THE EFFECTS OF CLIMATE ON MICROALGAE GROWTH IN ARCTIC WASTEWATER STABILIZATION PONDS

by

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A thesis submitted to the Department of Civil Engineering
In conformity with the requirements for
the degree of Master of Applied Science

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Abstract

The research presented in this thesis consists of work conducted in two studies designed to evaluate temperature effects on microalgal growth in Arctic wastewater stabilization ponds (WSPs). These systems are commonly used to treat municipal wastewater in small arctic communities, however, summer temperatures that rarely exceed 20°C could potentially limit microalgal growth. The presence of microalgalae in these systems has been suggested to enhance wastewater treatment efficiency due to the synergistic relationship between microalgae and heterotrophic bacteria, supporting the need to develop a better understanding regarding the effects of climate and summer temperatures on microalgal growth for areas north of the 54th parallel.

The first study investigated the effects of temperature on the growth of the microalgal strain *Chlorella vulgaris*. Experimental data were subsequently compared against three models developed to predict algae growth as a function of temperature. Growth rates were evaluated for a range of 1-23°C and experimental results showed a strong correlation between maximum microalgal growth rates and temperature, where maximum microalgal growth rates were found to be at their lowest at 1°C and peaked at 23°C. The experimental data were then compared to the maximum growth values predicted by three mathematical models: the Eppley Curve, an Arrhenius Relationship and the Peeters & Eilers model. The latter was deemed to be the most suitable model for microalgal growth rates in arctic WSPs, as it predicted the maximum growth rates of *C. vulgaris* reliably for the temperature range of 1-23°C. The second study involved the modification of an existing numerical model which was developed to simulate biochemical processes in WSPs. The Peeters & Eilers growth equation was incorporated into this numerical model, along with historical weather data from Pond Inlet, NU in order to characterize the effects of arctic temperatures on microalgal growth in WSPs from year to year.

The results of these studies suggest that microalgal growth in Arctic WSPs is highly susceptible to temperature, and solar radiation fluctuations, and that the presence of microalgae in these systems may contribute to wastewater treatment efficiency.
Co-Authorship

Rami Maassarani conducted the work presented in this thesis, with the assistance of co-authors who provided technical guidance and feedback with experimental work and results, reviewed manuscripts, and offered editorial comments and revisions for the various chapters. Chapters 3 and 4 are intended for publication in peer-reviewed journals or conference proceedings. Authorship and publication details are provided as follows:

- Chapter 3: Rami Maassarani, Pascale Champagne, Geof Hall
  - Manuscript to be submitted, in full, for publication in the Journal of Ecological Modelling, Elsevier

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  - Manuscript to be submitted, in full, for publication in the Journal of Ecological Modelling, Elsevier
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<td>A</td>
<td>Arrhenius constant</td>
</tr>
<tr>
<td>$a_s$</td>
<td>solar azimuth angle</td>
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<td>BMa</td>
<td>microalgae basal metabolism rate</td>
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<td>BOD</td>
<td>biochemical oxygen demand</td>
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<td>iron</td>
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<tr>
<td>$gC.m^{-3}$</td>
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<tr>
<td>H</td>
<td>hydrogen</td>
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<tr>
<td>$H^+$</td>
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<td>$h_s$</td>
<td>solar hour angle</td>
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$h_{sr}$  solar hour angle at sunrise

$h_{ss}$  solar hour angle at sunset

$H_2S$  hydrogen sulfide

HRAP  high rate aerobic ponds

$I_{\text{atm}}$  solar radiation intensity at the top of the atmosphere

$I_{\text{direct}}$  direct solar radiation intensity

$I_{\text{diff}}$  diffuse solar radiation intensity

$I_o$  photosynthetic active radiation intensity

$I_{\text{tot}}$  total solar radiation

$J$  joule

$G_a$  microalgae growth rate

$K$  potassium

$K_e$  wastewater light attenuation coefficient specific to microalgae

$K_e$  wastewater light attenuation coefficient

$K_m$  half saturation coefficient

KHN  nitrogen half-saturation concentration

KHP  phosphorous half-saturation concentration

$L$  liter

$\text{Lat}$  latitude

$\text{LN}$  limiting nutrient

$M$  air mass ratio

$m$  meter

$\text{min}$  minute

$\text{Mg}$  magnesium

$\text{mol}$  mole
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<td>nitrate</td>
</tr>
<tr>
<td>NPS</td>
<td>national performance standards</td>
</tr>
<tr>
<td>O</td>
<td>oxygen</td>
</tr>
<tr>
<td>$OH^{-}$</td>
<td>hydroxide ion</td>
</tr>
<tr>
<td>Opt$_{pH}$</td>
<td>optimal pH</td>
</tr>
<tr>
<td>P</td>
<td>phosphorous</td>
</tr>
<tr>
<td>$P_2O_7$</td>
<td>polyphosphate</td>
</tr>
<tr>
<td>PAR</td>
<td>photosynthetically active radiation</td>
</tr>
<tr>
<td>PAO</td>
<td>phosphorous accumulating organisms</td>
</tr>
<tr>
<td>$PO_4^{3-}$</td>
<td>orthophosphate</td>
</tr>
<tr>
<td>PRa</td>
<td>microalgae predation rate</td>
</tr>
<tr>
<td>Q</td>
<td>wastewater flow</td>
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<tr>
<td>R</td>
<td>universal gas constant</td>
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<tr>
<td>r</td>
<td>radius</td>
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<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>S</td>
<td>sulfur</td>
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<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>Si</td>
<td>silicon</td>
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</tbody>
</table>

xiii
\(S_o\)  
solar constant  

\(T\)  
temperature  

\(T_0\)  
temperature at which microalgae growth no longer occurs  

\(TC\)  
total cloud cover  

\(T_{opt}\)  
optimum temperature for microalgae growth  

\(TSS\)  
total suspended solids  

\(V Sa\)  
microalgae settling velocity  

\(\forall\)  
volume  

\(W\)  
watt  

\(WSER\)  
wastewater systems effluent regulations  

\(WSP\)  
wastewater stabilization pond  

\(\alpha\)  
solar altitude angle  

\(\beta\)  
shape factor  

\(\delta_s\)  
solar declination angle  

\(\tau_b\)  
radiation diffusion coefficient for direct radiation  

\(\tau_d\)  
radiation diffusion coefficient for diffuse radiation  

\(\mu\)  
growth rate  

\(s\)
Chapter 1

Introduction

1.1 Background

1.1.1 Municipal Solid Waste and Wastewater Streams

Since the industrial revolution began in the 18th century, a dramatic increase in manufacturing and urbanization has created an unprecedented production of Municipal Solid Waste (MSW). Reports estimate that in the last decade, the generation of urban waste has increased from 0.68 billion tonnes per year to 1.3 billion tonnes per year, with predictions that this figure may go up to 2.2 billion tonnes per year in the next twenty five years (Hoornweg & Bhada-Tata, 2012). The liquid portion of this waste is commonly referred to as wastewater and can be defined to include a combination of liquid or water-carrying wastes from residential, commercial or industrial sources. The chemical and biological composition of wastewater has been shown to stimulate the growth of aquatic plant life, potentially affecting the balance of any ecosystem into which it may be discharged. In order to protect the environment and public health, it is therefore important to have an understanding of the constituents of a wastewater stream, as well as the impacts that these constituents may have on a receiving environment, in both the long and short term (Tchobanoglous et al. 2014a).

1.1.2 Wastewater treatment

Two main approaches are typically used in wastewater treatment processes: active and passive treatments. Active treatment of wastewater involves the use of energy intensive physical and chemical processes that can be designed to meet specific treatment goals in a rapid and effective manner. Passive treatment systems are typically much less expensive than their active counterparts, with lower initial and operational costs as well as lower maintenance requirements, yet natural attenuation is the only approach that may be considered to be completely passive (Tchobanoglous et al. 2014a).
The definition of passive treatment can however be broadened to include naturalized systems such as constructed wetlands, biological filters and waste stabilization ponds (WSPs).

WSPs rely on chemical, physical and biological processes to break down nutrients and organic matter from the wastewater, and are dependent on environmental conditions such as temperature, wind and sunlight (Amengual-Morro et al. 2012). They have been proven to be successful applications in Canada and the USA (Heaven et al. 2007).

