{2.5}
\]

2. In the second scenario, there is a complete transfer of the electron from the photosensitizer to the ground state oxygen molecule, producing reactive superoxide. It should be noted that ground state oxygen is in triplet state. Superoxide can go on to form more oxygenated products by reacting with the substrate. In this case, the photosensitizer is preserved for future photochemical reactions.

\[
S^- + ^3O_2 \rightarrow S_0 + O_2^- \tag{2.6}
\]

\[
O_2^- + A \rightarrow A_{ox} \tag{2.7}
\]

Type II photosensitization occurs when energy, rather than electrons, is transferred to a ground state oxygen molecule producing the highly reactive singlet oxygen:

\[
^3S + ^3O_2 \rightarrow ^1O_2 + S_0 \tag{2.8}
\]

\[
^1O_2 + A \rightarrow A_{ox} \tag{2.9}
\]

Singlet oxygen has two excited states and the more common state has a longer lifespan. Singlet oxygen will react with substrate, such as lipids and proteins to generate oxidized products, or it can form peroxides (Smith, 2013; Curtis, 1990).

As such, the pathway favoured in photo-oxidation reactions in WSPS is of interest.
Type I and II reactions are dependent on substrate and oxygen concentrations. The high concentrations of oxygen and low concentrations of substrate expected in WSPs will favour Type II reactions where oxygen is the vehicle for energy transfer between the sensitizer and the substrate. In endogenous photo-oxidation, however, Type I may be favoured because of higher availability of substrate and $NADH^+$ (Curtis, 1990).

### 2.3.5 Modelling disinfection

The objective of modelling WSP disinfection is to predict performance and to optimize design in order to meet performance standards and mitigate the risks to public and environmental health. Disinfection in WSPs is commonly represented by a first order decay equation such as Equation 3.10. Relationships between variables and disinfection rates are derived deductively, by developing statistical relationships using
experimental or field data. Understanding the mechanisms of disinfection is secondary to the predicting function of the model; most models simply describe a numerical relationship between the chosen predictive variables and the disinfection rate. These variables and their parameter values are chosen based on statistical correlation, and correlation does not always imply causation. There are two factors that need to be considered when modelling WSP performance: the water quality parameter of interest (in this case disinfection) and the hydraulic conditions in the pond (Sah et al., 2012).

There has been some debate on the appropriate hydraulic model for disinfection modelling. There are basically three models that are used to recreate the hydraulic conditions in a WSP: the plug flow reactor (PFR) model, continuously stirred tank reaction (CSTR) and the dispersed flow model, which is a compromise between the two models previously mentioned. It is suggested that hydraulic models be evaluated on a case-by-case basis, as one model may not be appropriate for all situations. For example, for ponds with a more elongated geometry, the PFR model is suggested, while for those with a more square geometry, a CSTR model is recommended (Von Sperling, 2005). A study on hydraulic model approaches in predicting coliform removal in WSPs found that dispersed flow consistently underpredicted fecal coliform concentrations in ponds, especially as it approached a PFR approximation. In fact, the study showed a difference of up to 1-2 log units between CSTR and dispersed flow models, which can lead to drastically different designs (Buchauer, 2007). The CSTR model gave conservative estimates of coliform removal and many studies, including the commonly used WSP disinfection model by Marais (1974), have found that a CSTR model more accurately represents the hydrodynamic situation in WSPs (Marais, 1974;
2.3. DISINFECTION IN WSPS

Torres et al., 1999; Environmental Protection Agency USA, 2000; Buchauer, 2007).

First order models have been developed to represent disinfection in WSPs. They employ first order kinetics, as defined by Chick’s Law:

\[
\frac{dC}{dt} = -k(t)C
\]  

(2.10)

Where \(C\) is the indicator organism concentration, \(t\) is time and \(k\) is the mortality constant (Andrianarison et al., 2010). Models have been developed to predict the reaction constant or mortality rate constant values, which are affected by various parameters selected by the author. Generally, the equation for the mortality constant is of the form:

\[
k = k_d + k_s I
\]  

(2.11)

where \(k_d\) and \(k_s\) are the dark and the irradiance only disinfection rates; \(I\) is the total solar irradiance incident upon the pond surface. The mortality constant is measured in \(h^{-1}\).

A brief overview of photo-oxidation disinfection models

Marais (1974) developed the first disinfection model for WSPs. It strictly considered temperature to predict the mortality constant (Shilton, 2005). It is first order, hence the disinfection rate is directly proportional to the concentration of pathogens in the pond. It also assumes the pond hydraulics can be approximated by a completely stirred reactor, meaning that the effluent has the same concentration as a sample taken from anywhere in the pond. Maturation ponds have been traditionally designed for disinfection using this model. The equation for the mortality constant in the Marais
(1974) model is as follows:

\[ k = k_{20}\theta^{(T-20)} \]  

(2.12)

where \( T \) is the air temperature in °C, \( k_{20} \) is the die-off coefficient at 20°C and \( \theta \) is a temperature coefficient. Marais (1974) proposed \( k_{20} = 2.6 \ d^{-1} \), and \( \theta = 1.19 \).

Despite showing correlation in some studies, the role of temperature in disinfection has been debated, as aforementioned. As more information regarding the underlying processes participating in naturalized disinfection in WSPs, the parameters in first order models can be chosen by more than correlation alone. More recent studies have focused on developing models representing sunlight-mediated disinfection, as they have been suggested to be the most important pathogen removal mechanisms in WSPs. These are mainly variables that contribute to oxygen-independent disinfection and photo-oxidation including sunlight irradiance, pH, and dissolved oxygen, among others. The Curtis et al. (1992b) model was the first attempt at predicting sunlight mediated disinfection, especially photo-oxidation:

\[ k = -6.355 + 0.7437pH + 0.163[O_2] + 0.001027I \]  

(2.13)

Where \( I \) is irradiance in \( W/m^2 \) and the concentration of \( O_2 \) is measuring in \( mg/L \).

The Ouali et al. (2014) disinfection model used similar parameters as Curtis et al. (1992b) model. However, they included the influence of temperature in addition to the variables Curtis et al. (1992b) considered. Curtis et al. (1992b) conducted a disinfection study at temperatures between 30 and 40°C and found the results were not significantly different. However, in Ouali et al. (2014) experiments showed a large
increase in the mortality constant when the temperature increased from 13 to 28 °C.

\[ k = (-0.53 + 0.085pH + 0.0018[O_2] + 0.0138I)\theta^{(T-20)} \]  

(2.14)

Where \( \theta \) is the sensitivity coefficient with a value of 1.066. Note that neither the Curtis et al. (1992b) and the Ouali et al. (2014) models have been validated with field data at this time, as these models were developed in a laboratory setting.

**Modelling irradiance in WSPs**

An important consideration in WSPs is how light is to be modelled in the pond. A number of models simply consider the irradiance incident on the surface of the pond, leaving the which did not consider the ability for light to reach its targets (ie. pathogens or humic substances) (Curtis, 1990; Craggs et al., 2004; Ouali et al., 2014). A model that accounts for sunlight by simple surface irradiance makes the inherent assumption that suspended solids or turbidity is the same in every WSP. This is obviously not the case, as not all communities have equivalent systems; many communities in the Canadian North rely on a single WSP for treatment, containing wastewater that is extremely turbid when compared to maturation pond wastewater. The important thing to consider when modelling sunlight mediated disinfection is the effectiveness of the sunlight reaching the target. This necessarily implies that light attenuation is an important factor that should be taken into account.

Light can only penetrate to a certain depth before it is absorbed, scattered or reflected. An attenuation coefficient, \( K \) measured in \( m^{-1} \), can be used to quantify the light attenuation in a pond. The attenuation coefficient can easily be calculated by using a spectrometer to measure the irradiance along the depth profile of a WSP.
2.4 SUMMARY

Using software, such as the curve fitting tool in MATLAB and Equation 2.15, one can find the best fitting exponential decay curve, and thus $K$:

$$I = I_0e^{-Kz}$$

(2.15)

Where $I$ is the irradiance ($W/m^2$), $I_0$ is the surface irradiance ($W/m^2$), $K$ is the attenuation coefficient ($m^{-1}$) and $z$ is the depth ($m$).

If the total irradiance over the pond were considered by integrating Equation 2.15 from the surface to the depth, $Z$, one obtains the following:

$$I = \frac{I_0}{KZ}(1-e^{-KZ})$$

(2.16)

This value represents the depth-averaged irradiance, and considers the portion of the WSP that is actually contributing to sunlight mediated disinfection. This takes into account the depth of the pond ($Z$) and the effective penetration of sunlight by the attenuation, $K$ and thusly accounts for different wastewaters with different turbidity conditions (Mayo, 1989; Auer and Niehaus, 1993; Xu et al., 2002; Maïga et al., 2009).

2.4 Summary

WSPs are widely used in the Canadian Arctic due to their ease of operation, along with their low cost and maintenance requirements. Disinfection in these systems is highly dependent on environmental factors such as solar radiation and temperature for mechanisms such as photo-oxidation to be effective. In arctic climates, these factors may not meet the necessary conditions for adequate pathogen removal, thus producing effluent that does not meet discharge criteria. This could pose a risk to
public and wildlife health.

NWB regulations for *E. coli* in domestic wastewater effluent is $10^4 - 10^6$ CFU/100mL, 2 to 4 log units higher than the provincial guidelines in Ontario. The monitoring of effluent in Nunavut communities is irregular, but recent studies conducted by Queen’s and Dalhousie Universities have demonstrated that wastewater treatment inconsistently meets the NWB criteria in the communities that were monitored.

Currently, disinfection performance in Arctic WSPs is not well understood. Unlike WSPs in temperate and tropical climates, studies on Northern WSPs concerning pathogen removal efficiency have been limited. Numerical modelling provides an opportunity to better understand the effect that certain environmental parameters could have on disinfection performance in cold climate WSPs which could inform design of these systems in the future.
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Chapter 3

Comparative analysis of existing disinfection models

3.1 Introduction

Disinfection in wastewater stabilization ponds (WSPs) can be highly effective, while minimizing cost and labour efforts to the end-user. This makes WSPs advantageous for small communities that may not have the population size to fund and expertise required for the building and operation of a full-scale treatment plant. In many instances, these eco-engineered systems operate with minimal energy inputs, depending exclusively on environmental conditions to reduce organics, nutrients and pathogens. In extreme conditions, such as those found in the Canadian North, many of these environmental conditions, especially temperature and sunlight, may not be adequate for natural biochemical treatment processes that have been shown to take place effectively in more temperate or warm climates within WSPs. Under these conditions, disinfection performance, along with organic compounds and nutrient removal, can be compromised.

Modelling disinfection can be useful for design considerations and for optimizing
performance in WSPs. Several models have been developed over the last 40 years to predict and optimize disinfection performance, each considering different combinations of environmental factors (Marais 1974; Curtis et al. 1992; Auer and Nienhaus 1993; Mayo 1995; Xu et al., 2001). The Marais (1974) model was the first WSP disinfection model presented and has traditionally been used for maturation pond design. To date, there appears to be a lack of disinfection models that have been developed specifically for cold climate WSP applications, as existing models were generally designed using data from temperate or tropical regions which may not be representative of cold climate WSPs.

Previous studies have reported a range of different environmental factors selections and combinations to predict the rate of pathogen removal, presumably based on the predominant disinfection mechanisms they believed to be affecting the studied WSP. Temperature, sunlight, pH, and dissolved oxygen (DO) have been the most commonly used parameters, along with attachment and sedimentation, hydraulic retention time. Temperature plays a critical role in microbial growth, as most microorganisms exhibit an optimum temperature range for growth. In terms of disinfection, there has been conflicting research. Some studies have suggested that temperature has a positive correlation with disinfection rate (Klock, 1971; Pearson et al., 1987; Xu et al., 2002; Ouali et al., 2014), while others have found a negative relationship (Mancini, 1978) and some studies have observed no correlation between disinfection rate and temperature (Auer and Niehaus, 1993; Curtis et al., 1992a). The mechanism for disinfection influenced by temperature is not entirely clear, as it has been suggested that it may be a confounding factor also involving nutrient availability and sunlight intensity (Auer and Niehaus, 1993). pH levels above 8 or 8.5 have been found to be effective
for pathogen removal (Curtis et al., 1992a; Davies-Colley et al., 1999; Ansa et al., 2011). Moreover, a number of studies have asserted sunlight dependent disinfection mechanisms as the predominant means of pathogen removal in WSPs (Curtis et al., 1992a; Davies-Colley et al., 1997, 1999; Craggs et al., 2004; Maïga et al., 2009; Ouali et al., 2014; Kadir and Nelson, 2014). However, a mechanistic understanding of the role of sunlight in disinfection is still being investigated. Work previously done by Davies-Colley et al. (1999), Curtis et al. (1992), Ouali et al. (2014) and Kadir et al. (2014) has presented three main mechanisms for sunlight-mediated disinfection:

1. Oxygen independent ultra-violet (UV) disinfection

2. Endogenous photo-oxidation

3. Exogenous photo-oxidation

In mechanism 1, UV-A or UV-B radiation directly damages the deoxyribonucleic acid (DNA) and/or other cell constituents in the pathogen cell (Kadir and Nelson, 2014). In contrast, photo-oxidation is highly synergistic and requires several different constituents for disinfection. These constituents are, but are not exclusive to, dissolved oxygen (DO), a pH levels above 8 or 8.5 and sunlight of wavelengths below 500nm (Davies-Colley et al., 1999; Kadir and Nelson, 2014). The presence of photosensitizers is another critical factor required to enable photo-oxidation. Photosensitizer molecules can absorb solar energy and can, subsequently, either directly damage different cell constituents through redox reactions or indirectly through the transfer of absorbed solar energy to ground state oxygen, which then promotes the formation of reactive oxygen species (ROS) (Curtis et al., 1992b). ROS production is catalyzed by the presence of photosensitizers, and these reactive oxygen species
are toxic to pathogens, as they will damage the cellular membrane, or internal constituents of the organism through redox reactions (Curtis et al., 1992a; Davies-Colley et al., 1999; Kadir and Nelson, 2014). In endogenous photo-oxidation, the photosensitizers are various pigments, such as porphyrin derivatives and flavins, that absorb UV-A and UV-B radiation (Muela et al., 2002; Kadir and Nelson, 2014). In exogenous photo-oxidation, the photosensitizers are humic substances which are capable of absorbing UV-A, UV-B and visible light (Curtis et al., 1992b; Kadir and Nelson, 2014).