Depending on the type of waste stream, active and passive treatment processes can be combined into unit processes in series, which may be separated to perform different treatment functions known as preliminary, primary, secondary and tertiary treatment. Preliminary treatment removes large solids that might cause operational problems within the system, while primary treatment removes a large portion of the total suspended solids (TSS) and organic matter from the waste stream. Secondary treatment includes the treatment and removal of biodegradable organic material, while tertiary treatment typically involves the removal of any residual suspended solids by filtration, as well as nutrient removal (Tchobanoglous et al. 2014a)

1.1.3 Evolution of wastewater treatment policy

During the early 20th century, wastewater treatment primarily involved the removal of colloidal, suspended and floatable material, as well as the treatment of biodegradable organics and pathogenic organisms. In the early 1970’s, the implementation of the Clean Water Act (CWA) in the USA shifted wastewater treatment objectives to focus on aesthetic and environmental concerns. This included a reduction of biological oxygen demand (BOD), TSS and more stringent regulations in pathogenic organism removal. Furthermore, the removal of nutrients such as nitrogen and phosphorous were also included in the CWA for certain inland streams and lakes (Tchobanoglous et al. 2014a). Since then, wastewater treatment regulations have continued to evolve over time, with different countries developing governing bodies that would be put in charge of waste management and environmental protection.
In Canada, the Canadian Council of Ministers for the Environment (CCME) released the *Canadian Water Quality Guidelines* in 1987 to protect freshwater ecosystems and to standardize industrial, recreational and drinking water usage across the country (CCME 2001). This document was regularly updated and eventually led to the development of a *Canada-wide Strategy for the Management of Municipal Wastewater Effluent* in February of 2009 and the introduction of the Wastewater System Effluent Regulations (WSER) in 2012. This legislation requires that wastewater facilities across the country achieve minimum national performance standards (NPS) and manage site-specific effluent discharge objectives for various constituents of concern over a period of no longer than thirty years. However, the WSER was not ratified by the governments of the Northwest Territories, Nunavut, Quebec or Newfoundland and Labrador, due to the geographical and environmental constraints associated with treating wastewater in remote communities. These provinces and territories were therefore tasked with working with the federal government to assess the performance of existing wastewater treatment facilities located above the 54th parallel, a boundary set in the WSER, and develop performance standards as well timelines for their implementation (Canadian Council of Ministers of the Environment 2009; Government of Canada 2015)

1.1.4 Wastewater Treatment in Northern Canada

The cold climate and year round permafrost characteristic of the Canadian High Arctic make the installation of conventional treatment systems impractical from economic and environmental aspects. Furthermore, the lack of skilled labour in many smaller northern communities creates the need for systems that are both reliable and require little maintenance. As such, the use of WSPs is quite common because of their simplicity, reliability and cost effectiveness (Krkosek et al. 2012). The WSPs can also be used in conjunction with tundra wetlands that provide additional treatment to the wastewater before its discharge into a receiving body of water (Chouinard et al. 2014; Hayward et al. 2014).
1.1.5 Microalgae as a Passive Wastewater Treatment Method

High nutrient concentrations and long detention combined with ideal environmental conditions often lead to the presence of microalgae in WSPs. While the effects of microalgae in these types of systems have not yet fully been characterized, several strains have been shown to be effective in the removal of nutrients from wastewater streams due to their high uptake capacity for macronutrients such as nitrogen and phosphorous (Dickinson et al. 2013; McGinn et al. 2012; Wang et al. 2010).

1.1.6 Modelling WSP and Microalgae Kinetics

A number of models have been developed to simulate WSP dynamics in a variety of climates (Beran and Kargi 2005; Gehring et al. 2010; Heaven et al. 2011), but these models are not necessarily applicable to geographical locations above the Arctic Circle, where extreme temperatures and extended photoperiods are commonplace. Furthermore, models have also been developed to simulate microalgae growth kinetics under varying temperatures and light conditions (Tang et al. 1997; Yun and Park 2003), yet the behaviour of microalgae under summer arctic conditions has still yet to be extensively characterized.

1.2 Scope of Studies

This thesis examines the role of microalgae in cold climate wastewater treatment, specifically in communities located in the Canadian High Arctic where the use of wastewater stabilization ponds is extensive. The scope of work is separated into two themes. The first investigates the effects of cold temperatures on microalgae kinetics, specifically involving growth and substrate utilization rates, and is presented in Chapter 3. The second presents the modifications made to an existing model used to simulate WSP dynamics under various environmental conditions with an emphasis on microalgae dynamics within the modelled system and is presented in Chapter 4.
1.2.1 Literature Review

Chapter 2 of this thesis presents a comprehensive literature review of the challenges and regulations associated with wastewater treatment in Canada. WSP treatment mechanisms and performance issues in various climates are also considered, along with an overview of the biology of microalgal species, including the effects of factors such as nutrient availability, solar radiation and temperature on microalgal growth. Finally, an overview of the use of numerical methods to model biochemical WSP processes along with solar radiation will also be presented.

1.2.2 Growth Rates of the Microalgae Strain Chlorella Vulgaris at Cold Temperatures Compared to Three Microalgal Growth Models

Chapter 3 presents an investigation on the effects of cold temperatures on the growth rates of the green microalgae strain *Chlorella vulgaris*. Growth rates were evaluated at a temperature range of 1°C to 23°C in aerated photobioreactors (PBRs) under a continuous irradiance of 100 µmol photons m² s⁻¹. The collected experimental results were then compared to three different microalgae growth models: the Eppley Curve (Bissinger et al. 2008; Eppley 1972), the Arrhenius Relationship (Xin et al. 2011) and a model developed by Peeters and Eilers (Peeters and Eilers 1978). The latter was deemed to be the best model method to predict maximal microalgal growth rates at summer arctic temperatures and was later used in a numerical model that was developed to investigate the effects of varying climate on an Arctic WSPs, presented in Chapter 4.

1.2.3 Modelling Waste Stabilization Ponds in the Canadian High Arctic

Chapter 4 outlines the modification of an existing numerical model used which was then used to investigate the performance of WSPs under varying climatic factors, such as temperature and solar radiation. The model uses historical weather data from Pond Inlet, NU and also incorporates a solar radiation model that outputs radiation data at a given latitude for a desired period of time in order to include the effects of photoperiods of varying intensity and duration on a WSP. Numerical
equations were obtained from several pre-existing models and modified to account for the differences in microalgae growth and biological wastewater treatment rates under cold temperature conditions.

This numerical model could therefore be used to determine the effects of varying climate on the performance of arctic WSPs

1.2.4 Concluding Remarks and Additional Materials

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

Additional materials such as the MATLAB code used in the numerical model presented in Chapter 4, along with the values of the constants used in the numerical model can be found in Appendices A and B, respectively. Appendix C and D contain summary plots generated by the numerical model for the years 2011 and 2013, respectively.

1.3 References


Chapter 2

Literature Review

2.1 Introduction

Municipal wastewater is a product of human activity that can pose a risk to receiving aquatic environments if improperly treated before its discharge. These risks include threats to human health, acute and chronic toxicity to aquatic ecosystems, as well as nutrient imbalances that could cause eutrophication in bodies of water. In Canada, the introduction of the Wastewater Systems Effluent Regulations (WSER), a federal legislation introduced in 2012, required that treatment facilities across the country adhere to strict National Performance Standards (NPS) for the treatment of wastewater. However, the WSER were not ratified by the governments of the Northwest Territories, Nunavut, Quebec or Newfoundland and Labrador, who stated that the NPS would be too difficult to achieve in communities located above the 54th parallel due to environmental and geographical constraints. These provinces and territories were therefore tasked with working in collaboration with the federal government to develop appropriate performance standards by early 2015 (Canadian Council of Ministers of the Environment 2009).

Prior to the introduction of the WSER, wastewater stabilization ponds (WSPs) were considered to be an adequate method of wastewater treatment and were commonly used to treat municipal wastewater in small northern communities due to their effectiveness, operational simplicity and low maintenance costs. (Federation of Canadian Municipalities and National Research Council 2004; Krkosek et al. 2012). The principal treatment objectives of WSPs are to convert, transform and remove undesirable biodegradable constituents and nutrients, such as nitrogen and phosphorous, from wastewater (Tchobanoglous et al. 2014b). These biologically-mediated processes occur when microorganisms, such as bacteria and microalgae, oxidize particulate and dissolved carbonaceous organic matter into carbon dioxide and additional biomass. These same
microorganisms can be used to remove nitrogen and phosphorous from wastewater (Shammas et al. 2009). Disinfection is another treatment process that can occur within WSPs and involves sunlight in the ultraviolet range penetrating the water column and inactivating enteric pathogens (Curtis et al. 1992; Kohn and Nelson 2007).

Several factors influence the efficiency of WSPs. High nutrient loadings combined with long detention times may lead to the presence of high microalgae concentration within the system. Under these conditions, a bacterial-microalgal synergy can promote biological treatment. The ability of microalgae to photosynthesize can provide the pond with a constant supply of dissolved oxygen, allowing aerobic bacteria to oxidize organic matter and remove nutrients from the wastewater (Shammas et al. 2009). Ambient temperatures and incident solar radiation have been shown to affect microalgae growth in WSPs by influencing their metabolic activities, including cellular division, nutrient uptake rates and photosynthesis (Dermoun et al. 1992; Tchobanoglous et al. 2014b).

A number of models have previously been developed to simulate WSP dynamics in a variety of temperate climates (Beran and Kargi 2005; Gehring et al. 2010; Heaven et al. 2011). However, these models do not necessarily include parameters that account for environmental conditions experienced in WSPs located above the 54th parallel. Cold temperatures combined with extended photoperiods would require some considerations and recalibrations for these models to adequately simulate Arctic WSP dynamics.

2.1.1 Wastewater treatment in Canada’s Far North

2.1.2 Geographical and Environmental Challenges

The remote environment and cold climate of the Canadian Arctic creates a variety of operational, financial and technical constraints for the treatment of municipal wastewater (Yates et al. 2012). A report compiled by Infrastructure Canada identified several geographical challenges faced by Northern Communities located above the 54th parallel. First among these challenges is the transport of goods and labour between communities dispersed over a large area resulting in extremely
high costs and inflexible timelines. Furthermore, conventional needs are required to be met through unconventional technologies and small communities with significant needs are often required to be serviced without being able to offset costs by traditional means like taxation. Finally, the absence of power lines in many communities creates a dependence on other options, such as diesel generators, to provide electricity (Infrastructure-Canada 2012).

Environmental conditions create another set of challenges in the provision of infrastructure services to communities in Canada’s Far North. Arctic ecosystems are characterized as being low in diversity, lacking in nutrients and subject to large changes in photoperiods (Gunnarsdóttir et al. 2013; Tilsworth and Smith 1984). Furthermore extreme winter temperatures combined with a deep, year-round permafrost whose top layer experiences seasonal freeze-thaw cycles, makes the installation of above-ground or buried pipelines not only expensive, but also unstable. As such, water and sewerage services are provided through trucking systems that provide homes with drinking water and dispose of wastewater (Hayward et al. 2014; Infrastructure-Canada 2012; Yates et al. 2012).