In WSPs, both oxygen independent disinfection and endogenous photo-oxidation can be limited by UV attenuation. In fact, shortwave radiation can be rapidly attenuated at depths less than 5 centimeters (Curtis et al., 1992a). Visible light exhibits lower light attenuation and better depth penetration in WSPs due to its longer wavelengths. This would suggest that exogenous photo-oxidation could be more predominant than UV-disinfection and endogenous photo-oxidation in a WSP if water clarity is limited.

First order models have been developed using first order kinetics, by Chick’s Law:

\[
\frac{dC}{dt} = -k(t)C
\]

Where \(C\) is the indicator bacteria concentration in \(CFU/100mL\), \(t\) is time in \(h\) or \(d\) and \(k\) is the mortality constant in \(h^{-1}\) or \(d^{-1}\) (Andrianarison et al., 2010). Models have been developed to predict the reaction constant values, which are affected by various parameters selected by the researcher. Generally, the equation for the reaction constant is of the form:
\[ k = k_d + k_s I \] (3.2)

Where \( k_d \) and \( k_s \) are the dark and the irradiance only disinfection rates in \( h^{-1} \) or \( d^{-1} \); \( I \) is the total solar irradiance incident upon the pond surface in \( W/m^2 \) (Andrianarison et al., 2010).

It should be noted that these disinfection models were generally designed and calibrated with data collected in tropical and temperate regions, rather than colder climate or Arctic data. There has been limited focus on disinfection models developed specifically for cold climate applications. This chapter compares existing models developed to simulate fecal coliform (FC) removal by using data collected at a WSP in Pond Inlet, Nunavut. The models were evaluated on their ability to predict disinfection performance in a Northern WSP and suggestions for future model adaptation and/or development are offered, accordingly.

3.2 Materials and Methods

3.2.1 Study site

Field measurements were collected from a WSP in a community located on the North side of Baffin Island, Pond Inlet, Nunavut (72°4157 N, 77°5733 W). Pond Inlet is a small, pre-dominately Inuit community with a population of 1,549 people as of 2011.

Pond Inlet relies on a single-celled WSP for treatment of their municipal wastewater. The estimated volume of the pond is 100,000 \( m^3 \). Sewage haulers deliver raw sewage collected from individual homes to the WSP, filling and emptying their tanks multiple times per day. On average, approximately 100 \( m^3 \) of sewage is emptied into
the WSP on a daily basis. The bathymetry profile and plan views of the WSP with the sampling locations are shown in Figure 3.1. The treatment season extends 2-3 months, beginning in June when ice clears from the pond and ending in late August or early September when ice returns. At the end of the treatment season the pond is decanted directly into the Arctic Ocean. Raw sewage continues to be pumped into the pond during the winter months. Measurements and samples were collected in the summer of 2015 from July 25th-28th and August 29th-September 1st, two weeks prior to the end of season decant. This was completed around noon each day.

Figure 3.1: The plan view of the sampling plan and profile view of the bathymetry of the Pond Inlet wastewater stabilization pond.

### 3.2.2 Assessment of water quality

During each visit to Pond Inlet, a 5 days sampling regime was undertaken. Six sampling locations throughout the pond were identified for *in situ* environmental parameter measurements and the collection of grab samples. Raw wastewater samples were also collected from sewage trucks to represent the influent to the pond. Bathymetry measurements were performed at several additional locations.

Environmental parameters included downwelling irradiance measurements from
290 nm to 700 nm measured using the Jaz EL200-XR1 spectrometer suite manufactured by Ocean Optics (Florida, USA). In order to calculate the attenuation coefficient, light measurements were taken every 1 cm for the first 5 cm, followed by 5 cm increments until no signal was observed. Measurements were conducted at approximately noon on a daily basis, so the irradiance is assumed to be the peak value for each day. Bathymetry, DO, pH and temperature data were collected using the Hydrolab DS5 multiparameter data sonde manufactured by OTT Hydromet.

Disinfection performance of the wastewater treatment system was assessed through routine monitoring of the effluent for the presence of indicator organisms, such as *E. coli* or other coliform bacteria. These were measured in July and August 2015 to examine the disinfection performance of the WSP system at the beginning and end of the treatment season. Coliform enumeration was carried out via the membrane filtration method according to *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association (APHA), 2005). Chromocult coliform agar was used to prepare agar plates. Multiple dilutions were prepared for each sample using peptone solution to obtain 30-300 CFU per membrane filter for accurate counting. 100mL of diluted wastewater samples were filtered with a vacuum filtration kit using a manual pump. Membrane filters were removed with sterile forceps and placed on agar plates. The plates were cultured at 37 °C for 24 hours. Subsequently, *E. coli* and coliform colonies were counted. Note that measurements were performed in triplicates, yielding 27 measurements per sampling day: 6 sampling locations in WSP plus the raw wastewater sample in triplicate.
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3.2.3 Disinfection modelling

Interpolation was used to extend the data sets for comparison with predictions from existing models. This was accomplished using simple linear regression. For pH, DO and temperature data, the lines of best fit were calculated using the ordinary least squares (OLS) method. Random noise from a normal distribution was generated using the standard deviation of the data to provide artificial variability. The resulting data sets are shown in Figure 3.2. As a result of the nearly 24 hours of sunlight expected at this latitude (72°N), photosynthetic activity was assumed to be fairly constant throughout the day and therefore diurnal variation of these parameters was assumed to be minimal. The values of pH, DO and temperature were assumed to be constant for the day.

![Figure 3.2: pH, DO, and temperature data interpolated between July 26th to September 1st from the data collected in Pond Inlet in the summer of 2015.](image)
Hourly irradiance data was synthesized in two steps. Firstly, the daily peak surface irradiance (W/m²), assumed to occur at noon hour each day, was calculated by using OLS to generate a line of best fit for the downwelling surface irradiance data, collected at the beginning and end of the 2015 treatment season at the WSP in Pond Inlet. Random noise was generated using a normal distribution with a mean equal to zero and a standard deviation of 90 W/m², the standard deviation of the downwelling irradiance data collected in Pond Inlet. This was used to simulate daily variation for the peak irradiance. Secondly, hourly variation of irradiance had to be generated. During Arctic summers, the hours of daylight extend to nearly 24 hours a day. However, there is still hourly variation in irradiance, and this variation increases in magnitude toward the end of the treatment season, as the number of sunlight hours decrease. Hourly variation in sunlight at the pond surface was approximated by Equation (3.3).

\[ I_o = S_i \sin(\pi \frac{t_{ij}}{24}) \]  

(3.3)

Where \( i \) is the day, \( j \) is the hour, \( I_o \) is the downwelling irradiance in W/m² measured at the surface of the pond, \( S_i \) is the daily peak irradiance in W/m² previously calculated by interpolation, \( t_{ij} \) is hours of daylight, collected from Time and Date’s Sunrise, Sunset, and Daylength database (Time and Date, 2015). If there is sunlight at \( j \)th hour of the \( i \)th day, then \( t_{ij} = t_{ij} \). If there is no sunlight at \( j \)th hour of the \( i \)th day, then \( t_{ij} = 0 \). The synthesized irradiance at the surface of the WSP is shown in Figure 3.3.
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Figure 3.3: Hourly sunlight irradiance data at the surface of the WSP in Pond Inlet, NU. Data was interpolated using OLS, and hourly variation was added using Equation 3.3.

The attenuation coefficient ($K$) is an indication of how easily light penetrates the water column at depth. In this study, the attenuation coefficient was determined by collecting depth profiles at each of the six sampling locations in the pond on each sampling day. Subsequently, Beer Lambert’s Law, Equation (3.4), was fit to the data using non-linear regression (Curtis et al., 1994).

$$I = I_0 e^{(-Kz)}$$

The average attenuation coefficient for the photosynthetically active region of light (PAR) was 29 $m^{-1}$, while the averaged attenuation coefficient for UV light was 80 $m^{-1}$. The averaged depth profile for both PAR and UV light is shown in Figure 3.4. Note that the large variation in the first few centimetres is due to the daily fluctuation of surface irradiance. This effect is less noticeable at greater depths where the light is strongly attenuated.
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Figure 3.4: Spatial and temporal average of light attenuation for PAR and UV light respectively in Pond Inlet, NU.

Depth-averaged irradiance was calculated using the following equation (Curtis et al., 1994):

\[ I = \frac{I_o}{KZ}(1 - e^{-KZ}) \]  

(3.5)

Where \( I \) is the depth-averaged irradiance in \( W/m^2 \), \( I_o \) is the surface irradiance, and \( Z \) is the depth of the WSP in metres. This relationship takes into consideration both the turbidity and depth of the pond as being factors to consider for sunlight-mediated disinfection.

A simulation of disinfection in the WSP was run from July 26th to September 1st for a total of 38 days. The measured bathymetry of the pond is shown in Figure 3.1, however the geometry was approximated to Figure 3.5 to simplify calculations.
3.2. MATERIALS AND METHODS

The initial depth of the pond was 1.44 metres and it had a daily inflow of 100 m$^3$. The approximate volume of the pond was 100,000 m$^3$. The controlled discharge WSP had no outflow, such that the water level increased throughout the course of the treatment season, making this a non-steady state hydraulic model. The volume of wastewater at a given point in time ($V_i$ in m$^3$) was given by Equation 3.6.

\[ V_i = V_{i-1} + (Q_{in} - Q_{out}) \Delta t \]  

(3.6)

Where $i$ is the day, $Q_{in}$ is the flow rate into the pond in m$^3$/d, $Q_{out}$ is the wastewater outflow, also with units of m$^3$/d, and $\Delta t$ is the time step of 1 day. $Q_{out}$ is zero, as the WSP is a controlled discharge pond. It was assumed the daily inflow was pumped into the pond as a single event, daily at noon. Equation 3.6 simplifies to Equation 3.7.

\[ V_i = V_{i-1} + (Q_{in}) \Delta t \]  

(3.7)

The WSP gains and losses due to precipitation and evaporation for the 2015 treatment season were calculated. Daily evaporation rates were estimated using a modified version of the Penman equation (Valiantzas, 2006). Historical weather data
recorded at the Pond Inlet Airport was used for the calculation (Weather Spark, 2015). The ratio between net loss (precipitation minus evaporation) of water from the WSP over the treatment season and total volume of wastewater influent delivered by the sewage haulers was less than 3%. Therefore, changes due to evaporation and precipitation were considered negligible. Changes in volume due to infiltration were not included and outside the scope of this study due to lack of available data.

The increased volume occurred as an increase in depth, as the x and y dimensions of the WSP were fixed, increasing the depth of the WSP. The changing depth was important to calculate as it affected the models that included depth-averaged irradiance. This can be shown by the Equation (3.8).

\[
d_t = \frac{V_i}{\pi r^2}
\]  

(3.8)

Where \( r \) is the radius in m, approximately 150m. Minimal spatial variation were observed for the wastewater parameters in the WSP, as such a continuously stirred tank reactor (CSTR) model was used as the hydraulic model. Additionally, Von-Sperling (2005) suggested WSPs with low length to width ratios, like the Pond Inlet WSP, could be represented by a CSTR model. The CSTR model has been shown to yield more conservative estimates of disinfection performance (Buchauer, 2007). The disinfection predictions for a CSTR are expressed as:

\[
C_{i+1} = \frac{(C_{in}V_{in} + C_iV_i e^{-t})}{V_{i+1}}
\]  

(3.9)

Where \( C_{in} \) is the fecal coliform concentration in raw sewage \( CFU/s/100mL \), \( V_{in} \) is the daily discharge volume in \( m^3/day \), \( k \) is the calculated mortality coefficient in
3.2. MATERIALS AND METHODS

Table 3.1: Past models that predict fecal coliform reduction in WSPs. These models were used in the comparative analysis.

<table>
<thead>
<tr>
<th>Author</th>
<th>$k_d$</th>
<th>$k_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marais (1974)</td>
<td>$2.6<a href="d%5E%7B-1%7D">1.19(T-20)</a>$</td>
<td>N/A</td>
</tr>
<tr>
<td>Curtis et al. (1992)</td>
<td>$-6.355 + 0.7437pH + 0.163<a href="h%5E%7B-1%7D">O_2</a>$</td>
<td>$0.001027(W^{-1}m^2)$</td>
</tr>
<tr>
<td>Auer and Nienhaus (1993)</td>
<td>$0.73 + \frac{v}{Z}(d^{-1})$</td>
<td>$\frac{(0.00824(1 - e^{-KZ}))}{KZ}(cal^{-1}cm^2)$</td>
</tr>
<tr>
<td>Mayo (1995)</td>
<td>$0.135pH(d^{-1})$</td>
<td>$\frac{5.67 \times 10^{-4}}{Z}(cal^{-1}cm^2)$</td>
</tr>
</tbody>
</table>

Table 3.2: Notation employed for equations presented in Table 3.1.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>Mortality coefficient</td>
<td>$h^{-1}$ or $d^{-1}$</td>
</tr>
<tr>
<td>$k_d$</td>
<td>Dark disinfection rate</td>
<td>$h^{-1}$ or $d^{-1}$</td>
</tr>
<tr>
<td>$k_s$</td>
<td>Irradiance disinfection rate</td>
<td>$h^{-1}$ or $d^{-1}$</td>
</tr>
<tr>
<td>$K$</td>
<td>Irradiance attenuation coefficient</td>
<td>$m^{-1}$ or $cm^{-1}$</td>
</tr>
<tr>
<td>$[O_2]$</td>
<td>Dissolved oxygen concentration</td>
<td>$mgL^{-1}$</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature</td>
<td>$^oC$</td>
</tr>
<tr>
<td>$Z$</td>
<td>Water depth</td>
<td>$m$ or $cm$</td>
</tr>
<tr>
<td>$v$</td>
<td>settling velocity</td>
<td>$md^{-1}$</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Irradiance coefficient</td>
<td>$J^{-1}cm^2d$</td>
</tr>
</tbody>
</table>

$h^{-1}$ or $d^{-1}$ (Andrianarison et al., 2010). A variety of models have been employed to calculate disinfection coefficients, as shown in Table 3.1. The notation for these models is described in Table 3.2. It should be noted that the models evaluated in this paper used fecal coliform concentrations (CFU/100mL), which included E. coli, rather than E.coli concentrations alone to quantify disinfection performance.
Table 3.3: Influent and effluent indicator organism concentrations for the Pond Inlet WSP.