2.1.3 Current treatment technologies

The most common wastewater treatment method used by communities in the Canadian High Arctic involves the use of single or multiple celled WSPs that are conveyed to into natural tundra wetlands, with sixteen out of twenty five Nunavut communities utilizing systems with this type of configuration. These systems typically operate under facultative conditions (Section 2.3) and can either be engineered or naturally occurring “lake lagoons” (Krkosek et al. 2012). The primary purpose of WSPs in arctic communities is to store frozen wastewater during the winter months, and to provide treatment during the summer season when biological activity can occur, albeit at a slower rate than WSPs located in more temperate climates due to lower temperatures (Hayward et al. 2014; Krkosek et al. 2012). In certain communities, WSPs discharge into tundra wetlands that serve as an additional form of effluent treatment before its discharge into receiving bodies of water like the Arctic Ocean. While the exact transformative and removal processes of tundra wetlands have yet to
be fully identified, these systems have been shown to augment treatment efficiency for facilities that rely solely on WSPs as their only form of wastewater treatment (Chouinard et al. 2014; Yates et al. 2012). Tundra wetlands are different than conventional constructed wetlands in a number of ways. These natural systems have not been designed to address specific treatment objectives or to achieve certain treatment standards. They also differ from one another in terms of site specific hydrology, porosity, flow paths and vegetation (Chouinard et al. 2014).

More conventional treatment plants are less commonly used due to high operational costs and lack of technically skilled personnel available in remote locations, but may be found in certain larger communities such as Iqaluit (Gunnarsdóttir et al. 2013; Hayward et al. 2014).

2.1.4 Canadian regulations

In 2012, Environment Canada and the Canadian Council of Ministers of the Environment introduced a new federal regulation under the Fisheries Act known as the Wastewater Systems Effluent Regulations. The WSER (SOR/2012-139) is a Canada-wide legislation which applies to wastewater systems that collect, on average, 100 m$^3$ or more of wastewater per day and stipulates that all facilities achieve minimum National Performance Standards for common wastewater pollutants. Specifically, the NPS includes maximum effluent concentrations of 25 mg/L five day carbonaceous biochemical oxygen demand (cBOD$_5$), 25 mg/L total suspended solids (TSS), 0.2 mg/L average total residual chlorine and 1.25 mg/L un-ionized ammonia (NH$_3$-N). Facilities are also required to develop site specific effluent discharge objectives to address specific substances that are of concern to a particular discharge environment (Government of Canada 2015). However, the NPS does not apply to wastewater systems located in the Northwest Territories, Nunavut or north of the 54th parallel in Quebec or Newfoundland and Labrador, where extreme climatic conditions were deemed to impede treatment (Canadian Council of Ministers of the Environment 2009; Government of Canada 2015).

The governments of the Northwest Territories, Nunavut, Quebec, Newfoundland and Labrador were tasked to work in collaboration with the federal government over a five year period to:
assess the performance of existing wastewater facilities; develop northern performance standards along with a timeline for their implementation; adapt an environmental risk assessment approach for the Far North; and adapt monitoring and reporting requirements. The results from this collaboration are expected to be released in early 2015. It should be noted that while communities located in the Yukon face similar challenges, the territorial government of the Yukon was tasked with developing a similar, yet separate, provincial-territorial agreement over a period of three years (Canadian Council of Ministers of the Environment 2009).

2.2 Wastewater Stabilization Ponds

2.2.1 Introduction

WSPs are one of the simplest forms of biological treatment processes and serve many basic purposes, ranging from storage of wastewater, settling and removal of total suspended solids (TSS), aeration and evaporation (Shammas et al. 2009). These systems are widely used in Canada and are considered to be advantageous due to their low operational and maintenance costs and their ability to realize additional capacity and provide effective treatment with minimal threat to the environment when operated properly. A survey conducted by Environment Canada identified over 504 WSPs in Canadian municipalities with populations of over 1,000 inhabitants. (Federation of Canadian Municipalities and National Research Council 2004). However, WSPs require a relatively large amount of space and have been shown to occasionally overflow during periods of heavy precipitation, cause offensive odors and be unaesthetically pleasing (Federation of Canadian Municipalities and National Research Council 2004; Heaven et al. 2003).

2.2.2 Types and characteristics

Wastewater stabilization ponds are used for the treatment of wastewater using natural processes and can be classified under four different categories based on the presence of oxygen in the
system. These categories are: aerobic ponds; anaerobic ponds; facultative ponds; and tertiary-maturation ponds (Tchobanoglous and Burton 1991).

Aerobic WSPs are large shallow basins that use both microalgae and microorganisms in suspension to treat the wastewater. The shallow nature of these systems allows for the diffusion of oxygen from the atmosphere into the system, along with the production of oxygen by microalgae (Tchobanoglous and Burton 1991). Microalgae provide heterotrophic aerobic microorganisms with oxygen, allowing them to aerobically degrade organic matter. This degradation process in turn provides the microalgae with nutrients and carbon dioxide, creating a synergistic cycle, as illustrated in Figure 2-1 (Muñoz and Guieysse 2006; Shammas et al. 2009). The presence of particular microalgal and bacterial species in aerobic WSPs is dependent on factors such as organic loading, pond mixing, pH, nutrients, sunlight and temperature, with the latter having a profound effect on treatment efficiency (Tchobanoglous and Burton 1991).

Aerobic ponds can be further sub-categorized into two types based on their depth. High-rate aerobic ponds (HRAP) are typically limited to a depth of 150 to 450 mm (0.5 to 1.5 ft) and their primary purpose is to produce high concentrations of microalgae that are easily settled in subsequent ponds (Sah et al. 2012). Light penetration is therefore essential for microalgal growth, since the relationship between the microalgae and bacteria in the system directly affects BOD and nutrient removal. BOD removal in these systems has been shown to be good, even when subject to very high organic loading. The second type of aerobic pond is designed to maximize oxygen production. These systems are typically deeper than HRAPs, with depths of up to 1.5m (5 ft) being used. Pumps and surface aerators are commonly used to achieve the best results for aerobic ponds by providing the system with a constant supply of oxygen (Shammas et al. 2009; Tchobanoglous and Burton 1991).
Facultative ponds receive lower organic loadings than their aerobic counterparts and can reach depths of up to 2.5 m (just under 8 ft). An aerobic zone exist near the surface of these systems due to photosynthetic activity and surface reaeration, while the bottom typically consists of an anaerobic zone in which sludge accumulates. A transitional zone exists between the aerobic and anaerobic layers and is commonly referred to as the facultative layer. The major source of oxygen in facultative WSPs is either through mechanical aeration or photosynthetic oxygenation, with surface reaeration generally considered to be insignificant (Shammas et al. 2009). Significant research has been conducted on the characterization of facultative ponds and the development of models for their designs (Beran and Kargi 2005; Heaven et al. 2011; Sah et al. 2012). However, due to the high variability of the processes that affect these systems, such as prevailing windy conditions affecting effluent quality due to mixing processes, no universal design exists. Facultative WSPs are therefore typically designed based on loading factors that have been determined from previous field experience relevant to a given site (Tchobanoglous and Burton 1991).

Tertiary-maturation ponds are used for seasonal nitrification and polishing of secondary effluents. Long detention times of 18 to 20 days have been suggested as the minimum period required to allow for the removal of residual TSS and in order to maintain aerobic conditions in the system. Biological treatment processes in these systems are similar to those in aerobic and facultative ponds, with the oxygen required for aerobic processes in these systems being provided by surface

Anaerobic ponds are used to enhance the removal of solids and organic matter from a wastewater stream and are typically used in conjunction with other treatment methods. In order to minimize the surface area exposed to the atmosphere, these systems can reach depths of up to 9.1m (30 ft). This minimizes heat and oxygen transfer to the atmosphere and maintains anaerobic conditions in the system while conserving heat energy (Sah et al. 2012; Tchobanoglous and Burton 1991).

WSPs can also be classified depending on their discharge frequency (Heaven et al. 2003; Krkosek et al. 2012). Continuous discharge systems are common for ponds in warm climates, where treated effluent is continuously discharged into a receiving environment at a rate proportional to the inflow. Intermittent discharge systems are more common in cold and extreme climates (Krkosek et al. 2012). Due to slower treatment rates, these systems release effluent once or twice per year, in spring or autumn, allowing for longer wastewater detention times. Furthermore, the ice cover on receiving bodies of water during an extended portion of the year will also hinder effluent discharge. Finally, WSPs that do not discharge into a body of water can be found in areas where evaporation rates are greater than loading and precipitation rates. These systems are rare in North America but have commonly been used by industrial enterprises in the former Soviet Union (Heaven et al. 2003).

2.2.3 Treatment Mechanisms

Several treatment mechanisms for the removal of organic matter, TSS, nitrogen and phosphorous occur in WSPs as can be seen in Figure 2-2. Facultative ponds, in particular, provide a wide range of conditions well suited for the degradation of organic matter and uptake of nutrients through biological processes due to the aerobic, facultative and anaerobic zone located throughout the system. Settleable organic solids accumulate in the anaerobic zone of the pond where they are broken down by anaerobic bacteria into dissolved organics and gases such as CO₂, H₂S and CH₄.
These gases are either oxidized by bacteria in the aerobic and facultative zones or vented to the atmosphere (Tchobanoglous et al. 2014b). In the aerobic zone of the pond, organic material breakdown and nutrient conversion is accomplished by heterotrophic organisms. These organisms require an adequate supply of DO, which can either be generated by microalgae through photosynthesis, or obtained from reaeration at the interface of the water surface and the atmosphere. Finally, the bacteria located in the facultative zone of the WSP are capable of breaking down the organic matter generated in the aerobic zone (Sah et al. 2012; Shammas et al. 2009; Tchobanoglous et al. 2014b).

Figure 2-2- Schematic representation of the treatment processes and nutrient cycles in facultative Wastewater Stabilization Ponds (adapted from (Tchobanoglous and Burton 1991))
2.2.3.1 Organic Matter

Organic matter in WSPs represents the carbon based biodegradable material that comes from plants, humans or animals and is removed either by bacterial oxidation, sedimentation or anaerobic conversion. In aerobic and facultative ponds, bacterial consortia oxidize soluble organic material in the aerobic zones of the system. While aerobic ponds have been shown to reduce soluble BOD$_5$ concentrations by up to 95%, the resulting effluent may contain high concentrations of bacteria and microalgae that may exert a higher oxygen demand on the receiving environment than the original waste. The removal of these microbiota from the system’s effluent is therefore necessary before its discharge into a receiving environment (Federation of Canadian Municipalities and National Research Council 2004; Tchobanoglous and Burton 1991).

The removal of organic matter in anaerobic ponds is accomplished through a combination of sedimentation and anaerobic degradation, through hydrolysis, acidogenesis, acetogenesis and methanogenesis. BOD removal rates of 70% are typically achievable and can be as high as 85% if the system is operating under optimal conditions. (Sah et al. 2012; Tchobanoglous and Burton 1991).

2.2.3.2 Suspended Solids

Organic matter, nutrients and microalgae can all account for total suspended solids concentrations in wastewater streams. TSS and organic matter concentrations have been shown to increase in WSPs, resulting an effluent that may not meet its discharge target. This can be attributed to the accumulation of microalgal cells in the system during treatment and may account for up to 50-60% of TSS in aerobic ponds which need to be removed from the effluent before discharge.

Microalgae removal can occur either through settling within the WSP, or through filtration of the effluent. (Camargo Valero et al. 2010; Shammas et al. 2009). The removal of TSS in facultative WSPs occurs through sedimentation into the anoxic zone, where they are broken down by anaerobic bacteria. In the aerobic zone of WSPs, aerobic bacteria consume organic matter and TSS reduction occurs through endogenous respiration (Tchobanoglous and Burton 1991)
2.2.3.3 Nitrogen

The presence of nitrogen in wastewater is of particular concern due to its role in the eutrophication process and its potential toxicity to aquatic species depending on its form. It can be found in many forms in solution and can undergo transformations that convert undesirable nitrogen species, like ammonia, into others that can be more easily removed from wastewater. The most important forms of inorganic nitrogen in municipal and domestic wastewater are ammonia, nitrite ($\text{NO}_2^-$), nitrate ($\text{NO}_3^-$), nitrous oxide ($\text{N}_2\text{O}$) and dissolved or gaseous elemental nitrogen ($\text{N}_2$). Ammonia can exist in solution as either ammonia ($\text{NH}_3$) or ammonium ($\text{NH}_4^+$), its ionic form, depending on water temperature and pH, as per Equation 2.1 (Kadlec and Wallace 2008).

$$\text{NH}_3 + \text{H}_2\text{O} \leftrightarrow \text{NH}_4^+ + \text{OH}^- \quad (2.1)$$

This is of particular importance due to the different characteristics of both species. Un-ionized ammonia is typically found in solution as a dissolved gas and is highly volatile due to its high vapour pressure, while ammonium can only be present in an aqueous form, making it non-volatile. The equilibrium between these two forms is an acid-base relationship and a function of pH and temperature. Un-ionized ammonia accounts for a higher percentage of total ammonia at warmer temperatures and at a higher pH, while ammonium is dominant at cooler temperatures and in more acidic solutions. Total ammonia is the sum of ammonia and ammonium and is referred to as ammonia-nitrogen for the purpose of this thesis.

The removal of nitrogen in WSPs can occur either through ammonia volatilization, or biochemically through assimilation or nitrification-denitrification. Microbial populations can assimilate nitrogen, in the form of ammonia, and incorporate it into their biomass, with a portion of the assimilated ammonia being returned to the solution upon cell death (Camargo Valero et al. 2010; Kadlec and Wallace 2008; Tchobanoglous, H. David Stensel, et al. 2014).

The removal of nitrogen from a wastewater stream is accomplished through nitrification and denitrification. Nitrification is typically facilitated by two bacteria genera, *Nitrosomonas* and
Nitrobacter. The process can be broken down into two steps, with the first involving the oxidation of ammonia to nitrite by Nitrosomonas (Equations 2.2), followed by the conversion of nitrite to nitrate by Nitrobacter (Equation 2.3).

\[ 2 \text{NH}_4^+ + 3 \text{O}_2 \rightarrow 2 \text{NO}_2^- + 4 \text{H}^+ + 2 \text{H}_2\text{O} \quad (2.2) \]
\[ 2 \text{NO}_2^- + \text{O}_2 \rightarrow 2 \text{NO}_3^- \quad (2.3) \]

Stoichiometrically, based on Equations 2.2 and 2.3, 3.43 grams of oxygen are required to fully oxidize 1 gram of ammonia to nitrite, while 1.14 grams of oxygen are required to fully oxidize 1 gram of nitrite to nitrate, resulting in a total of 4.57 grams of oxygen (Tchobanoglous et al. 2014b).

The growth and nitrogen uptake rates of Nitrosomonas and Nitrobacter are highly susceptible to a variety of factors in their environment. Elevated concentrations of ammonia and nitrous oxide have been shown to inhibit growth, along with cold temperatures and pH values outside of the range of 7.5 and 8.6. Finally, sufficient DO needs to be present in the system for nitrification to occur. If concentrations decrease below 1 mg/L, oxygen becomes the limiting nutrient and nitrification rates diminish or stop entirely (Tchobanoglous et al. 2014b).

Denitrification is the second step in the nitrogen removal process in which nitrate is biologically converted to nitrogen gas under anoxic conditions. The term anoxic is used because the biochemical pathways used in the denitrification process are not purely anaerobic but rather modified aerobic pathways since the facultative heterotrophs that carry out the reaction can either use oxygen or nitrate as terminal electron acceptors. Several genera of bacteria are capable of converting nitrate to nitrogen, including Archomobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter Bacillus, Chromobacterium, Corynebacterium, Flavobacterium, Halobacterium, Hypomicrobium, Methanomonas, Moraxella, Neisseria, Paracoccus, Propionibacterium, Pseudomonas, Rhizobium, Rhodopseudomonas, Spirillum and Vibrio (Tchobanoglous et al. 2014b). In this reaction, nitrate is first converted to nitrite which is in turn converted to nitrogen gas as seen in Equations 2.4(a) and 2.4(b).

\[ 6\text{NO}_3^- + 2 \text{CH}_3\text{OH} \rightarrow 6 \text{NO}_2^- + 2 \text{CO}_2 + 4 \text{H}_2\text{O} \quad (2.4.a) \]
6 \text{NO}_2^- + 3 \text{CH}_3\text{OH} \rightarrow 3 \text{N}_2 + 3 \text{CO}_2 + 3 \text{H}_2\text{O} + 6\text{OH}^-

As with the nitrification process, denitrification is susceptible to variations in wastewater constituents, particularly DO concentration, pH and temperature. The presence of dissolved oxygen will suppress the enzymes required for denitrification, with DO concentrations as low as 0.09 mg.L$^{-1}$ having been shown to decrease denitrification by more than 35% (Plósz et al. 2003). Furthermore, pH values outside of the optimum range of 7 and 8, depending on the bacterial species, have also been shown to have adverse effects on denitrification. Cold temperatures have been shown to negatively affect nitrogen removal rates following an Arrhenius relationship, with inhibitory effects being observed below 10°C (Bachand and Horne 2000; Illies and Mavinic 2001; Kadlec and Reddy 2001; Tchobanoglous et al. 2014b).

2.2.3.4 Phosphorus

Phosphorous may be present in wastewater as either orthophosphate (PO$_4^{3-}$); polyphosphate (P$_2$O$_7$), commonly found in detergents; or organically bound phosphorous, which is typically bound or tied up to autotrophs. The latter two components can account for up to 70% of the influent phosphorous in a waste stream (Barsanti and Paolo 2006). Phosphorous is considered to be the limiting nutrient in freshwater aquatic systems along with nitrogen, implying that autotrophic growth will cease should either of these two nutrients become biologically unavailable (Barsanti and Paolo 2006). Orthophosphates are readily available for biological processes and are found in sewage or agricultural run-off. Finally, polyphosphates and organic phosphorous both need to be converted to orthophosphate before being able to be uptaken by autotrophs. (Barsanti and Paolo 2006). Certain microorganisms, referred to as phosphorous accumulating organisms (PAOs), utilize phosphorous during cell synthesis and energy transport, resulting in removal rates of 10 to 30% of the influent phosphorous in secondary biological wastewater treatment (Tchobanoglous et al. 2014b). Biological phosphorous removal approaches must therefore be designed to promote the growth of biological populations with a high cellular phosphorus content. Microorganisms within the \textit{Acinetobacteria}
genera may uptake phosphorous under aerobic conditions, which occurs in the upper zone of facultative ponds, while anoxic conditions, found in the sludge layer of a WSP, may cause the release of phosphorous from decaying cell mass. The sludge containing excess phosphorous should then be removed from the system and treated separately. (Obaja et al. 2003; Tchobanoglous et al. 2014b)

The release and absorption of phosphorous in the sediment of WSPs also plays an important role in its removal and is affected by the reduction potential and the pH of the system’s sediment. Under anaerobic conditions, increases in reduction potential (Eh) have been assumed to be simultaneous with increases in phosphorous absorption into the sediment, with the greatest binding capacity occurring at a pH range of 7-8 (Peng et al. 2007).