<table>
<thead>
<tr>
<th>Indicator organism</th>
<th>Trip 1 (July)</th>
<th>Trip 2 (August)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent</td>
<td>Effluent</td>
</tr>
<tr>
<td><em>E. coli</em> (CFU/100mL)</td>
<td>5.1 x 10^6</td>
<td>8.9 x 10^5</td>
</tr>
<tr>
<td>Fecal coliform (CFU/100mL)</td>
<td>2.3 x 10^7</td>
<td>3.7 x 10^6</td>
</tr>
</tbody>
</table>

3.3 Results and Discussion

3.3.1 Treatment performance in Pond Inlet WSP

Overall, disinfection in the Pond Inlet WSP was limited, as shown by the data presented in Table 3.3. The collected data showed an approximate log unit reduction in fecal coliform levels. Under optimum pond conditions, up to 3-4 log units of reduction can be observed (Ouali et al., 2014). Moreover, the WSP was shown to be above the maximum discharge limit of 10^6 CFU/100mL, as mandated by the Nunavut Water Board. The concentration of fecal coliform increased throughout the course of the treatment season. This was an expected result; the conditions in July were more conducive for disinfection, with nearly 24 hours of sunlight, higher temperatures and therefore higher algal activity (Maassarani, 2015). It is likely that sunlight-mediated disinfection was limited in the WSP, based on the observed levels of pH, DO, and light attenuation (Ouali et al., 2014; Kadir and Nelson, 2014; Ouali et al., 2016; Maraccini et al., 2016). Throughout the treatment season, oxygen concentrations were very low, limiting both photo-oxidation mechanisms for pathogen removal (Davies-Colley et al., 1999; Kadir and Nelson, 2014).
3.3. RESULTS AND DISCUSSION

3.3.2 Disinfection modelling

Models were evaluated based on two important factors: their ability to accurately predict concentrations of fecal coliforms in the WSP, and their ability to replicate important trends in the observed data, particularly the increasing concentration in fecal coliforms throughout the course of the treatment season. In general, the models over-predicted the disinfection performance in the WSP by at least one order of magnitude and showed variable success in replicating increases in fecal coliform concentration, as shown in Figure 3.5. This included the Marais (1974) model, which is commonly used for designing WSPs for disinfection. This was important because one order of magnitude could be the difference between complying or failing to meet regulatory discharge requirements. The over-prediction likely resulted from:

1. The extrapolation of the mortality rate outside of the range of environmental parameters for which the model was designed.

2. The use of surface irradiance for quantifying the effect of sunlight rather than depth-averaged irradiance.
3.3. RESULTS AND DISCUSSION

Figure 3.6: Observed fecal coliform concentrations (CFU/100mL) compared with predicted concentrations by various disinfection models throughout the treatment season.

These models were designed and calibrated with data that was collected in both tropical and temperate regions, rather than Arctic data. In some cases, the parameter values in Pond Inlet, particularly temperature and sunlight irradiance, were much lower than those used to develop the models. Hence, the models were extrapolated outside their calibrated range. For example, the irradiance values used to calibrate the Curtis et al. (1992) model were between 429 -1096 W/m², while the average daily peak surface irradiance in Pond Inlet was 250 W/m². Extrapolation of models can be challenging, particularly when the data employed is outside the range for which the model was originally developed. The Xu et al. (2002) model most closely predicted the performance of the Pond Inlet WSP. This model was developed using year-round
data from a pond in Noirmoutier, France. The winter climate in Noirmoutier is comparable to the summer treatment season in Pond Inlet, especially in terms of temperature and irradiance (Xu et al., 2002).

The Curtis et al. (1992) model only considers surface irradiance and was designed using relatively clear tertiary wastewater. As such, it tends to over predict sunlight-mediated disinfection rates in the Pond Inlet primary wastewater which has a much higher suspended solids content. Hence, this model may be impractical for a number of communities that only have single or two staged WSP systems with higher turbidity wastewater.

The fecal coliform concentration in the Pond Inlet WSP increased between the July and August sampling events by nearly an order of magnitude. The decrease in pH, sunlight and temperature could be responsible for this trend in conjunction with daily increases in coliform from daily raw sewage input. This increase in concentration should correspond to decreasing mortality rates, k. The predicted mortality rates are displayed in Figure 3.6. The Xu et al. (2002) and Auer and Nienhaus (1993) models showed an increase, rather than decrease, in mortality rate over the course of the treatment season. The input of sewage and a zero mortality rate alone could not account for the increasing disinfection rate. A negative mortality rate, or a net positive growth rate, would be necessary to account for the increased concentration. Otherwise, the Xu et al. (2002) model would have more accurately predicted the concentration, as the mortality rate was essentially zero, as shown in Figure 3.6.

The models that incorporated both sunlight and pH values predicted decreasing mortality rates, which was the observed trend in the WSP. Moreover, the Curtis (1992) model predicted negative mortality rates in the second half of the treatment
season. It became unstable and predicted concentrations magnitudes higher than observed concentrations. This model represents sunlight mediated disinfection and does not incorporate attachment and sedimentation, which was likely the predominant disinfection mechanism in the WSP at Pond Inlet (Mayo, 1995; Maynard et al., 1999). This tends to suggest sunlight-mediated disinfection mechanisms were minimal in the pond. Moreover, physical removal via attachment and sedimentation did not yield high enough mortality rates necessary to reduce fecal coliform concentrations in the WSP. Hence, sunlight mediated disinfection, especially exogenous photo-oxidation, which requires sunlight irradiance as well as a high pH, could be an important disinfection mechanism to achieve adequately treated wastewater effluent from Northern WSPs. Sunlight-mediated disinfection under Arctic environmental conditions should be investigated further.
Figure 3.7: FC mortality rate constants ($k$) predicted for the 2015 treatment season in Pond Inlet, NU, using 5 different disinfection models.
3.4 Conclusion

The comparative analysis of disinfection models showed that current models can have limitations in representing disinfection performance in WSPs. This is likely due to their development with calibration data from tropical and temperate locations. Hence, existing models would need to be adapted or a new model developed specifically for Arctic and other higher latitude locations that consider relevant parameters (ie. sunlight, pH, dissolved oxygen, temperature) and is calibrated with appropriate ranges of these parameters. Moreover, it is apparent that sunlight mediated disinfection mechanisms were limited under the conditions observed in Pond Inlet’s WSP, and could have compromised treatment performance. Further investigation of sunlight-mediated disinfection mechanisms under Arctic-like conditions should be completed in order to understand how to improve treatment performance. Sunlight should be quantified using depth averaged irradiation in order to make the model more widely applicable and to adequately consider turbidity and water clarity. In addition, further field testing of a wider variety of cold climate WSP systems should be conducted for the purposes of adequately validating a cold climate disinfection model.


Time and Date (2015). Pond Inlet, Nunavut, Canada - Sunrise, Sunset, and Daylength.


Weather Spark (2015). Weather History for CYIO.

Chapter 4

Disinfection model for cold climate WSPs

4.1 Introduction

Sunlight-mediated disinfection mechanisms have been demonstrated to be the most effective pathogen removal processes in wastewater stabilization ponds (WSPs) (Mayo, 1990; Curtis et al., 1992b; Davies-Colley et al., 1997, 1999; Craggs et al., 2004; Maïga et al., 2009; Ouali et al., 2014; Kohn et al., 2016; Young et al., 2016). Various disinfection models have been developed over the past 25 years to further predictive capabilities of disinfection performance. This has been accomplished through both bench-scale experiments and field testing. However, these studies have generally been conducted in temperate or tropical regions, and their applicability to cold climate WSPs may be limited. This is while many Northern Indigenous communities across Canada utilize WSPs as their sole wastewater treatment method. Their widespread use is due to their practicality for small remote communities: they have very low operational costs and require very little maintenance. WSP performance, however, is variable in cold climates, which may pose a risk to public health. Inadequately or untreated wastewater can contaminate drinking water sources such as groundwater.
or surface water, acting as a vector to spread disease. For example, land spreading poorly treated wastewater could conceivably contaminate groundwater. Some pathogens can survive up to 120 days in freshwater, including groundwater, providing a window of opportunity to reach groundwater or surface water receivers (Fong and Lipp, 2005). There is a growing need to extensively investigate the treatment performance of WSPs, particularly as it relates to disinfection, under cold climate conditions, as this could be highly relevant to source water protection (SWP). This should be undertaken in efforts to mitigate public and wildlife health hazards. In order to better understand cold climate disinfection, sunlight-mediated disinfection mechanisms need to be assessed under Arctic environmental conditions, as these mechanisms are heavily reliant on ambient conditions.

There are three reported mechanisms for sunlight mediated disinfection:

1. Oxygen independent UV disinfection
2. Endogenous photo-oxidation
3. Exogenous photo-oxidation

These three mechanisms are thought to be the primary contributors to disinfection. Oxygen independent ultraviolet (UV) disinfection, mainly attributed to UV-A and UV-B radiation, directly damages deoxyribonucleic acid (DNA) material in pathogen cells (Kadir and Nelson, 2014). When UV light is absorbed by DNA, molecular lesions on the double helix structure can form inhibiting replication, transcription and translation, ultimately resulting in cell death. This is the only pathway that involves direct interaction between sunlight and pathogens.
4.1. INTRODUCTION

The next mechanism is endogenous photo-oxidation, where UV-A and UV-B light indirectly kills the pathogens. UV-A and UV-B light are absorbed by an endogenous photosensitizer within the pathogen cell. A photosensitizer is a molecule that can absorb light and change the chemical or physical properties of another chemical (Curtis et al., 1992b; Curtis, 1990; Davies-Colley et al., 1999; Muela et al., 2002; Benchokroun et al., 2003; Ouali et al., 2014; Kadir and Nelson, 2014; Kohn et al., 2016). The chlorophyll pigment in plant cells is an example of a photosensitizer. Porphyrins and riboflavins have also been shown to be related to endogenous photo-oxidation, and are thought to be common endogenous photosensitizers found in a wide variety of bacterial pathogens (Sammartano and Tuveson, 1987; Curtis, 1990). Subsequently, reactive oxygen species (ROS) are produced by the photo-excited photosensitizer transferring its energy to a dissolved oxygen (DO) molecule. Examples of ROS are singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radicals (Curtis et al., 1992a). Many cell constituents can be damaged by ROS, including DNA (Davies-Colley et al., 1999). Endogenous sensitization was first discovered by Hollaender in 1943, where there was a marked difference between the measured absorption spectra of DNA in *Escherichia coli* (*E. coli*) and the action spectra that resulted in cell death (Hollaender, 1943). Subsequent studies showed that DNA light absorbance decreased above 320nm, but aerobic inactivation peaks at 340, 365, 410 and 500nm, demonstrating that processes other than oxygen-independent mechanisms could contribute to disinfection (Webb and Brown, 1979).

The third mechanism is referred to as exogenous photo-oxidation, and it occurs primarily outside of the cell. Exogenous photo-oxidation was first reported by Raab in 1900, who observed the removal of *Paramecium* in solution with acridine orange,
a cationic dye used for cell-cycle determination. The process is very similar to en-
dogenous photo-oxidation but there are a few important distinctions. UV-A, UV-B
and visible radiation (390-700nm) are absorbed by an exogenous photosensitizer. In
wastewater, this photosensitizer involves a class of organic matter called humic sub-
stances (HS). Porphyrins, flavins and similar molecules are likely present in the water,
but at much lower concentrations than humic substances. In addition, the insolubil-
ity of chlorophyll in water may limit its influence as a photosensitizer (Curtis et al.,
1992b). ROS production is catalyzed by the photosensitizer, which will damage ex-
ternal structures of pathogens, such as their cellular membrane. This renders the cell
more sensitive to external factors that could impact survivability, such as pH and
salinity. The effects of dissolved oxygen (DO) and pH in exogenous photo-oxidation
will be discussed further.

HS concentrations in wastewaters of WSPs have been found to vary between 10
and 30 mg/L and have been shown to consist of up to 60% of the chemical oxygen
demand (COD) typically found in secondary wastewater (Asano et al., 2008; Kliau-
gaita et al., 2013). The concentration of HS has been shown to increase in membrane
bioreactions (MBRs) with increased solids retention time (Liang et al., 2007). HS
concentration and its direct influence on sunlight-mediated disinfection has yet to be
investigated. This study explores cold climate WSP disinfection and the role of hu-
mic substances in disinfection. The research is motivated by the following question:
Are there any simple methods of improving WSP disinfection without the need for
conversion to a full-scale conventional treatment plant?
4.2 Materials and methods

This chapter presents the disinfection of \textit{E. coli} in a simulated cold climate WSP environment under controlled pH, DO, temperature and depth-averaged irradiance. The experimental setup is shown in Figures 4.1 and 4.2.

Fisherbrand 50mL falcon tubes containing synthetic wastewater were loaded into holsters made of PVC tubing inside a controlled atmosphere chamber (CAC). The holsters ensured irradiance entered strictly through the surface of the samples. The CAC served as a closed environment allowing for DO control. This was accomplished by flowing nitrogen gas into the chamber to displace oxygen, and measuring the atmospheric oxygen concentration at the outflow with a Pro O$_2$ analyzer by Nuvair. Atmospheric oxygen concentrations were adjusted according to Equation 4.1.

\[
\frac{20.9\%}{DO_{s\text{aturation}}} = \frac{X}{DO_{t\text{arget}}} \tag{4.1}
\]

Where 20.9\% represents the percent composition of oxygen in the atmosphere, $DO_{s\text{aturation}}$ is the saturated DO concentration (mg/L) under atmospheric conditions at a given temperature, X represents the target atmospheric percentage of oxygen, and $DO_{t\text{arget}}$ is the required dissolved oxygen concentration (mg/L) in the synthetic wastewater samples. After target atmospheric oxygen levels were reached, samples were allowed to equilibrate for at least 12 hours. A YSI model 57 oxygen meter was used to measure DO concentrations in the synthetic wastewater. Samples that did not meet the target DO concentration after the given equilibration period, were sparged with nitrogen gas. The CAC was then resealed and adjusted to the target atmospheric oxygen concentration before beginning the experiment.
4.2. MATERIALS AND METHODS

Figure 4.1: Experimental setup consisting of a controlled atmosphere chamber (CAC) with valves controlling the inflow of nitrogen gas and outflow of air within the chamber.

Figure 4.2: Schematic representation of the experimental setup. 15 vessels containing synthetic wastewater were organized in triplicates each having the same concentration of humic substances.
4.2. MATERIALS AND METHODS

Sunlight intensity, turbidity, and depth of the WSP all control the effectiveness of sunlight-mediated disinfection. These three factors are incorporated into the depth-averaged irradiance calculation, which represents the amount of effective sunlight penetration in the WSP as shown in Equation 4.2.

\[ I = \frac{I_o}{KZ} (1 - e^{-KZ}) \]  \hspace{1cm} (4.2)

Where \( I_o \) is the downwelling irradiance at the surface of the pond, measured in \( W/m^2 \), \( K \) is the attenuation coefficient in \( m^{-1} \), and \( Z \) is the depth of the WSP in m (Curtis et al., 1994). The lighting system in the experimental setup consisted of an Orphek Atlantik aquarium lighting system to provide the photosynthetically active region (PAR) of the light spectrum, and the UV sunlight was simulated with two 25 watt fluorescent UV lights. The irradiance emitted from the system was approximately 135 \( W/m^2 \) and entered through the top of the vessels. This system was found to best simulate sunlight measured at a WSP in Pond Inlet, NU during the summer treatment season of 2015. Further details concerning the design and setup of the lighting system can be found in Appendix A.

The attenuation coefficient was adjusted using whey protein isolate according to Equation 4.3.

\[ K_{PAR} = 2.7x + 3.7 \]  \hspace{1cm} (4.3)

Where \( x \) is the concentration of whey protein isolate in g/L. The method describing the derivation of Equation 4.3 can be found in Appendix A. It should be noted the synthetic wastewater mixture was tested for bacterial contamination prior to the
Table 4.1: Chemical composition of the synthetic wastewater used for the disinfection experiments.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>160</td>
</tr>
<tr>
<td>Meat extract</td>
<td>110</td>
</tr>
<tr>
<td>Urea</td>
<td>30</td>
</tr>
<tr>
<td>$K_2HPO_4$</td>
<td>28</td>
</tr>
<tr>
<td>$NaCl$</td>
<td>7</td>
</tr>
<tr>
<td>$CaCl_22H_2O$</td>
<td>4</td>
</tr>
<tr>
<td>$MgSO_47H_2O$</td>
<td>2</td>
</tr>
</tbody>
</table>

The pH of the samples was adjusted with 0.1 M NaOH and 0.1 M HCl solutions using a Fisher Scientific Accuser 1001 pH analyzer. The experimental setup was contained in a VWR BOD low temperature refrigerated incubator with temperature control. Prior to the experiment, the target temperature was set, and the samples were allowed to equilibrate for at least 12 hours.

HS concentrations were adjusted to test UV independent and photo-oxidation disinfection mechanisms and their effects on overall disinfection performance. HS concentrations in the synthetic wastewater were adjusted using the previously prepared 250 mg/L stock solution. The synthetic wastewater composition, excluding HS, is shown in Table 4.1.

Please refer to Appendix A for details on the development of the experiment apparatus and for additional details.
4.2. MATERIALS AND METHODS

4.2.1 Preparation of humic substances

HS, which are a hydrophobic constituent of the dissolved organic matter (DOM) in wastewater, were isolated from 60 L of secondary wastewater effluent collected from Cataraqui Bay Wastewater Treatment Plant. Two stage filtering through 1.5 um CFP3 cellulose filter paper and 0.45 um nylon membrane filters by Sterlitech was conducted to remove suspended solids. Adsorption and ion-exchange chromatography were used to isolate and prepare the HS according to the procedure endorsed by the International Humic Substances Society (IHSS) (Aiken et al., 1992; Thurman and Malcolm, 1981).

DAX-8 adsorption resin was cleaned using various solvents according to Thurman and Malcolm (1981). 1 L of wet DAX-8 resin was packed into a 1.2 L glass column, rinsed with 0.1 M NaOH and then 0.1 M HCl three times, following with a rinse using 3 bed volumes of deionized water prior to sample application. The pH of the filtered wastewater was lowered to 2.0 and was then pumped through the packed column using a Masterflex L/S peristaltic pump manufactured by Cole Parmer at a rate of 125-250 mL/min. The effluent was discarded, and 1.8 L of 0.1 M NaOH solution was pumped into the column in reverse at 80 mL/min. The eluate was immediately acidified to prevent oxidation of the HS. The concentration of HS in the eluate was high with humic acids (HA) precipitating out of solution. Reconcentration on a smaller DAX-8 column was deemed unnecessary, avoiding the risk of clogging the column. The eluate pH was adjusted to 1 and the precipitated HA was allowed to settle over 24 hours. Subsequently, the eluate was centrifuged at 8000 rpm in the Centrifuge 5804R by Eppendorf, separating HA and fulvic acid (FA). The solution containing FA was decanted, and the HA pellet was washed with deionized water.
4.2. MATERIALS AND METHODS

and centrifuged at 8000 rpm. A silver nitrate test was completed on the decanted water that had been separated from the pellet to determine whether the pellet had been adequately cleaned. The procedure was repeated until the silver nitrate test results were negative. The FA solution was pumped through 5 mL of hydrogen form AGMP-50 ion exchange resin in a 8 mL glass column at a rate of 1.25 mL/min. The effluent was collected and stored in a refrigerator at 5 °C. Another AGMP-50 column was prepared by packing a 1.2 L glass column with 1 L of resin. The precipitated HA was freeze-dried and dissolved in 0.1 M NaOH solution to yield a concentration of 100 mg/L. It was pumped through the AGMP-50 column at a rate of 1.25 mL/min. The effluent was collected and was stored in a refrigerator at 5°C. Subsequently, both the HA and FA solutions were freeze-dried. Freeze-dried HS were used to make 1.5 L of 250 mg/L stock solution.

4.2.2 Preparation of E. coli

Microbiological sample preparation were employed based on Public Health Ontario’s best practices under the guidance of the laboratory staff at Public Health Laboratories (PHL) in Kingston, Ontario. American Type Culture Collection’s (ATCC) E. coli 112299 strain was used for all experiments. This strain was recommended by ATCC as it is commonly used as an indicator organism in disinfection studies. An initial volume of 1 mL of BD Difco’s nutrient broth, previously autoclaved, was transferred into a vial containing freeze dried stock of ATCC 11229, rehydrating the pellet. The rehydrated pellet was then aseptically transferred into a 5mL broth tube containing nutrient broth. Two of Oxoid’s blood agar (BA) plates were loop inoculated with this culture, as a backup in case of future bacterial contamination of the stock solution.
Both the stock solution and the BA plates were incubated at 37 °C for 24 hours.

A new culture was prepared daily for experiments from the stock solution. The night before the experiment, a 5 mL broth tube filled with 3 mL of nutrient broth was inoculated with 300uL of the stock culture. The broth tube was then placed on a shaker table over night at a temperature of 20 °C. In the morning, before the experiment, another broth tube was filled with 3 mL of nutrient broth that had been previously warmed to 37 °C. 300 uL was extracted from the overnight culture, and the 5 mL broth tube was placed on a shaker table inside a VWR 1915 Reach-In incubator. The culture was given 30 minutes to reach the log growth phase. Afterward, optical density of the culture at 600 nm was measured using a Nanodrop 2000c spectrophotometer by Thermoscientific, to ensure target concentrations were reached. The conversion of an $OD_{600}$ value of 1 is equivalent to $1.0 \times 10^9$ was used as a rough guide to ensure concentrations were at the targeted level (\(?\)). This method consistently produced concentrations of $5 \times 10^8$ CFU/100mL ($+/−2 \times 10^8$) measured by the spectrophotometer, matching the average concentration of $E. coli$ measured in Pond Inlet’s raw sewage.

$E. coli$ enumeration was carried out via the membrane filtration method according to Standard Methods for the Examination of Water and Wastewater (American Public Health Association (APHA), 2005). Merck millipore’s 0.45 um 47 mm gridded membranes were used to filter sample previously diluted in 10% peptone solution. Synthetic wastewater samples were diluted by a factor of $10^{-2}$, $10^{-3}$, and $10^{-4}$. For initial $E. coli$ concentrations, 9 samples of 10 mL each were filtered. For final $E. coli$ concentrations, after the experiment had finished, 45 samples were filtered. 10 150 mm agar plates were prepared with Oxoid’s m-FC dehydrated agar with aniline blue.
4.2. MATERIALS AND METHODS

After filtering, membrane filters were placed on the plates and incubated at 37 °C for 24 hours.

4.2.3 Disinfection experiment procedure

Triplicate vessels (50 mL Fisherbrand falcon tubes) containing synthetic wastewater with different HS concentrations (0, 10, 20, and 30 mg/L) were inoculated with 100 uL of previously prepared *E. coli* culture. Synthetic wastewater was used in order to ensure consistent initial HS concentrations. Note that each concentration of HS was tested in triplicates. In addition, triplicates of a dark control covered in aluminum foil with a HS concentration of 10 mg/L were included. The dark control provided the disinfection rate of mechanisms that occur independent of light. Variables (pH, DO, temperature, irradiance) were previously adjusted according to the sample plan, which is presented in detail in Appendix A and summarized in Table 4.2. After inoculation, samples were extracted from 3 sample vessels to obtain an average initial concentration. The experiment was performed over 2 hour period. Subsequently, samples were taken from each of the 15 vessels. Samples were then membrane filtered as described above. As previously mentioned, dilution series were used to ensure the samples would be in the enumerable range of 30-300 CFUs per membrane filter (Breed and Dotterrer, 1916). Note that more detailed laboratory procedures can be found in the Appendix.

4.2.4 Statistical analysis

Disinfection was quantified by mortality rate and was calculated by rearranging Chick’s Law, shown in Equation 4.4.
4.2. MATERIALS AND METHODS

\[
k = -\frac{ln(C)}{C_o}t
\]  \hspace{1cm} (4.4)

Where \(C\) is final \(E. coli\) concentration in CFU/100mL, \(C_o\) is initial concentration in CFU/100mL, and \(t\) is time in hours (Andrianarison et al., 2010). Please note that in this study, growth and death rates refer to the number of new \(E. coli\) cells or deaths, respectively, while net growth rate, disinfection, or mortality rate refer to the overall rate, i.e., the death rate minus the growth rate.

A \(2^k\) factorial design was implemented to ensure that interactions between sunlight, pH, DO, temperature and HS could be delineated (Montgomery, 2013). The \(2^k\) factorial experimental design is an efficient way of collecting data, while enabling examination of interaction effects of the independent variables (pH, DO, temperature, and depth-averaged irradiance) on the dependent variable (mortality rates). The two levels employed for each of these parameters are shown in Table 4.2, and the different combinations of these parameter levels for the experiments can be seen in Table 4.3. These ranges were determined from literature values and from field testing of a WSP in Nunavut.

Multiple linear regression including interactive terms was used to describe the relationship between the predictor and response variables. Depth-averaged irradiance, pH, DO, and temperature were tested in different combinations of additive and multiplicative relationships. The best models were determined through stepwise regression using ordinary least squares. Backward elimination was used, starting with all potential variables and interaction terms and testing the deletion of each (Quinn and Keough, 2002). The adequacy of a model fit was evaluated on the basis of four criteria:
Table 4.2: Parameter ranges for $2^k$ factorial design of experiments. The high and low value of these parameters were tested in 16 different combinations.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low (-)</th>
<th>High (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>DO ($mg/L$)</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Depth-averaged irradiance ($W/m^2$)</td>
<td>40</td>
<td>90</td>
</tr>
<tr>
<td>Temperature ($^oC$)</td>
<td>7</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 4.3: Combinations of parameter testing levels for experiments according to $2^k$ factorial design.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>pH</th>
<th>DO</th>
<th>Depth-averaged irradiance</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
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<td>14</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1. p-value of regression coefficients from null hypothesis test

2. p-value of the model

3. Adjusted Pearson correlation coefficient for the model fit

4. Explanation of underlying mechanisms

At each step, p-values were evaluated to determine which predictor terms could be removed, and the adjusted $R^2$ value was noted before and after the term was
removed. Predictors and interaction terms were eliminated if they scored a p-value above 0.10. As opposed to Pearson’s squared correlation coefficient which favours models with more predictor terms, the adjusted correlation coefficient is a non-biased indicator of model fitting by incorporating the number of predictor variables in its calculation (Quinn and Keough, 2002).