2.2.3.5 Environmental Effects

The effects of temperature on biological treatment processes in WSPs can be mathematically represented using a modified Arrhenius relationship (Shammas et al. 2009), as seen in Equation 2.5:

$$k_T = k_{20} \theta^{T - 20}$$

(2.5)

where $k_T$ (d$^{-1}$) is the reaction rate at a given temperature T (°C), $k_{20}$ (d$^{-1}$) is the reaction rate at 20°C and $\theta$ is the temperature correction coefficient (unitless). The value for the temperature correction coefficient is dependent on the type of system that the equation is meant to represent. Aerobic and anaerobic processes have different temperature correction coefficients, with the latter being more sensitive to changes in temperature and therefore requiring a higher $\theta$, as can be seen in Table 2.1.

Table 2-1 - Arrhenius relationship temperature correction coefficient values for WSPs (Shammas et al. 2009)

<table>
<thead>
<tr>
<th>Type of stabilization</th>
<th>Suggested $\theta$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic, high-rate aerobic, tertiary WSPs</td>
<td>1.03 – 1.04</td>
</tr>
<tr>
<td>Facultative, aerobic-anaerobic WSPs</td>
<td>1.05 – 1.06</td>
</tr>
<tr>
<td>Anaerobic WSPs</td>
<td>1.06 – 1.085</td>
</tr>
</tbody>
</table>
Another method of accounting for temperature effects in WSPs is the $Q_{10}$ factor, which measures the ratio of removal rates at intervals of 10°C (Kadlec and Reddy 2001) as seen in Equation 2.6.

$$Q_{10} = \frac{k_{T+10}}{k_T} = \theta^{10}$$  \hspace{1cm} (2.6)

where $Q_{10}$ (unitless) is the ratio of removal rates, $k_T$ (d$^{-1}$) is the reaction rate at a temperature $T$ (°C), $k_{T+10}$ (d$^{-1}$) is the reaction rate at a temperature of $T+10$ and $\theta$ is the temperature correction coefficient (unitless). Typical $Q_{10}$ values for biological reactions vary between 2 and 3, meaning that with every 10°C increase in temperature, reaction rates double or triple (Reyes et al. 2008).

The amount of incident solar radiation on a WSP will also indirectly affect treatment rates by affecting microalgae growth, and therefore dissolved oxygen concentrations. As discussed in Sections 1.4.2 and 1.5.2, the amount of radiation available for photosynthesis will govern microalgal growth and photosynthetic activity, and hence, affect the amount of DO in the system. The availability of oxygen plays an important role in biological treatment by allowing aerobic species to oxidize organic material and nutrients. (Concas et al. 2014; Dauta et al. 1990; Moisan et al. 2002; Yun and Park 2003).

2.3 Microalgae

2.3.1 Species

Microalgae refers to a highly diversified group of microorganisms that can be broadly defined as photosynthetic prokaryotes, single-celled organisms that derive their energy from sunlight, and can be found in aquatic and terrestrial habitats ranging from Antarctic lakes to hot desert soils (Barsanti and Paolo 2006). It should be noted that the term microalgae is distinct from macroalgae, which refers to larger, multicellular organisms found in marine environments. It has been estimated that between one and ten million species of macro and microalgae exist on Earth, with the latter
including the majority of this estimate (Barsanti and Paolo 2006). This thesis deals exclusively with microalgal species, specifically with unicellular chlorophytes like *Chlorella vulgaris*.

### 2.3.2 Microalgae Growth

The majority, up to 99.9%, of the cellular make up of microalgae can be accounted for by the six major elements, with carbon (C), Oxygen (O), hydrogen (H), nitrogen (N), sulfur (S) and phosphorous (P), as well as calcium (Ca), potassium (K), sodium (Na), chlorine (Cl), magnesium (Mg), iron (Fe) and silicon (Si) (Barsanti and Paolo 2006; Mandalam and Palsson 1998). Therefore, the presence of these nutrients, along with the appropriate environmental conditions, are required for microalgal growth to occur. Liebig’s “Law of the Minimum” was introduced to establish a relationship between the yield of a crop and the elemental composition of that crop’s substrate, meaning that if one nutrient was deficient, plant growth would be negatively affected. Nutrient limited growth can be modelled with a Monod equation (Barsanti and Paolo 2006), as see in Equation 2.7:

\[
\mu = \frac{\mu_m [LN]}{[LN] + K_m}
\]  

(2.7)

where \( \mu \) (d\(^{-1}\)) is the specific growth rate of the population as a function of the concentration of the limiting nutrient (LN, mg.L\(^{-1}\)), \( \mu_m \) (d\(^{-1}\)) is the maximum population growth rate under optimal conditions and \( K_m \) (mg.L\(^{-1}\)) is the half saturation coefficient, which corresponds to the concentration at which \( \mu \) is exactly one half of its maximum. Microalgae growth rate can be calculated based on the change in biomass concentration over time (McGinn et al. 2012), as seen in Equation 2.8:

\[
\mu = \frac{\ln C_2 - \ln C_1}{t_2 - t_1}
\]  

(2.8)

where \( \mu \) (d\(^{-1}\)) is the specific growth rate, \( C_2 \) is the biomass concentration at time \( t_2 \) (d), and \( C_1 \) (cells.L\(^{-1}\) or mg.L\(^{-1}\)) is the biomass concentration at time \( t_1 \) (d). Biomass concentration can either be expressed as dry-mass (mg.L\(^{-1}\), cell number (cells.L\(^{-1}\)) or chlorophyll a concentration (mg.chl.a.L\(^{-1}\)).
As with most photosynthetic organisms, microalgal growth is limited by nitrogen and phosphorous concentrations. A continuous supply of these nutrients combined with the proper environmental conditions, will sustain maximal microalgae growth rates, leading to a phenomenon referred to as eutrophication. This process can cause oxygen depletion in aquatic environments, greatly reducing biodiversity and damaging ecosystems (Abdelaziz et al. 2014; Barsanti and Paolo 2006).

Microalgal growth in an environment can be broken down into six successive phases: lag, acceleration, exponential, retardation, stationary and decline. In the lag phase, microalgae newly introduced to a system will begin to acclimatize to their new surroundings but will be unable to divide, meaning that the growth rate in this phase is negligible. In the acceleration and exponential phases, growth rates continuously increase until they reach their maximum value and cell density increases according to the exponential equation, as seen in Equation 2.9

\[ C_2 = C_1 \cdot e^{\mu} \]  

(2.9)

where \( C_2 \) and \( C_1 \) (cells.L\(^{-1}\),mg.L\(^{-1}\)) are the cell concentrations at two successive times and \( \mu \) (d\(^{-1}\)) is the growth rate.

Exponential growth is followed by the retardation and stationary phases, where cell concentrations remain constant at their maximum values until the water quality declines and microalgae cultures begin to die off in the decline phase (Barsanti and Paolo 2006).

2.3.3 Photosynthesis

Microalgal photosynthetic activity accounts for roughly more than half of global photosynthesis and converts solar radiation into biologically useable energy (Barsanti and Paolo 2006). The shortwave radiation that makes its way from the sun through the Earth’s atmosphere can be broken down into three spectra: ultraviolet (approximately 300 nm to 400 nm), visible (400 nm to 700 nm) and infrared (700 nm to 4000 nm). Microalgae derive their biologically usable energy from the visible light spectrum, also known as Photosynthetic Active Radiation (PAR), which typically
accounts for less than half of the total solar energy that reaches the Earth’s surface (Barsanti and Paolo 2006). The ratio of PAR to the entire spectrum of solar irradiance (SI) has been shown to vary between 0.34 and 0.499, with cloud cover further affecting these values by preferentially absorbing different wavelengths of solar radiation (Papaioannou et al. 1993).

In the photosynthetic process, carbon is converted from its highest oxidized form, CO\textsubscript{2}, to strongly reduced compounds like carbohydrates as demonstrated in Equation 2.10:

\[ n\text{CO}_2 + n\text{H}_2\text{O} + \text{light} - \text{chlorophyll } a \rightarrow (\text{CH}_2\text{O})_n + n\text{O}_2 \quad (2.10) \]

where light, water and CO\textsubscript{2} are reactants, chlorophyll \( a \) is a catalytic agent and \((\text{CH}_2\text{O})_n\), the organic matter which has been reduced to the level of carbohydrate, and \( \text{O}_2 \) are the products. It is possible for the reduced organic matter to be re-oxidized to \( \text{CO}_2 \) in the respiration process (Barsanti and Paolo 2006).

A number of models have been proposed to predict the amount of oxygen produced by microalgae during the photosynthetic process (Aguilera et al. 1999; Beran and Kargi 2005; Peeters and Eilers 1978; Yun and Park 2003). The first method of modelling oxygen production during photosynthesis takes into states that the amount of oxygen produced depends on the form of nitrogen utilized for growth as per Equations 2.11 and 2.12 (Beran and Kargi 2005):

\[ 106\text{CO}_2 + 16\text{NH}_4^+ + \text{H}_2\text{PO}_4^- + 106\text{H}_2\text{O} \rightarrow C_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P} + 106\text{O}_2 + 15\text{H}^+ \quad (2.11) \]
\[ 106\text{CO}_2 + 16\text{NO}_3^- + \text{HPO}_4^{2-} + 122\text{H}_2\text{O} + 17\text{H}^+ \rightarrow C_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P} + 138\text{O}_2 \quad (2.12) \]

where the product \( C_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P} \) is protoplasm.