For the HS experiments, unpaired two sample t-tests were used to assess significant differences of mortality rates with 10, 20, and 30 mg/L of HS with the control, 0 mg/L of HS. A Pearson’s Chi-Square goodness of fit test was used to ensure the normality assumption of the t-test was satisfied.

4.3 Results and Discussion

4.3.1 Cold climate model

Observed data

The mortality rates measured in the experiments ($k_{meas}$) are shown in Figure 4.3. They compared reasonably with previous studies focusing on *E. coli* disinfection (Ouali et al., 2014; Kadir and Nelson, 2014). Rates were typically lower ($-0.8198$ to $1.1057 \text{ h}^{-1}$) when compared with previous studies such as Ouali et al. (2014) ($0.047$ to $1.81 \text{ h}^{-1}$). This was expected, as pH, DO, irradiance and temperature were at lower ranges in the experiments conducted for this work compared to previous studies. Interestingly, the highest disinfection rate of $1.1057 \text{ h}^{-1}$ was observed at maximum levels for pH, DO and depth-averaged irradiance, but at the lowest level for temperature, as shown in Figure 4.3. It was expected that the maximum disinfection rate would be observed when each parameter was set to a maximum. The lowest disinfection rate (or net growth rate), $-0.8198 \text{ h}^{-1}$, did not occur when all parameters were
4.3. RESULTS AND DISCUSSION

Table 4.4: The model and parameter values determined by stepwise regression. Details of the t-test on individual model parameters and the overall model are included, as well as the adjusted $R^2$ coefficient.

$$k_{\text{calc}} = k_d + k_T \theta(T-20) + ((k_{\text{pH,DO,I}} DO + k_{\text{pH,I}})I + k_{\text{pH,T}} \theta(T-20))pH$$

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimate</th>
<th>p-value</th>
<th>$R^2$</th>
<th>Model p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_d$</td>
<td>0.035768</td>
<td>&lt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_T$</td>
<td>-3.0262</td>
<td>&lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{\text{pH,DO,I}}$</td>
<td>$6.7838 \times 10^{-5}$</td>
<td>&lt; 0.1</td>
<td>0.60</td>
<td>$&lt; 5 \times 10^{-10}$</td>
</tr>
<tr>
<td>$k_{\text{pH,I}}$</td>
<td>$-1.5961 \times 10^{-4}$</td>
<td>&lt; 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{\text{pH,T}}$</td>
<td>0.32719</td>
<td>$&lt; 5 \times 10^{-12}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

set to a minimum; it was observed when all parameters, other than pH, were at their maximum level. These results are discussed in further detail in the section below.

Multiple linear regression

Initially, 4 predictor variables and 16 interaction terms were included in the analysis, representing all possible interactions of predictor variables. The stepwise regression reduced the variables and interaction terms and yielded Equation 4.5. Detailed results, including p-values of the model fit and the parameters, are shown in Table 4.4.

$$k_{\text{calc}} = k_d + k_T \theta(T-20) + ((k_{\text{pH,DO,I}} DO + k_{\text{pH,I}})I + k_{\text{pH,T}} \theta(T-20))pH$$  (4.5)

Where $k_{\text{calc}}$ is the calculated mortality rate in $(h^{-1})$, $k_d$ is the dark disinfection rate in $h^{-1}$, $\theta$ is the unit-less sensitivity coefficient set to 1.19, $T$ is the temperature in $^\circ C$, and $DO$ is dissolved oxygen in $mg/L$.

In Table 4.4, it can be seen the model fit was statistically significant with a p-value of $< 5 \times 10^{-10}$. A scatterplot of the observed and predicted results, displayed in Figure
4.3. RESULTS AND DISCUSSION

4.3. shows the model fit the data relatively well, furthermore, it was conservative in its estimate of the mortality rate when compared to the observed data. The $R^2$ value indicates a good fit, however, some of the variability not captured by the model could be due to the different HS concentrations and physical disinfection processes not included in the model. HS concentration was not used as a predictor in the model for future validation and potential use. Including HS in the model would make it impractical for future use, as the monitoring of HS in wastewater can be time consuming and costly. However, the relationship between HS and disinfection rates will be investigated further in section 4.3.2. Although physical settling processes likely play a more important role when sunlight-mediated disinfection is limited by low temperatures, light and pH (Mayo, 1995; Maynard et al., 1999), they were not measured in the experiment and therefore not included in the model.
4.3. RESULTS AND DISCUSSION

Figure 4.3: Mortality rates measured in the disinfection experiments plotted with mortality rates predicted by the fitted cold-climate model, in units $h^{-1}$. The observed mortality rates are an average of triplicate measurements.

Figure 4.4: A residual plot of mortality rates predicted by the model subtracted from observed mortality rates in $h^{-1}$. The observed mortality rates are an average triplicate measurements.
The residual plot shown in Figure 4.4 and the histogram in Figure 4.5, supports the principal assumptions justifying the use of multiple regression for the purposes of inference or prediction were satisfied. The random distribution of residuals with no evident trends (such as a wedge-shaped pattern) indicated heterogeneity of variance. There are no clear outliers in the data, indicating that the parameter estimates of the model were not being disproportionately influenced by extreme observations. In addition, there is no skewness in the residual histogram. Error should be random with a normal distribution as this demonstrates there are no systematic errors affecting the regression coefficient estimates (Quinn and Keough, 2002).

The model reflects that pH is crucial in *E. coli* disinfection, as it interacts with every other predictor variable. In Equation 4.5, the multiplicative relationship of pH with DO, depth-averaged irradiance and temperature would suggest its importance.
in sunlight-mediated disinfection. Figure 4.3 displays the strong influence of pH on mortality rates that were observed in the experiments. Previous literature suggested that after the membrane of a pathogen cell has been damaged by an ROS, a high pH in the wastewater could kill a pathogen by raising its internal pH of outside of its viable range (Curtis et al., 1992b; Davies-Colley et al., 1999; Craggs et al., 2004; Ouali et al., 2014). The pH level in wastewater essentially acts as an on and off switch for \textit{E. coli} disinfection. Past studies have found that \textit{E. coli} disinfection was greatly enhanced at pH above 8.5 (Davies-Colley et al., 1999; Ouali et al., 2014; Kadir and Nelson, 2014), while below 8.5, disinfection was minimal. This pattern was also observed in the two levels of pH tested in the experiment.

The relationship between temperature and disinfection has been difficult to describe explicitly by previous models, and there have been a number of conflicting findings, as noted in Chapter 2 (Marais, 1974; Klock, 1971; Flint, 1987; Mancini, 1978; Pearson et al., 1987; Curtis, 1990; Auer and Niehaus, 1993; Mayo, 1995; Ouali et al., 2014). In this study, the data and the model suggested that temperature played multiple roles in disinfection. From a biological and chemical process perspective, temperature likely serves as a positive influence in disinfection by increasing the reaction rates between the interactions of light, HS, DO and pH. This may be the underlying mechanism captured by $k_{pH,T}$ in Equation 4.5. However, at higher temperatures approaching 37 °C the metabolism of mesophilic bacteria such as \textit{E. coli}, also increases and as long as nutrients are readily available, will enhance their growth rates. The growth of \textit{E. coli} might be explained by $k_T$ in Equation 4.5. This would support the observations in the measured data. The highest mortality rate was observed when temperature was set to a minimum, but all other parameters (pH,
DO, depth-averaged irradiance) were at their maximum level. This would suggest that in the operating ranges of a cold-climate WSP, a colder temperature that would inhibit the growth of bacteria, in combination with sunlight-mediated disinfection mechanisms, would produce the highest overall mortality rate. Note that to the author’s knowledge, this is the first WSP disinfection model that includes both growth and death rates. An increase in *E. coli* concentration, rather than a reduction, was observed in Pond Inlet’s WSP over the course of the treatment season, as noted in Chapter 3. The increase in concentration could not be attributed to the addition of sewage and a zero disinfection rate alone, or the Xu et al. (2002) model, which essentially predicted a mortality of zero, would have more accurately captured the concentrations as shown in Figure 3.5 and 3.6 (Xu et al., 2002). The increase in *E. coli* concentration was likely due to growth, rather than simply a mortality rate of zero.
Figure 4.6 illustrates different conditions that should be considered for WSPs operated in cold climates. Scenario A displays ideal conditions in a Northern WSP, likely observed in mid-treatment season when pH, DO, and irradiance are at a maximum. The model predicts peak mortality rates when depth-averaged irradiance, pH, DO and temperature are at a maximum. However, this does not correspond with the
maximum disinfection rate that was observed in the experimental data when temperature was at a minimum but all other parameters were set to their maximum level. Scenarios B and C represent conditions in a cold climate WSP at the beginning of the treatment season after spring thaw or at the end of the treatment season before freeze-up. The surface plot shows that adequate disinfection can occur under cold conditions, as long as low turbidity, and high pH and DO are maintained. This could be influenced by high algae concentrations for promoting disinfection in WSPs (Ansa et al., 2012). Scenario D represents a case with low DO, but moderate pH. This could represent a situation where BOD is high, contributing to the low DO concentration, but there is algal activity keeping the pH at a moderate level. This scenario was observed in Pond Inlet at the beginning of the 2015 treatment season. As shown in Figure 4.6, a clear pond, with low turbidity, would maximize the disinfection rate in this scenario. Moreover, it would likely increase algal growth, increasing pH and DO in the process.

Figure 4.7 displays the results of the comparative analysis presented in Chapter 3 including the cold climate model derived in this chapter. Overall, the model did not accurately predict fecal coliform concentrations in the WSP. This was expected, as the model in this study focused on environmental parameters contributing to sunlight-mediated disinfection mechanisms specifically, which were likely minimal in this pond due to limited sunlight penetration. This may have led to an under prediction of disinfection performance. Physical treatment mechanisms, like attachment and sedimentation, should be incorporated into models for predicting performance in single-stage WSP systems, like the system in Pond Inlet, as these are likely the
predominant disinfection mechanisms (Mayo, 1995; Maynard et al., 1999). In addition, the model was developed using synthetic wastewater with a single strain of *E. coli*, and may not accurately represent real wastewater samples containing a range of fecal coliform species and other organisms. For instance, the synthetic wastewater did not contain any predators or competitors that would have increased the value of the dark disinfection rate \( k_d \). It is expected that this model would predict coliform concentrations more accurately in an Arctic WSP functioning as a maturation pond. For future validation of this or other cold climate models, more data collection at a variety of WSP systems would be beneficial and is highly recommended.

Figure 4.7: Observed fecal coliform concentrations (CFU/100mL) in the WSP in Pond Inlet, NU, compared with predicted concentrations by various disinfection models, including the cold climate model derived in this study.
4.3.2 Humic substances and disinfection mechanisms

HS concentrations were adjusted to explore their effect on treatment performance. Figure 4.8, Table 4.5 and 4.6 show six of the most important scenarios to consider based on the results of this study. It should be noted that in each scenario, the intercept represents mortality rate resulting from the UV Oxygen independent, endogenous photo-oxidation and any other dark disinfection mechanisms, as there were no HS to facilitate exogenous photo-oxidation. At 10, 20, and 30 mg/L a combination of the 3 sunlight-mediated disinfection mechanisms were prevalent, along with the dark disinfection mechanisms.
Figure 4.8: Humic substances vs. mortality rate under various levels of pH, DO, temperature and depth averaged irradiance. The mortality rates were measured in triplicates. The error bars are 2 x the standard deviation.

It would appear that HS relationship with disinfection rate is much more complex than previously suggested in the literature (Curtis et al., 1992b; Davies-Colley et al., 1999). Initially, it was believed that increasing HS concentration would simply increase disinfection rate, as there would be more sunlight energy captured for photo-oxidation. The results shown in Figure 4.8 and Table 4.5, illustrate this was not always the case. The effect of HS concentrations on mortality rate seems to be
influenced by the other environmental conditions affecting photo-oxidation (pH, DO, depth-averaged irradiance, and temperature).

Table 4.5: The simple linear regression coefficients and results of t-tests for Scenarios A through F presented in Figure 4.8. Testing levels of parameters (pH, DO, temperature, depth-averaged irradiance) are also provided. Refer to Table 4.2 for the exact value for each level.

<table>
<thead>
<tr>
<th>Level</th>
<th>Coefficient</th>
<th>Intercept</th>
<th>$R^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>pH + T + I + DO +</td>
<td>0.019173</td>
<td>0.2008</td>
<td>0.86</td>
</tr>
<tr>
<td>B</td>
<td>pH + T + I - DO +</td>
<td>-0.0051156</td>
<td>0.4255</td>
<td>0.87</td>
</tr>
<tr>
<td>C</td>
<td>pH - T + I + DO +</td>
<td>-0.0069604</td>
<td>-0.64768</td>
<td>0.90</td>
</tr>
<tr>
<td>D</td>
<td>pH + T + I + DO -</td>
<td>-0.010132</td>
<td>0.5576</td>
<td>0.53</td>
</tr>
<tr>
<td>E</td>
<td>pH + T + I + DO +</td>
<td>-0.0035428</td>
<td>0.93203</td>
<td>0.06</td>
</tr>
<tr>
<td>F</td>
<td>pH - T + I - DO -</td>
<td>0.00067523</td>
<td>-0.048644</td>
<td>0.22</td>
</tr>
</tbody>
</table>

In Scenario A, pH, temperature, depth-averaged irradiance and DO were at their maximum value (10, 20 °C, 90 W/m², and 8 mg/L, respectively). Under these conditions, 30mg/L of HS had a significant ($p < 0.01$) positive effect on disinfection performance when compared to the control as shown in Table 4.6. However, concentrations of 10 and 20 mg/L were found to have no statistically significant effect on disinfection performance.
4.3. RESULTS AND DISCUSSION

Table 4.6: The statistics from an unpaired t-test between the control (0 mg/L HS) and samples containing 10, 20 and 30 mg/L HS for each Scenario. Significant results ($p < 0.10$) are bolded.

<table>
<thead>
<tr>
<th>HS Concentration</th>
<th>10 mg/L</th>
<th>20 mg/L</th>
<th>30 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.26</td>
<td>-1.12</td>
<td>-5.93</td>
</tr>
<tr>
<td>B</td>
<td>0.69</td>
<td>0.42</td>
<td>1.11</td>
</tr>
<tr>
<td>C</td>
<td>0.28</td>
<td>2.15</td>
<td>2.34</td>
</tr>
<tr>
<td>D</td>
<td>0.99</td>
<td>0.84</td>
<td>2.52</td>
</tr>
<tr>
<td>E</td>
<td>-0.59</td>
<td>-1.08</td>
<td>1.62</td>
</tr>
<tr>
<td>F</td>
<td>0.27</td>
<td>0.12</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

In Scenarios B, C, and D, the effect of HS on disinfection was not found to be consistent to the behaviour observed for Scenario A. In each of these Scenarios, one environmental parameter important for exogenous disinfection (pH, DO, depth-averaged irradiance, and temperature) was set to a minimum, thus limiting the anticipated success of exogenous photo-oxidation. Other studies have suggested that the presence of HS may be able to counteract the negative effect of turbidity on disinfection (Curtis et al., 1992b). This is because HS absorb light in the visible spectrum in addition to UV, and these wavelengths can penetrate deeper into a turbid WSP (Curtis et al., 1994; Craggs et al., 2004). However, in Scenario B, when the attenuation coefficient was high ($K_{PAR}$), and therefore depth-averaged irradiance low, HS were found to have no significant effect on mortality rate at any concentration.

In Scenario C, the pH was set to a minimum value and the growth rate of $E. coli$ was found to be higher than the death rate. Higher HS concentrations were noted to have a significant negative effect on disinfection at 20 and 30 mg/L as shown in Table
4.3. RESULTS AND DISCUSSION

4.6, as the success of exogenous photo-oxidation was greatly reduced at pH below 8.5. It is likely that HS was absorbing light to produce ROS, but with the pH below 8.5, *E. coli* could survive the damage to their cytoplasmic membranes. The disinfection rate at 0 mg/L was also much lower than the previous scenarios. This could have been due to a higher growth rate of *E. coli*, since in Scenario C, ideal conditions for growth were provided. Alternatively, it is possible that the higher pH played a role in disinfection other than exogenous photo-oxidation (Curtis et al., 1992b).

In Scenario D, limited DO availability likely restricted both of the photo-oxidation mechanisms. HS was noted to have a significant negative effect on disinfection performance at 30 mg/L (*p < 0.1*). With limited DO, production of ROS would be restricted as excited HS molecules that previously absorbed solar radiation would have a lower chance of colliding and transferring energy to a DO molecule. In this case, HS would emit the absorbed energy as heat returning to ground state (Smith, 2013). Therefore, UV energy absorbed by HS would be diverted away from oxygen independent UV disinfection effectively decreasing the mortality rate. Despite this, the disinfection rate was notably higher than in Scenario C, indicating some successful disinfection via endogenous and exogenous photo-oxidation.

Scenario E was shown to exhibit a particular advantage cold climate ponds have for disinfection. In this Scenario, the cold temperatures likely limited growth rates of *E. coli* but there were still adequate conditions (high pH, DO, depth-averaged irradiance) for sunlight-mediated disinfection mechanisms, increasing the mortality rate. However, HS concentrations were found to have no significant effect on disinfection. Reaction rates are generally limited at colder temperatures, by limiting the
number of collisions of molecules. Therefore, these results could indicate that photooxidation is restricted by cold temperatures, as there are several reactions between several compounds that are contingent for successful disinfection by this pathway.

In Scenario F, when all environmental parameters were set to a minimum, the disinfection rate was noted to be negligible, and no significant relationship with HS concentrations was observed. In this Scenario, it is likely that the colder temperatures were limiting reaction rates of exogenous and endogenous photo-oxidation, limiting death rates, but also limited the growth rate \( E. \text{coli} \). UV attenuation in these turbid conditions would likely limit oxygen independent disinfection as well. Hence the mortality rate was essentially zero.

This study demonstrated that higher HS concentrations can increase disinfection performance under appropriate environmental conditions for exogenous photo-oxidation. Therefore, controlling HS concentrations in cold climate WSPs could be useful in improving disinfection performance. Past studies have demonstrated that there is a positive correlation between HS and solids retention time (SRT) in activated sludge systems (Liang et al., 2007; Michael et al., 2015). It would be useful to investigate whether a similar relationship exists between HRT and HS concentrations in WSPs and if so, there should be a focus on defining a relationship between HRT and HS concentrations for WSPs. In addition, the relationship between HS concentrations should be studied at concentrations higher than 30 mg/L.

### 4.4 Suggested WSP configuration

Based on the Scenarios presented above, ideal WSP setup to maximize disinfection would include two WSPs in a treatment train. The first WSP would be a deep
primary pond to reduce turbidity via sedimentation, increasing wastewater clarity for
treatment in the second WSP. The final stage would be a shallow maturation WSP
with a large surface area, to maximize depth-averaged irradiance and temperature in
the pond. Additionally, this would serve as an ideal environment for algae growth,
which would increase pH and DO concentrations.

If increasing HRT increases HS concentrations, larger pond volumes should be
considered in the design of the two WSPs to improve disinfection. However, based on
the findings in this study, the ideal conditions for photo-oxidation would have to be
satisfied in order for increased HS concentrations to improve disinfection performance
(i.e. high water clarity, and high pH and DO). However, these conditions may not al-
ways be present in a cold climate pond. Alternatively, a holding pond with controlled
inflow from the primary settling pond, and controlled outflow into the maturation
pond could be installed. This could prove useful as a method to increase HS concen-
trations, as shown in Figure 4.9. This could add multiple levels of control. Firstly,
HS concentrations could be controlled by the HRT in the holding pond. Secondly,
disinfection performance could be controlled by the rate of wastewater inflow from
the holding pond into the maturation pond. For example, HS concentrations could be
increased in the maturation pond when algae concentrations (and therefore pH and
DO) are adequately high for exogenous photo-oxidation. Alternatively, inflow from
the holding pond could be stopped when conditions are not optimal for exogenous
photo-oxidation. This configuration could optimize disinfection performance. Hence,
pilot-scale testing using this configuration should be investigated further.
4.5 Conclusion

The results of the experiment showed that the effectiveness of photo-oxidation is greatly influenced by pH, DO, temperature and light. Light attenuation could be reduced by reducing turbidity to improve photo-oxidation in WSPs. Temperature was shown to have a more complex role in disinfection than previously reported, affecting both growth and death rates of *E. coli* ATCC 11229. Low temperatures were shown to improve net mortality rate, likely by decreasing *E. coli* growth rate.

Figure 4.9: A schematic of a suggested WSP arrangement to potentially increase disinfection performance.
In addition, it was determined that future cold climate disinfection models should be developed with real wastewater samples and should incorporate physical treatment processes for predicting treatment performance in poor functioning WSPs.

HS concentrations were shown to significantly affect photo-oxidation at concentrations of 30 mg/L, and could be employed to improve disinfection performance if the environmental parameters (pH, DO, depth-averaged irradiance) are within the range that would promote exogenous photo-oxidation. Further investigation into the relationship between SRT, HRT, and HS concentrations, as well as the effect of HS concentrations on disinfection performance at the pilot scale should be pursued. Developing an understanding of these relationships could eventually provide a low-cost solution to those Northern Indigenous communities that are having difficulty in adequately treating their wastewater to yield safe effluent.
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Davies-Colley, R. J., Donnison, a. M., Speed, D. J., Ross, C. M., and Nagels,


Chapter 5

Summary and Conclusions

5.1 Summary

The research in this thesis evaluated the effects of cold climate conditions and humic substances on naturalized disinfection performance in Arctic wastewater stabilization ponds (WSPs) by:

1. Evaluating the ability of existing models to represent disinfection in cold climate WSPs, comparing their predictions with observed data collected at a WSP in Pond Inlet, NU, during the 2015 treatment season.

2. Investigating sunlight-mediated disinfection mechanisms under cold climate conditions to better understand the influence of pH, dissolved oxygen (DO), depth-averaged irradiance, and temperature on disinfection performance. This culminated in a new empirical model describing sunlight-mediated disinfection in cold climate WSPs.

3. Studying the effect of varying humic substances (HS) concentrations on sunlight-mediated disinfection mechanisms.
The purpose of this work was to better understand sunlight-mediated disinfection in cold climate WSPs, as they are widely utilized by Indigenous communities in Northern Canada (Neegan Burnside, 2011). A deeper understanding of naturalized disinfection could help in making evidence-based recommendations supporting the more effective use of WSPs in these communities.

The first study compared the ability of existing models in describing cold climate disinfection performance by comparing their predictions of mortality rates with rates observed in a single stage WSP in Pond Inlet, NU during the 2015 treatment season. The results of this study demonstrated that current models can have limitations in representing disinfection performance in Arctic WSPs, stemming from the fact that they were developed and calibrated using data from temperate and tropical locations. The models either exhibited difficulties forecasting trends or replicating concentrations of fecal coliform bacteria observed in the WSP, or both. Existing models would need to be adapted or a new model developed specifically for Arctic and other higher latitude locations that consider relevant parameters (ie. depth-averaged irradiance, pH, dissolved oxygen, temperature) and are calibrated with appropriate ranges of those parameters. It was noted that the environmental and operational conditions in Pond Inlet’s WSP could be limiting sunlight-mediated disinfection, which has been reported as the predominant disinfection mechanism in well-functioning WSPs (Curtis et al., 1992; Davies-Colley et al., 1997, 1999; Craggs et al., 2004; Maïga et al., 2009; Ouali et al., 2014; Kohn et al., 2016; Young et al., 2016). This could have been the explanation for the poor overall disinfection performance observed for the WSP.

The results from the first study were considered in the design of the experiment and for developing a sunlight-mediated disinfection model for cold climate WSPs. A
novel experimental setup was developed explicitly for simulating the conditions in an Arctic WSP at a bench-scale level by controlling pH, DO, depth-averaged irradiance and temperature. The experiments were based on a $2^k$ factorial design, which was a different approach in the investigation of WSP disinfection. $2^k$ factorial designs are an efficient way of collecting data, enabling examination of interaction effects of independent predictor variables on the dependent variable (Montgomery, 2013).

The numerical model presented in this thesis was developed using multiple linear regression. The resulting model fit to the data from the experiment was significant ($p < 5 \times 10^{-10}$), and showed a reasonably good fit, with an adjusted $R^2$ value of 0.60. The model was used to better describe and summarize sunlight-mediated disinfection of WSPs in high latitude locations. From this study, the following conclusions were surmised:

- It was confirmed that pH has a large influence on mortality rates of *E. coli*.

- Turbidity could be controlled in WSPs to improve photo-oxidation, by increasing sunlight penetration.

- The presence of algae likely has an important role in providing sufficient pH levels and DO concentrations to promote successful exogenous photo-oxidation. Therefore, disinfection performance may be closely linked to algae growth.

- Temperature has a more complex relationship with disinfection than previously thought, likely affecting both growth and death rates of *E. coli*. Algae growth is affected by temperature, which could add to the complexity of this relationship (Maassarani, 2015).
In addition to the above, the effect of HS concentrations on disinfection was investigated. Higher HS concentrations were found to improve disinfection performance when the conditions supporting exogenous-photo-oxidation were present (i.e., high pH, DO, and depth-averaged irradiance). However, under non-ideal conditions for exogenous photo-oxidation, HS concentrations had varying and less predictable effects on disinfection performance. In some cases, higher HS concentrations were found to reduce the mortality rates of *E. coli*.

### 5.2 Future work

The disinfection performance of WSPs appears to be closely linked to climatic conditions, which could explain inconsistencies and reliability issues. However, identifying and enhancing controllable variables could be useful in improving the treatment performance of these systems. One recommendation to improve disinfection performance would be that single stage WSP designs be minimized in the future. The turbidity in single stage WSPs wastewater reduces the potential for successful sunlight-mediated disinfection; adding another WSP into the configuration would reduce turbidity, increasing depth-averaged irradiance, thereby improving disinfection. In addition, this may increase algal growth which would likely increase pH and DO levels in the process, further improving disinfection performance.

The model presented in Chapter 4 was developed using bench-scale tests. This was useful in providing insight into sunlight-mediated disinfection under cold climate conditions, but its applicability for predicting performance in full-scale systems may be limited. In the future, field testing focusing on the disinfection performance of
a variety of temperate and Arctic WSP systems should be undertaken. A predictive model could be designed, calibrated and validated using the field data, while considering the findings of this study. Some important factors that should be considered and incorporated into a disinfection model with broader application are physical pathogen removal, competition, predation, and starvation. Physical removal via attachment and sedimentation, may be the predominant disinfection mechanisms in poorly functioning or single-stage WSPs (Mayo, 1995; Maynard et al., 1999). For these situations, incorporating physical removal into cold-climate WSP models may be useful for predictive purposes. Using real samples of wastewater, rather than synthetic wastewater, would inherently capture the effect of competition and predation embedded in the "dark disinfection rate" term, as real wastewater would likely include competitors and predators of the indicator organisms. Additionally, there is a knowledge gap concerning the effect of nutrient availability on disinfection, specifically as it relates to the role of temperature in disinfection. Under WSP conditions where organic compounds and nutrients are limited, the effect of temperature on disinfection may increase by promoting faster consumption of these limited resources by organisms (Auer and Niehaus, 1993). Further experiments could be completed to delineate the effects of nutrient availability and temperature on pathogen survival.