Equation 2.11 shows that one mole of oxygen is produced for every mole of carbon dioxide consumed when ammonia is the nitrogen source; as opposed to 1.3 moles of oxygen being produced per mole of carbon dioxide consumed when nitrate is the nitrogen source as shown in Equation 2.12.

Alternatively, photosynthetic activity can also be modelled as a function of light dependence of microalgal photosynthesis as described by Yun and Park (2003). This study reported that microalgal photosynthetic activity is based on the local photon flux density (LPFD), or the number of
photons per second per unit area, and that each microalgal cell responds to the LPFD at its position. This model is considered to be more accurate due to the fact that the attenuation of light as it moves through a water column is taken into account. As such, microalgae cells located deeper in a water column would not have access to the same LPFD as cells located closer to the surface and would, therefore, be less photosynthetically active.

2.3.4 Microalgae in wastewater

As discussed in Section 2.3.2, microalgae are often found in WSPs and have been shown to affect the treatment efficiency of these systems by providing heterotrophic microorganisms with a source of oxygen that allows them to aerobically convert organic matter into biomass and CO₂ (Camargo Valero et al. 2010; Shammas et al. 2009; Tilsworth and Smith 1984). However, certain microalgal strains have been found effectively contribute to the treatment of wastewater beyond their photosynthetic role. Studies have shown that several microalgal strains can efficiently remove nutrients like nitrogen and phosphorous from wastewater streams under a range of growth conditions, making them suitable microorganisms in secondary or tertiary wastewater treatment (Abdelaziz et al. 2014; Dickinson et al. 2013; McGinn et al. 2012; Wang et al. 2010). A study conducted by McGinn et al (2012) found that the strain *Scenedesmus sp* was capable of facilitating nitrogen and phosphorous removal rates of over 90% with a retention time of approximately 6.5 days while nearly depleting iron, zinc and cadmium concentrations from the wastewater. Similarly, the microalgal strain *Chlorella vulgaris* has been shown to achieve removal rates of up to 82% for nitrogen in the form of ammonia, 91% for phosphorous and 83% for COD (Wang et al. 2010).

The presence of microalgae has been shown to affect the pH of water and wastewater due to the utilization of CO₂ in the photosynthesis process and subsequent formation of OH⁻ ions as demonstrated in Equation 2.13 (Uusitalo 1996).

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{CO}_3^{2-} + 2\text{H}^+ \quad (2.13)
\]
The change in one carbon species in Equation 2.13 results in a compensation by the other carbon species through the aqueous carbonate system. For example, the uptake of \( \text{CO}_2 \) from solution by microalgae through photosynthesis will result in \( \text{HCO}_3^- \) forming \( \text{CO}_2 \) and \( \text{OH}^- \) ions in order to maintain equilibrium, increasing the pH of the medium in the process. As shown in Equation 2.1, ammonia is dominant in its un-ionized form at elevated pH, and thus more volatile. The presence of microalgae in wastewater streams can contribute to pH increases, consequently leading to higher rates of ammonia volatilization. A study conducted by Rockne and Brezonik (2006) examining nutrient removal rates in cold weather WSPs, noted that the onset of microalgae blooms and aerobic conditions in a Minnesota WSP coincided with the highest rates of ammonia volatilization. In addition to contributing to nutrient removal, microalgae have also been shown to transform, degrade and remediate organic compounds in wastewater. This is generally accomplished through the use of the contaminants as a carbon source to obtain energy for growth and other processes, thus transforming them into new biomass and dissolved oxygen (Combalbert and Hernandez-Raquet 2010; Ghasemi et al. 2011).

Finally, the recent surge in the research and development of microalgae as a source of biomass for biofuel production has created a need to develop large-scale microalgae operations that will not adversely affect the environment or food production (Wang et al. 2010; Xin et al. 2011). Studies have shown that many species of microalgae have the ability to effectively grow in wastewater while producing the high lipid contents that are desirable for biofuel production. Unlike the production of terrestrial bioenergy crops, ideal growth conditions for the cultivation of microalgae in wastewater are not exclusive to temperate climates and can be achieved in Canada (Abdelaziz et al. 2014; Hallenbeck et al. 2014).

2.3.5 Temperature Effects

Sub-optimal temperatures have been shown to negatively affect microalgal growth rates. A number of models and equations have been developed to predict the growth response of many
microalgae species to variations in temperature (Bissinger et al. 2008; Dauta et al. 1990; Mayo 1997; Moisan et al. 2002; Xin et al. 2011).

The Arrhenius equation is often used to express the temperature dependence of reaction rates and equilibrium constants and has commonly been used to describe the effects of temperature on microalgal growth (Beran and Kargi 2005; Tchobanoglous et al. 2014b). This equation predicts microalgal growth rates at a given temperature based on the growth rate at 20°C and a temperature correction coefficient, as seen in Equation 2.14:

$$\mu_T = \mu_{20}\theta^{T-20}$$  (2.14)

where $\mu_T$ (d$^{-1}$) is the predicted growth rate at a given temperature T (°C), $\mu_{20}$ (d$^{-1}$) is the growth rate at 20°C and $\theta$ (unitless) is the temperature correction coefficient. This equation implies an exponential relationship between temperature and growth and has been shown to be applicable in the temperature range of 10°C to 25°C (Xin et al. 2011).

The Eppley Curve is another common equation used to predict microalgal growth at various temperatures. Developed by Eppley (1972), this model represents the relationship between temperature and microalgal growth in marine and freshwater ecosystems (Bissinger et al. 2008; Moisan et al. 2002). The empirical equation was originally derived from growth curve data for a variety of microalgal species and can be expressed as follows in Equation 2.15:

$$\mu_T = 0.59e^{0.0633T}$$  (2.15)

where $\mu_T$ is the predicted growth rate (d$^{-1}$) at a given temperature T (°C). Similar to the Arrhenius equation, the Eppley curve also implies an exponential relationship between growth and temperature. However, a study by Moisan et al. (2002) involving more than 100 species of phytoplankton noted that the Eppley curve could overestimate growth rates at colder temperatures by up to 80%, resulting in significant variability in the predicted versus observed microalgal growth rates.

Other models have been suggested to account for the effects of sub-optimal temperatures as well as the effects of temperatures above those ideal for microalgal growth. Peeters and Eilers (1978)
developed a mathematical equation to describe the relationship between light intensity and photosynthesis. This model was also used to describe the relationship between microalgal growth and temperature (Dauta et al. 1990; Dermoun et al. 1992) and is expressed in Equation 2.16 as follows:

\[ \mu_T = 2\mu_{\text{max}}(1 + \beta) \left( \frac{x}{x^2 + 2\beta x + 1} \right) \]  

\[ x = \frac{T - T_0}{T_{\text{opt}} - T_0} \]  

(2.16a)  

(2.16b)

where \( \mu_T \) is the predicted growth rate (d\(^{-1}\)) at a temperature \( T \) (°C); \( \mu_{\text{max}} \) is the maximal growth rate (d\(^{-1}\)) occurring at the optimal temperature (\( T_{\text{opt}}, \) °C); \( \beta \) (unitless) is the shape factor. The general shape of the fitting curves generated by this equation for different microalgal strains can be seen in Figure 2-3:

![Figure 2-3 Variation in maximal growth rates of four microalgae strains (Chlorella vulgaris, Fragilaria crotonensis, Staurastrum pingue and Synechocystis minima) in relation to temperature using the Peeters-Eilers method. Adapted from Dauta et al. (1990)](image)

The main advantage of the Peeters and Eilers method is its use laboratory data combined along with curve fitting techniques to represent variations in growth rates for a specific microalgal
strain over a wide range of temperatures. This can be of particular interest in the modelling microalgal growth rates in Arctic WSPs, where temperatures can fluctuate between 0°C and 20°C.

2.3.6 Lighting Availability Effects

The effects of available solar radiation on microalgae are similar to those of temperature, with sub-optimal values negatively affecting growth rates (Dauta et al. 1990; Dermoun et al. 1992; Peeters and Eilers 1978). Growth rates for individual microalgal species at varying light intensities can be predicted using the Peeters and Eilers method discussed in Section 2.4.5 by replacing the temperature terms in Equation 2.16 by light intensity terms. The resulting growth curve exhibits the same behaviours as the temperature curves illustrated in Figure 2-3, with growth rates declining on either side of the optimum light intensity value (Dermoun et al. 1992; Peeters and Eilers 1978).

However, this method assumes that the light intensity is constant throughout a growth vessel or environment and therefore can only apply to small, bench scale conditions. Light attenuation throughout the water column, along with the depth of the water column, need to be taken into consideration when predicting microalgal growth rates at varying light intensities in large systems.