Finally, the effect of HS concentrations on disinfection performance should be studied at a pilot-scale level, as suggested in Chapter 4. Hydraulic retention time (HRT) in WSPs should be explored as well, as there is minimal literature focusing on the environmental and operational factors affecting HS concentration in wastewater.
5.3 Engineering contributions

The work presented in this thesis provides two contributions to the field of environmental engineering. Firstly, a better understanding of environmental parameters and their interactions leading to naturalized disinfection was described, culminating in a numerical model of sunlight-mediated disinfection for cold climate conditions. One of the most important outcomes arising from this research was a new, more detailed description of the role of temperature in disinfection, building on the work of previous studies. Secondly, an investigation into the role of HS concentrations on *E. coli* disinfection provided new insight into how this photosensitizer may enhance naturalized disinfection performance as a potential low-cost, easily implemented engineering solution. Another novel feature was the bench-scale experimental setup, particularly the controlled atmosphere chamber (CAC), which allowed for the control of environmental parameters such as pH, DO, depth-averaged irradiance, and temperature to simulate conditions in Arctic WSPs. It also made a $2^k$ factorial experimental design feasible, since each environmental parameter could be independently controlled. In the future, this setup could be used to simulate WSP conditions in different climates, especially when collecting field data is not viable.

The knowledge developed through this study could prove beneficial to helping Indigenous communities in Canada improve public health and environmental safety of wastewater effluent and ultimately protect their drinking water sources.
Bibliography


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Appendix A

Controlling wastewater disinfection variables in a bench-scale experiment

A.1 Introduction

Sunlight mediated disinfection mechanisms have been demonstrated to be the most effective pathogen removal processes in wastewater stabilization ponds (WSPs) (Mayo, 1990; Curtis et al., 1992b; Davies-Colley et al., 1997, 1999; Craggs et al., 2004; Maïga et al., 2009; Ouali et al., 2014; Kohn et al., 2016; Young et al., 2016). Developing disinfection models to further a practical understanding of how these sunlight mediated processes work has been studied over the past 25 years. This has been accomplished through both bench scale experiments and field testing. However, these studies have been conducted in temperate or tropical climates or conditions, and their applicability to cold climate WSPs may be limited. Many Northern Indigenous communities across Canada utilize WSPs as their sole wastewater treatment method. This is due to WSP’s practicality in small remote communities: they have very low operational costs and require very little maintenance. WSP performance is variable in cold climates which is a risk to source drinking water by potentially acting as a vector to spread
disease. For the purpose of source water protection (SWP), treatment performance, especially disinfection, in cold climate WSPs should be studied more extensively in the interest of public and wildlife health.

Field studies to measure disinfection performance in cold climate WSPs are logistically challenging and expensive, like most scientific studies conducted in the North. Moreover, there may not be enough variability of the parameters affecting disinfection over the course of the short treatment season to collect a useful data set for model calibration and validation. So recreating polar conditions in a lab setting may be the most practical and useful way to collect data, until there is a better understanding of cold climate disinfection. This way, one can delineate the effect of different variables on disinfection by independently controlling their intensity or concentration. An experiment recreating cold-climate WSP conditions was designed considering variables shown to be the most influential in past disinfection studies (Mayo, 1990; Curtis et al., 1992b; Davies-Colley et al., 1997, 1999; Craggs et al., 2004; Maiga et al., 2009; Ouali et al., 2014; Kohn et al., 2016; Young et al., 2016):

1. Depth-averaged irradiance
2. pH
3. Dissolved oxygen
4. Temperature

This section focuses on how the specifics of the experimental design, and how each variable was controlled to best recreate cold climate conditions.
Table A.1: Variable ranges for experiment as determined by Pond Inlet’s single-celled WSP

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7 – 10</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>2 – 8</td>
</tr>
<tr>
<td>Downwelling irradiance (W/m²)</td>
<td>150 – 300</td>
</tr>
<tr>
<td>Attenuation coefficient (K_{PAR})</td>
<td>29m⁻¹</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>7 – 20</td>
</tr>
</tbody>
</table>

A.2 Methodology

The appropriate testing ranges of the variables previously listed were determined by the results of a field study at a single-celled WSP in Pond Inlet, Nunavut, in the summer of 2015. When deciding on appropriate testing ranges, this WSP was considered to be a poorly functioning cold climate pond due to the climactic conditions and the particular single-celled design of this WSP system. Therefore the variable ranges measured here were considered to be the bare minimum for cold climate WSPs. Table A1 displays the variable ranges that were decided upon and therefore the constraints for the design of the experiment.

A.2.1 Dissolved oxygen (DO)

In the past, researchers have controlled dissolved oxygen (DO) through sparging–bubbling inert gas such as nitrogen into wastewater samples (Davies-Colley et al., 1999; Hassell et al., 2009). However, this method lacks precision and control, and can become costly depending on the gas used. Other researchers have used the aerobic
metabolism of microbes present in wastewater samples to control dissolved oxygen concentrations (Curtis et al., 1992a). For this particular experiment this was not an option. Humic substance (HS) concentrations, one of the variables in the experiment described in Chapter 5, are directly affected by microbial activity (Liang et al., 2007). HS concentrations are found to have a positive correlation with retention time in the presence of microbes.

Hassell et al. (2009) presented a novel approach to controlling DO in experiments (Hassell et al., 2009). Instead of directly bubbling nitrogen gas into samples, the authors controlled atmospheric oxygen concentrations thereby manipulating surface aeration to control DO concentrations. Their fundamental idea was incorporated into the design. A controlled atmosphere chamber (CAC), shown in Figure A.1, was built out of 1/4” acrylic glass to control atmospheric oxygen and dissolved oxygen concentrations.
concentration in samples. Nitrogen gas flows into the CAC from a compressed air cylinder, mixing with the air inside the chamber. On the opposite side of the chamber there is outflow with a valve, along with a 10 PSI pressure relief valve. The gas mixture flows out of the chamber into a Pro O$_2$ analyzer by Nuvair, a device used to measure nitrox mixtures in dive tanks. This is an inexpensive substitute for a lab oxygen analyzer.

The relationship between atmospheric oxygen and dissolved oxygen was assumed to follow Equation A.1. Atmospheric oxygen concentrations were adjusted according to this equation for a target DO concentration.

$$\frac{20.9\%}{DO_{satisfaction}} = \frac{X}{DO_{target}}$$  \hspace{1cm} (A.1)

Where 20.9\% represents the percent composition of oxygen in the atmosphere, $DO_{satisfaction}$ is the saturated DO concentration under atmospheric conditions at a given temperature, X represents the target atmospheric percentage of oxygen, and $DO_{target}$ is the required dissolved oxygen concentration in the synthetic wastewater samples. Once the CAC reached the required atmospheric concentration, samples were allowed to sit for at least 12 hours to equilibrate.

### A.2.2 Depth-averaged irradiance

Sunlight intensity, turbidity, and depth of the WSP all control the effectiveness of sunlight mediated disinfection. These three factors are incorporated into the depth-averaged irradiance calculation, which calculates the amount of effective sunlight penetration in the WSP shown in Equation A.2.
\[
I = \frac{I_o}{KZ}(1 - e^{-KZ}) \tag{A.2}
\]

Where \(I_o\) is the downwelling irradiance at the surface of the pond, measured in \(W/m^2\), \(K\) is the attenuation coefficient in \(m^{-1}\), and \(Z\) is the depth of the WSP in m (Curtis et al., 1994).

A lighting system had to be designed to recreate the spectrum of sunlight at the surface of the WSP \((I_o)\). Two different setups were tested:

1. The photosynthetically active region (PAR) of the spectrum (400-700nm) simulated by a commercial Orphek Atlantik aquarium lighting system and the UV-A sunlight simulated with two 25 watt fluorescent UV lights.


These were compared for their ability to recreate Arctic sunlight and their ease of use.

The simulated sunlight had to travel through the lid of the CAC with as little attenuation as possible in order to disinfect the synthetic wastewater samples. Three different materials were tested for transmission of the UV spectrum: polycarbonate, acrylic and glass. All three materials tested had a thickness of 1/4”. The material transmitting the highest percentage of UV light was considered the most ideal for the lid of the CAC. The transmission of light was measured by Ocean Optics’ Jaz Spectrometer.

In order to vary the depth-averaged irradiance for the experiments, there had to be a variable in Equation A.2 that could be adjusted. Surface irradiance and depth
A.2. METHODOLOGY

Table A.2: Synthetic wastewater chemical composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>160</td>
</tr>
<tr>
<td>Meat extract</td>
<td>110</td>
</tr>
<tr>
<td>Urea</td>
<td>30</td>
</tr>
<tr>
<td>$K_2HPO_4$</td>
<td>28</td>
</tr>
<tr>
<td>$NaCl$</td>
<td>7</td>
</tr>
<tr>
<td>$CaCl_2\cdot2H_2O$</td>
<td>4</td>
</tr>
<tr>
<td>$MgSO_4\cdot7H_2O$</td>
<td>2</td>
</tr>
</tbody>
</table>

were fixed for this setup, so the attenuation coefficient was adjusted. This was accomplished by using synthetic wastewater with pure whey protein isolate acting as suspended solids. The ingredient list for the synthetic wastewater are shown in Table A.2 (Vélez-Colmenares et al., 2011). A calibration curve of whey protein isolate concentration versus attenuation coefficient was constructed for concentrations between 0-14 g/L. The Ocean Optic’s Jaz spectrometer was used to measure the downwelling irradiance of sunlight at depths of 0, 2, 4, 7 and 20 centimeters in a 60L tank filled with 40L of synthetic wastewater. This test was repeated three times. Subsequently, the attenuation coefficient was calculated by using Beer Lambert’s law:

$$ I = I_0 e^{(-Kz)} $$  \hspace{1cm} (A.3)

where, $I$ is the irradiance at a given depth $z$, $I_0$ is the downwelling irradiance measured just below the surface of the WSP or synthetic wastewater (Curtis et al., 1994). $I_0$ should be measured just below the surface so that any light that is reflected and not transmitted into the synthetic wastewater is not included in the calculation of
### Table A.3: $K_{PAR}$, $K_{UV}$ from past studies.

<table>
<thead>
<tr>
<th>WSP description/Author</th>
<th>$K_{PAR}(m^{-1})$</th>
<th>$K_{UV}(m^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-celled Arctic WSP in Pond Inlet, NU (Liu et al., 2017)</td>
<td>29</td>
<td>80</td>
</tr>
<tr>
<td>Median of 11 domestic sewage lagoons in New Zealand (Davies-Colley et al., 1995)</td>
<td>13</td>
<td>N/A</td>
</tr>
<tr>
<td>Pilot scale WSPs in Kazakhstan and the UK (Heaven et al., 2005)</td>
<td>5 – 25</td>
<td>N/A</td>
</tr>
<tr>
<td>WSPs in Dakota (Bartsch, 1961)</td>
<td>5.9 – 10.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Pilot-scale high rate ponds for dairy farm wastewater (Craggs et al., 2004)</td>
<td>14</td>
<td>N/A</td>
</tr>
</tbody>
</table>

the attenuation coefficient. This could lead to an overestimation of attenuation. Non-linear regression is used to find the coefficient. The attenuation coefficient for PAR light is disproportionately reported in literature. Accordingly, the PAR attenuation coefficient was used for the calibration curve and for the depth-averaged irradiance calculation, rather than the UV attenuation coefficient. Past studies have shown that scattering plays a minimum role in the extinction of light in WSPs, so upwelling irradiance was not included in these measurements (Curtis et al., 1994). A table of wastewater attenuation coefficients are shown in Table A.3. From the past studies, it was determined that PAR attenuation coefficients between 5-30 $m^{-1}$ is an appropriate range for wastewater.

#### A.2.3 pH

pH was adjusted with 0.1N NaOH and 0.1N HCl solutions according to the reading on a pH meter. The synthetic wastewater pH change over the course of the experiment was expected to be minimal, so no buffer was used.
A.3. RESULTS AND DISCUSSION

A.2.4 Temperature

The experiment setup was housed inside a VWR BOD Low temperature refrigerated incubator with temperature control. The samples were given at least 12 hours to reach the target temperature before running the procedure.

A.3 Results and Discussion

A.3.1 Dissolved Oxygen

Through trial and error, Equation A.1 was shown to hold true, with minor adjustments. Target DO levels were achieved with the system, however, lower DO concentrations required a longer equilibration time to reach target levels. For those samples, pre-sparging with nitrogen gas and then placing the samples in the CAC accelerated equilibration. This method could achieve DO concentrations from 2 to 9 mg/L with an accuracy between +/- 0.5 mg/L.

A.3.2 Depth-averaged Irradiance

CAC lid material

UV transmission through acrylic, polycarbonate and glass were measured. The results are shown in Figure A.2. Note that the original light source emission spectra was between 280 and 400nm, therefore anything above 400nm in the graph is likely noise or stray light captured by the Jaz sensor. Glass majorly outperformed acrylic and polycarbonate, transmitting the largest range of UV (330nm and above) as well as the highest transmission for each wavelength. It transmitted approximately 80 percent of the light with wavelengths larger than 350nm. Therefore, glass was selected as the
material used for the lid of the CAC.