Beran and Kargi (2005) derived an equation for a light intensity factor to calculate microalgal growth rates in WSPs at varying light intensities, as can be seen in Equation 2.17:

$$ f(I) = \frac{2.718}{(K_e + K_n C_n) d_t} \left[ \exp \left( -\frac{I_n}{I_s} \right) \exp \left( -(K_e + K_n C_n) d_t \right) - \exp \left( -\frac{I_n}{I_s} \right) \right] $$

(2.17)

where $f(I)$ is the light intensity factor, $K_e$ is the wastewater light attenuation coefficient (m⁻¹), $K_n$ is the specific light attenuation coefficient of microalgae (m².g⁻¹), $C_n$ is the microalgae concentration (g.m⁻³), $d_t$ is the WSP depth (m), $I_o$ is the solar radiation acting on the WSP (W.m⁻²) and $I_s$ is the optimal light intensity for microalgae (W.m⁻²). The predicted growth rate is then calculated by multiplying the light intensity factor by the maximum growth rate for a specific microalgal species.
2.4 Solar Radiation

Solar radiation can be defined as the radiant energy emitted by the sun and is the universal source of energy in the biosphere. Total solar radiation is in the wavelength of 300 nm to 4000 nm and includes ultraviolet radiation (300-400 nm), visible light (400-700 nm) and heat, or infrared radiation (700-4000 nm). On average, the solar radiation intensity at top of the Earth’s atmosphere is 1.4 kW.m\(^{-2}\) and is made up of 8% ultraviolet radiation, 41% visible light and 51% infrared radiation. As it moves through the atmosphere, this energy is scattered, absorbed and reflected by air molecules in the atmosphere and will be typically be reduced to less than 1.0 kW.m\(^{-2}\) (3% ultraviolet, 42% visible light and 55% infrared) by the time it reaches the Earth’s surface (Barsanti and Paolo 2006). The amount of energy lost will depend on factors such as atmospheric and meteorological conditions, as well as the latitude, elevation and landscape of the location upon which the sun is shining (Barsanti and Paolo 2006; Grenfell and Perovich 2008). The total solar radiation acting on a given surface can be broken down into three components: direct, diffuse and reflected, as illustrated in Figure 2-4. Direct radiation (1 in Figure 2-4) is defined as the solar radiation travelling in a straight line from the sun to the incident surface, while diffuse radiation (2 in Figure 2-4) is the solar radiation that has been scattered by particles in the atmosphere but still indirectly reaches the surface of the earth (Liu and Jordan 1960). Finally, reflected radiation (3 in Figure 2-4) is mainly reflected from the terrain and is more prominent in mountainous areas (Kumar et al. 1997).
As discussed in Section 2.4.2, photosynthetic organisms derive their energy from light in the visible, or PAR, range. PAR radiation accounts for between 34 and 50% of total solar radiation, depending on factors such as site latitude and cloud cover (Papaioannou et al. 1993).

2.5 Numerical Modelling

2.5.1 Introduction

Numerical models are an essential tool used in most branches of engineering and science that require the computation of many differential equations to describe a phenomenon of interest (Allen et al. 1987). These mathematical models use a numerical time-stepping procedure to model the behaviour of a system over time and can be used to simulate realistic and complex situations (Datta and Sablani 2006). The advantages of using numerical models include: reductions in the required number of laboratory to bench-scale experiments along with the time and costs associated with
running them; more insight into the determination of processes behaviours that may not be possible solely through experimentation; process optimization; and predictive capability (Datta and Sablani 2006).

This thesis will focus on numerical models designed for WSPs (Beran and Kargi 2005; Gehring et al. 2010; Kayombo et al. 2000; Moreno-Grau et al. 1996) and solar radiation processes (Kumar et al. 1997; Luo et al. 2010; Woo and Young 1996; Young et al. 1995).

2.5.2 Waste Stabilization Ponds

While WSPs are considered to be relatively simple to design and operate, the biological processes that occur within these systems have yet to be entirely characterized (Sah et al. 2012). A number of studies have presented or investigated individual pond models and have typically focused on individual or a limited combination of different aspects of WSP operations, from hydraulic modelling to carbon and nutrient removal to microalgal growth (Beran and Kargi 2005; Gehring et al. 2010; Heaven et al. 2011; Moisan et al. 2002; Shilton and Mara 2005). These models can be used to optimize the design of WSPs by computing treatment rates under operating conditions such as loading rate, detention time, basin volume, eliminating the need for extensive experimental work. However, the hydrodynamic and biochemical components of WSPs can be extremely complex, resulting in individual models that focus on either the hydrology these systems while simplifying the biochemistry, or vice versa (Sah et al. 2012).

Understanding the hydraulic behaviour of WSPs is extremely important in characterizing the effectiveness of these systems. Either plug-flow or completely mixed conditions are assumed in WSP design, however, hydraulic conditions are typically variable and far from ideal (Sah et al. 2012). The presence of dead-zones in basins, areas where wastewater remains stagnant, can drastically affect hydraulic retention times and, as a direct result, treatment efficiencies. (Moreno 1990; Shilton and Mara 2005).
The biochemical processes that occur in WSPs along with the environmental conditions that these systems are exposed to also play an important role in treatment efficiency and pond performance (Sah et al. 2012). The biochemical processes described in section 2.3, including microbial and microalgal nutrient uptake or ammonia volatilization, can all be modelled by combining a series of differential equations (Beran and Kargi 2005; Gehring et al. 2010). However, these processes can either be directly or indirectly affected by environmental factors such as temperature, solar radiation, precipitation or wind. Changes in these environmental conditions over time therefore need to be taken into account when developing a dynamic numerical model in order to properly characterize their effects on WSP treatment performance (Sah et al. 2012). Tables 2-2 and 2-3 summarize the interactions between different WSP processes and their role on treatment performance as well as the effects of environmental factors on biochemical processes.

Table 2-2 - Overview of the major environmental factors that affect WSP performance (Adapted from Sah et al. 2012)

<table>
<thead>
<tr>
<th>Processes</th>
<th>Description</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advection and diffusion</td>
<td>Mechanisms for transport of dissolved substances and heat are gravitational movement, advection, molecular diffusion and turbulent diffusion</td>
<td>Facilitates mixing in the pond and is one of the main factors for pond performance</td>
</tr>
<tr>
<td>Sedimentation</td>
<td>Settling of particulate components whether organic or inorganic, dead algae and bacteria to the bottom</td>
<td>Pathway for BOD, nutrient and pathogen removal</td>
</tr>
<tr>
<td>Re-aeration</td>
<td>Physical process of air–water exchange of dissolved oxygen</td>
<td>Secondary role in contributing to DO concentration in the pond</td>
</tr>
<tr>
<td>Ammonium volatilization</td>
<td>High pH during day facilitates the conversion of NH₃⁻ ion into NH₃. Ammonia, being volatile, escapes from the pond into the atmosphere</td>
<td>Secondary pathway of nitrogen removal</td>
</tr>
<tr>
<td>Adsorption</td>
<td>Chemical process which depends upon pH, rots conditions, salinity, DO and temperature</td>
<td>Removal of inorganic phosphate and ammonia-nitrogen by adsorption to bottom sludge</td>
</tr>
<tr>
<td>Mineralization of organic matter by aerobic bacteria</td>
<td>Anaerobic bacteria in the presence of oxygen assimilate organic carbon and nutrients for growth. During this, nitrification occurs.</td>
<td>BOD and nutrients removal</td>
</tr>
<tr>
<td>Mineralization of organic matter by facultative bacteria</td>
<td>During growth facultative bacteria assimilate organic carbon and nutrients. Under anaerobic conditions, denitrification occurs.</td>
<td>BOD and nutrients removal</td>
</tr>
<tr>
<td>Mineralization of organic matter by anaerobic bacteria</td>
<td>Active in bottom sludge layer, assimilate organic matter and nutrients.</td>
<td>Carbon removal, sulphate removal.</td>
</tr>
<tr>
<td>Growth of algae</td>
<td>Algae use CO₂ and nutrients to fix carbon for growth via photosynthesis and in turn provide oxygen for aerobic bacteria</td>
<td>Main source of DO in the pond, involved directly or indirectly in nutrient removal and pathogen removal</td>
</tr>
<tr>
<td>Decay of algae and bacteria</td>
<td>Natural process of death</td>
<td>Contributes to sediments, a pathway for BOD and nutrient removal as part of non-biodegradable microbial biomass</td>
</tr>
</tbody>
</table>
Table 2-3 Overview of the major environmental factors that affect WSP performance (Adapted from Sah et al. 2012)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Description</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>Dynamic, can be considered as varying function of time, season, weather, time of the day and spatially (i.e. throughout the pond and over the depth)</td>
<td>Algal productivity influences DO and pH, pathogen removal, biochemical oxidation of organic matter</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>Oxygen dynamics is driven predominantly by photosynthesis, which is a function of light, light attenuation and organic loading. Consequently, oxygen shows variation diurnally, spatially (along length, breadth and depth) and between ponds with different organic loading. Important sources of oxygen in ponds are re-aeration and photosynthesis by photosynthetic algae.</td>
<td>Odour prevention, disinfection, biochemical oxidation of organic matter</td>
</tr>
<tr>
<td>Temperature</td>
<td>According to Beran and Kargi (2005), most of the processes are temperature-dependent and are assumed to follow Arrhenius-type temperature dependency. In addition, as cited by Stilton (2005), temperature affects the hydraulic properties of water by stratification and de-stratification under the influence of sun and wind. It also affects the solubility of different substances.</td>
<td>Important parameter controlling the rate of different biochemical reactions and governs the hydraulic properties affecting mixing conditions in the pond.</td>
</tr>
<tr>
<td>pH</td>
<td>Dynamic factor, the pH in the pond is controlled by bicarbonate buffering system. Since pH depends on photosynthesis and organic loading, it shows similar temporal and spatial variation like oxygen.</td>
<td>Pathogens and nutrient removal, odour control</td>
</tr>
<tr>
<td>Wind</td>
<td>Dynamic natural environmental factor</td>
<td>Driving force for re-aeration of ponds, speed and direction controls, hydraulic behaviour and, hence, performance of the ponds</td>
</tr>
</tbody>
</table>

2.5.3 Solar Radiation

Solar radiation data is often used for research in fields like ecology, hydrology and glaciology. However, it is often overlooked in many numerical models and simplified to account for general trends in the length of photoperiods and radiation intensity (Beran and Kargi 2005; Gehring et al. 2010; Kayombo et al. 2000). The effects of solar radiation have been shown to affect WSP performance by enabling microalgal photosynthesis and providing a source of DO to the system. Variations in photoperiod and radiation intensity can vary drastically based on location and time of year (Paltridge and Platt 1976) and should therefore be taken into account when modelling WSP systems.