![UV transmission test results with glass, polycarbonate and acrylic.](image)

**Figure A.2:** UV transmission test results with glass, polycarbonate and acrylic.

**Lighting system**

The 150 watt mercury light’s UV output far exceeded the requirements of the experiment. In addition, the high heat output from the lights made it extremely difficult, if not impossible, to control the temperature within the incubator. For these reasons, the mercury bulb proved to be impractical for the setup. The 100 watt Orphek Atlantik system augmented with fluorescent 25 watt fluorescent UV bulbs were found to give the closest UV and PAR spectral irradiance output when compared with the measured Arctic spectrum. The distance of the UV lights were adjusted to get the ideal irradiance. The UV irradiance of the system was 15W/m², which was exactly the average UV irradiance measured in the Arctic at noon in July and August. Moreover, the quality of the UV spectral irradiance was similar to the Arctic spectrum, as shown in Figure A.3.
The PAR irradiance from the system was $120\text{ W/m}^2$, which is below the 30th percentile of PAR sunlight irradiance measured in the Arctic in the summer of 2015. There are two reasons why this was acceptable for the design. Firstly, lower irradiance will produce conservative estimates of disinfection. Secondly, the Orphek Atlantik’s PAR output peaked at about 460nm, as shown in Figure A.4. This could have a biasing effect on the results, as UV radiation and the visible light below 500nm are thought to have a more pronounced effect on disinfection (Curtis et al., 1994; Davies-Colley et al., 1999). Increasing the irradiance of the Orphek Atlantik system (by decreasing the distance between the CAC and the lights) would have amplified this issue. Note the irradiance measurements were taken beneath the glass lid, so the attenuation from glass is included.
Attenuation coefficient

The linear regression of attenuation coefficient versus protein isolate concentration yielded a calibration curve with an $R^2$ value of 0.84, and is shown in Figure A.5 with the residual graph. Studentized t-residuals were used to eliminate any outliers in the data set. Outliers were considered to be any data points beyond 3 standard deviations. The relationship between attenuation coefficient and whey protein isolate concentration is given by Equation A.4. The residual plot shows non-homogeneity in the variance of the attenuation coefficients, especially toward higher concentrations of protein isolate. This was likely due to settling between the addition of the isolate and the measurement. A wait-time was necessary because froth formed along the surface of the synthetic wastewater from mixing in the isolate. This froth caused overestimates of the attenuation coefficient. The wait-time was inconsistent, based on the time required for the froth to dissipate and likely causing variability in the
concentration of suspended solids. It is expected that this effect would be more pronounced at higher concentrations of protein isolate. Weighted-least squares, a robust regression method, was used to minimize this effect on the regression estimation.

\[ K_{PAR} = 2.7x + 3.7 \]  \hspace{1cm} (A.4)

Where \( x \) is the concentration of whey protein isolate in g/L. The relationship is able to predict the required concentrations of protein powder for attenuation coefficients within the required range of 5-30\( m^{-1} \).

Figure A.5: Linear regression of the PAR attenuation coefficient versus concentration of whey protein isolate. The studentized residual plot reveals no outliers in the data.

A.3.3 pH

pH was found to remain stable through the 2 hour experiment run time. There were no observable changes noted between the initial and final pH.
A.3.4 Temperature

The greenhouse effect made temperature control within the CAC difficult. Despite the temperature in the incubator being at or below the target, the internal temperature of the CAC tended to be higher than the target temperature at the end of the 2 hour experiment. At the end of the experiment, the temperature was found to increase by approximately 5°C. Therefore the recorded temperature within the chamber is an average of the initial and final temperature reading of the synthetic wastewater. In the future, a water bath inside the CAC may help counteract this effect. The temperature change did not affect the DO content of the samples between the initial and final measurement.

A.4 Conclusion

As demonstrated, the experiment setup is capable of providing simulated Arctic conditions for the environmental variables of interest. This setup will provide the means to better understand cold climate disinfection in WSPs, which could inform design for new WSPs and upgrades to existing systems. This setup provides an alternative to the logistical challenges and costs associated with field studies on cold climate WSPs. Successful bench-scale experiments could help guide future field studies on cold-climate disinfection, improving time and cost efficiency.
Bibliography


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Appendix B

Laboratory procedure: Sunlight-mediated disinfection experiment

B.1 Materials

- N2 Compressed Air Tank
- Orphek Atlantik Lighting System
- UV lights
- Jaz Spectrophotometer
- Pipet/pipet tips
- Autoclave
- Filtration kit
- Membrane filters
- Standard plate counts
B.2 Procedure

At the beginning of each week, prepare synthetic wastewater and HS mixture according to the sample plan. Sterilize the synthetic wastewater before adding the HS (unless we determine the HS is contaminated).
1. Ensure all materials have been adequately washed and sterilized before begin-
ning.

2. Prepare culture for inoculation. If a culture could not be prepared the previous
night, note that inoculating with an old sample will take approximately 2 hrs
to reach target concentrations for sample.
   
   (a) Take 300ul of sample from the mother culture prepared previous day and
   add to 3mL of nutrient broth solution.

   (b) Take 100ul samples from subculture and add to cuvette. Measure the
   population growth with OD600 with a spectrophotometer at half an hour
   intervals (make sure to calibrate spec with blank before taking a mea-
   surement). The culture should enter log growth within the first half an
   hour.

   (c) Once the culture is in log growth and the target concentration of 10^7-10^8
   CFU/mL (target OD of max 0.05 b/c of dilution by 10) is met according
   to the OD600 readings, begin inoculating of samples. This should occur
   after a half an hour to 40 minutes.

3. While the culture is growing on the shake table, prepare microtubules for dilu-
   tion series with 900uL of 0.1% peptone solution (9 tubes for initial concentration,
   30-45 for final concentration). Also prepare 9 falcon tubes with 5mL of 0.1%
   peptone solution as a vector for membrane filtration of initial concentrations.

4. Open the chamber, inoculate 100uL into each sample of synthetic wastewater
   that was previously placed into the controlled air chamber in the incubator the
   night before to equilibrate and reach target temperature. Make sure to shake
the broth tube periodically while distributing to falcon tubes to ensure equal concentration. Make sure to stir the falcon tubes.

5. Quickly measure pH, temperature, and DO. Record these values in sample tubes C-1, 2-2 and 4-3. Wipe off DO probe afterward.

6. Take a 100uL sample from tubes C-1, 2-2 and 4-3. Do this as quickly as possible as to not alter the dissolved oxygen concentrations. Inoculate corresponding microtubules. Seal the chamber. Make sure there is no condensation on the glass lid.

7. Immediately turn on nitrogen tank and reach the target O2 atmospheric concentration with Nuvair’s Pro O2 meter. Make sure to consider the dependence of oxygen solubility on temperature.

8. Ensure the apparatus is positioned in the marked location and according to the layout (ie. dark control group on the left and sample group 4 on the right). Ensure the UV lights are placed correctly over the sample vessels.

(a) For temperature of 20°C, allow the incubator to reach 12 or 13°C before starting the experiment to help regulate temperature inside the CAC.

(b) For temperature of 5°C allow chamber to reach 0 to 1°C before beginning.

9. Turn on the Orphek atlantik light and UV lights

10. Allow samples to run for 2 hours.

11. While sample is running, prepare dilution series for the initial concentration (10-1, 10-2 and 10-3, possibly 10-4).
12. Vacuum filter 9 initial samples onto to 0.45 um mixed cellulose filters. Place the filters on the BCIG w/ analine blue plates. Incubate @ 37C for 18-24 hrs. Count colonies.

13. Prepare the 30-45 falcon tubes for membrane filtration. Use 5mL of peptone solution in each tube as a transfer solution. Add the 10-2 and 10-3 (possibly 10-4) dilutions.


15. After 2 hours, take 100uL sample from each vessel and inoculate corresponding dilution tube. Complete a dilution series for the final concentration (10-1, 10-2 and 10-3). Use previously prepared microtubules filled with 900uL of peptone solution. Transfer 100uL from each tube into the following dilution.

16. Measure the pH, temperature, and dissolved oxygen concentration reading immediately upon removing the sample vessel from the incubator. Sterilize and wash pH and DO/temp probe again.

17. Vacuum filter 30-45 final samples onto to 0.45 um mixed cellulose filters. Place the filters on the BCIG w/ analine blue plates. Incubate @ 37C for 18-24 hrs. Count colonies.

18. Distribute 35mL of synthetic wastewater with the appropriate HS concentration for next sample run.

   (a) Adjust the pH using 0.1N NaOH or 0.1N HCl and a pH meter. Record pH value.
19. Place new samples into the CAC. Place CAC into the incubator in the desired location under the lighting system. If run requires low DO concentrations, sparge each sample and place into the chamber (may not be required if samples are kept in controlled air chamber over night).

20. Attach gas cylinder, and open valve. Use the oxygen meter to reach target atmospheric oxygen concentration. Allow the samples to equilibrate to the target temperature and dissolved oxygen concentration overnight.

21. Adjust the temperature on the incubator to 5°C below the target temperature for the next sample run.

22. Wash falcon tubes and autoclave at 120°C for 20 minutes.

23. Prepare culture for the next day. 300uL in 3mL of nutrient broth solution, the shake table on low at room temperature overnight.
### B.2. PROCEDURE

<table>
<thead>
<tr>
<th>Run/Range</th>
<th>humic substances</th>
<th>pH</th>
<th>Dissolved oxygen</th>
<th>Attenuation Coefficient (K)</th>
<th>Surface Irradiance</th>
<th>Depth-averaged Irradiance</th>
<th>Temperature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30mg/L</td>
<td></td>
<td></td>
<td>7 to 10</td>
<td>2 to 8mg/L</td>
<td>3.7-30 m⁻¹·1</td>
<td>Orphek+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control, 0, 10, 20, 30</td>
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Factorial design considering 4 factors: pH, DO, sunlight and temperature. Humic substances are kept at consistent concentrations every sample run, so they are not included in the factorial design. Note, when anaerobic conditions are present, we are testing the Oxygen-Independent disinfection (UV disinfection). This can be seen in samples 9 through 16. In addition, when the HS = 0mg/L samples are a measurement of Oxygen-independent disinfection and Endogenous-photo-oxidation. Runs 1 through 8 are testing the cumulative disinfection of Oxygen Independent, Endogenous and Exogenous photo-oxidation. The control is to measure the dark disinfection rate which could include effects from pH and temperature. Triplicates are prepared for every concentration of humic substances per run, including the control.
Appendix C

Abbreviations and nomenclature
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AHS</td>
<td>aquatic humic substances</td>
</tr>
<tr>
<td>BAS</td>
<td>biologically activated sludge</td>
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<tr>
<td>BOD</td>
<td>biological oxygen demand</td>
</tr>
<tr>
<td>CAC</td>
<td>controlled atmosphere chamber</td>
</tr>
<tr>
<td>CBC</td>
<td>Canadian Broadcasting Company</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming units</td>
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<tr>
<td>cBOD</td>
<td>carbonaceous biological oxygen demand</td>
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<tr>
<td>CCME</td>
<td>Canadian Council of Ministers of the Environment (CCME)</td>
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<td>CSTR</td>
<td>continuously stirred tank reaction</td>
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<tr>
<td>DO</td>
<td>dissolved oxygen</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>DWA</td>
<td>drinking water advisory</td>
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<td>E. coli</td>
<td>Escherichia coli</td>
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<tr>
<td>FA</td>
<td>fulvic acid</td>
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<td>HA</td>
<td>humic acid</td>
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<td>HS</td>
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<td>INAC</td>
<td>Indigenous and Northern Affairs Canada</td>
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<tr>
<td>MBR</td>
<td>membrane bioreactor</td>
</tr>
<tr>
<td>NIRB</td>
<td>Nunavut Impact Review Board</td>
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<tr>
<td>NLCA</td>
<td>Nunavut Land Claims Agreement</td>
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<tr>
<td>NPC</td>
<td>Nunavut Planning Commission</td>
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<td>NWB</td>
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<td>PAR</td>
<td>photosynthetically active region</td>
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<td>PFR</td>
<td>plug flow reaction</td>
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<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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<td>SMP</td>
<td>soluble microbial products</td>
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<td>SRT</td>
<td>solids retention time</td>
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<td>source water protection</td>
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<tr>
<td>TSS</td>
<td>total suspended solids</td>
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<td>UV</td>
<td>ultraviolet</td>
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<td>VBNC</td>
<td>viable but non-culturable</td>
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<td>WSER</td>
<td>Wastewater Systems Effluent Regulations</td>
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<td>WSP</td>
<td>wastewater stabilization pond</td>
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A  substrate
$A^+$  oxidized substrate
$A_{ox}$  oxygenated substrate product
$C$  indicator bacteria concentration
$CO_2$  carbon dioxide
$h$  hour
$H^+$  hydrogen ions
$HCO_3$  bicarbonate
$H_2CO_3$  carbonic acid
$hv$  photon
$I$  Depth-averaged irradiance
$I(z)$  Irradiance at a depth z
$I_o$  total solar irradiance incident upon the WSP surface
$k$  mortality constant
$k_{20}$  mortality constant at $20^\circ C$
$k_d$  dark disinfection rate
$k_s$  irradiance only disinfection rate
$K$  light attenuation coefficient
$K_{PAR}$  light attenuation coefficient for PAR light
$K_{UV}$  light attenuation coefficient for UV light
$L$  Litre
$m$  metre
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<th>Symbol</th>
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<tr>
<td>$^3O_2$</td>
<td>triplet oxygen</td>
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<tr>
<td>$S_0$</td>
<td>ground state photosensitizer</td>
</tr>
<tr>
<td>$S^-$</td>
<td>reduced photosensitizer</td>
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<td>$^1S$</td>
<td>singlet state photosensitizer</td>
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<td>$S_{ox}$</td>
<td>oxygenated photosensitizer product</td>
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<td>$T$</td>
<td>temperature</td>
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<td>$t$</td>
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<td>Irradiance coefficient</td>
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<td>sensitivity coefficient</td>
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