Furthermore, in certain remote regions, like the Canadian High Arctic, the availability of weather stations to record solar activity can be limited, creating a need for solar radiation models that can produce this data based on limited information such as cloud cover (Young et al. 1995). A number of models have been developed to estimate the amount of PAR radiation acting on a surface based on latitude, time of year and cloud cover (Kumar et al. 1997; Luo et al. 2010; Wyser et al. 2007; Young et al. 1995). The combination of these works with solar radiation models could
therefore assist in depicting a more accurate representation of the effects of environmental conditions on WSPs based on their location, at different periods during the year.

2.6 Summary

The introduction of the WSER in 2012 set stringent nation-wide effluent standards for wastewaters being discharged into receiving environments. These regulations were not ratified by the governments of the Northwest Territories, Nunavut, Quebec or Newfoundland and Labrador due to the geographical and environmental challenges faced by communities located above the 54th parallel. These provinces and territories were tasked with working with the federal government to assess the current state of municipal wastewater treatment in the Arctic, along with setting and meeting adequate discharge standards.

Currently, WSPs are extensively used to treat municipal wastewater in communities located in the Canadian High Arctic due to their ease of operation, along with their low cost and maintenance requirements. However, the performance of these systems is closely linked to environmental factors such as temperature and solar radiation. If exposed to the right conditions, microalgae growth can be promoted in arctic WSPs and create aerobic conditions within the system, affecting their performance by providing oxygen to aerobic bacteria which in turn use it to oxidize organic material and reduce nutrient concentrations.

Numerical modelling can be a very powerful tool in determining the performance of WSPs operated under specific external environmental factors. However, to date, most models have been designed for systems located in temperate climates, with very few studies investigating WSPs in arctic locations, where cold temperatures and extended photoperiods during the summer treatment season could significantly affect the synergistic microbial/microalgal relationships in these biological systems, and hence the overall performance of these WSP systems.
2.7 References


Chapter 3

Growth Rates of the Microalga Strain Chlorella Vulgaris at Cold Temperatures Compared to Three Microalgal Growth Models

3.1 Abstract

The presence of microalgae in wastewater stabilization ponds (WSPs) has been suggested to enhance the treatment efficiency of these systems by creating a synergistic relationship between microalgae and heterotrophic microbial populations. WSPs are commonly used in Arctic communities to treat municipal wastewater, however, summer temperatures that rarely exceed 20°C could potentially limit microalgal growth within these systems. The effects of cold temperatures on the growth of Chlorella vulgaris were investigated and three microalgal growth models were compared to experimental data. The growth rates of C. vulgaris were evaluated for a temperature range of 1°C to 23°C in aerated photobioreactors under a continuous irradiance of 100 µmol photons m−2 s−1. Microalgal growth rates were reduced at colder temperatures with maximum growth rates of \( \mu_{\text{max}} = 0.26 \text{ d}^{-1} \) at 1°C, compared to \( \mu_{\text{max}} = 2.1 \text{ d}^{-1} \) at 23°C. The experimental data was then compared to \( \mu_{\text{max}} \) values predicted by the Eppley Curve, Arrhenius Relationship and a model developed by Peeters & Eilers to determine which model would be most applicable for the growth of C. vulgaris in Arctic WSPs. The Eppley curve was found to overestimate maximum growth rate values by up to 44% at temperatures below 5°C. The Arrhenius relationship was more accurate in predicting maximum growth rates but required the use of different constants at temperature ranges of 1-10°C and 10-23°C. The Peeters & Eilers model was deemed to be the most suitable model for microalgal growth rates in Arctic WSPs, accurately predicting \( \mu_{\text{max}} \) values at temperatures of 1-23°C.

3.2 Introduction

For decades, naturalized and biological wastewater treatment systems have been considered to be effective methods for the treatment of wastewaters in communities located in northern climates.
The low cost and low maintenance requirements of these systems, along with their minimal dependence on mechanical instrumentation, make them ideal systems for the lower volumes of wastewater typically generated by small communities (Heaven et al. 2003; Jenssen et al. 1994).

The main objectives of biological treatment systems are to transform biodegradable constituents into end products considered acceptable for discharge into receiving environments, as well as to reduce nutrient (nitrogen and phosphorous) concentrations from the wastewater (Tchobanoglous et al. 2014b). Particulate and dissolved carbonaceous organic matter are oxidized into simple end products by microorganisms such as microalgae and bacteria, which also participate in the uptake of nitrogen and phosphorous from the liquid waste stream. Nitrogen removal from wastewater typically occurs through nitrification and denitrification. In the nitrification process, aerobic bacteria in the genera of *Nitrosomonas* and *Nitrobacter*, oxidise nitrogen, mostly in the form of ammonia, to nitrite and nitrate in a two-step reaction. Nitrate is then converted to nitrogen gas through a biologically anoxic reaction with several genera of bacteria being capable of conducting this process, including, but not limited to, *Archomobacter, Bacillus, Methanomonas, Moraxella* or *Neisseria*. The complete removal of nitrogen from WSPs therefore requires both aerobic and anaerobic conditions (Sah et al. 2012; Tchobanoglous et al. 2014b; Yi et al. 2009). The biological removal of phosphorous occurs by its incorporation into the cellular structure of the biomass present in a wastewater treatment system, which is subsequently removed from the sludge. The composition of ordinary heterotrophic microorganisms is on the order of 0.015g P/g.VSS, resulting in typical phosphorous removal rates of 10-20% (Tchobanoglous et al. 2014b). However, the presence of phosphorous accumulating organisms, a group of bacteria that accumulate relatively larger amounts of polyphosphates within their cells, can result in phosphorous removal rates of up to 80% (Blackall et al. 2002; Tchobanoglous et al. 2014b).

Wastewater stabilization ponds (WSP) are a simple form of biological treatment (Shammas et al. 2009) and are commonly used to store and treat municipal domestic wastewater in Arctic
communities (Krkosek et al. 2012). During certain periods of the treatment seasons, high microalgal concentrations can sometimes be observed in WSPs, affecting the pH and dissolved oxygen concentration of the wastewater, as well as the concentration of total suspended solids (TSS) in the effluent of the system (Camargo Valero et al. 2010; Hayward et al. 2014; Shammas et al. 2009). While their role in WSPs has yet to be fully characterized, microalgae have been shown to promote the growth of aerobic microorganisms by providing them with a constant supply of dissolved oxygen through the photosynthetic process (Kohn and Nelson 2007; McGinn et al. 2012; Tang et al. 1997). The metabolic activities of these microorganisms, in turn, supply the system with CO$_2$ as an end product of the oxidation process, creating a synergistic relationship with the microalgae (Shammas et al. 2009). A number of microalgal strains have also been shown to be effective in the removal of nutrients from wastewater due to their high uptake capacity for nitrogen and phosphorous (Dickinson et al. 2013; Ge et al. 2013; Wang et al. 2010). These factors suggest that the presence of microalgae in WSPs should beneficially contribute to treatment efficiency, provided that the microalgae is removed from the effluent prior to its discharge into a receiving environment. However, the presence of microalgae in WSPs located in the Canadian High Arctic is highly dependent on temperature and solar radiation availability (Shammas et al. 2009). Developing an understanding of how these environmental factors affect microalgal growth could assist in assessing the reliability of WSPs as a primary method of wastewater treatment in Arctic communities.

Temperature has been shown to be one of the main environmental factors that affects microalgal growth in WSPs (Beran and Kargi 2005; Heaven et al. 2007; Kayombo et al. 2000), with microalgal photosynthesis and cellular division reported at temperatures below 0°C and over 30°C (Bartosh and Banks 2007; Moisan et al. 2002). Most microalgal species have an optimal temperature, or range of temperatures, at which maximum growth rates occur, typically between 15-20°C (Bouterfas et al. 2002; Xin et al. 2011). Growth rates generally decrease as temperatures shift from the optimal range before eventually reaching a point where cellular division is no longer possible. For
example, the microalgal strain *Chlorella vulgaris*, which is considered to be a representative species of microalgae in WSPs (Shammas et al. 2009), is unable to grow at water temperatures below 0°C but has been shown to survive at temperatures below -20°C (Bartosh and Banks 2007).

A number of models have been developed to predict maximum microalgal growth rates at specific temperatures (Bernard and Rémon 2012; Bissinger et al. 2008; Xin et al. 2011) in order to identify conditions that are favorable or limiting to microalgae growth. However, the majority of these studies have been conducted within temperature ranges of 10-30°C, and can therefore not be considered to necessarily reflect the temperature conditions of Arctic WSPs. The aim of this study was to characterize the growth rates of *C. vulgaris* within Arctic summer temperature ranges, between 1°C and 23°C, and compare the effectiveness of three different models at predicting microalgal growth rates at these temperatures.

### 3.3 Material and Methods

#### 3.3.1 Stock microalgal cultivation

The microalgal strain *Chlorella Vulgaris* (UTEX, B1803) was selected for this experiment as it has previously been reported to be robust in wastewater environments under cold climate conditions, and is considered to be a representative species in WSPs (Bartosh and Banks 2007; Muñoz and Guieysse 2006). The microalgae were initially cultivated in a standard Bold’s Basal Medium (BBM) in a 26cm(H) x 10cm(D) UTEX photobioreactor (PBR) and maintained in an exponential growth phase at ambient temperature as noted in McGinn et al. (2012). These two-liter cylindrical glass containers were aerated using an air diffuser and illuminated from the side using LED strips, which provided a constant light output of 100 μmol photons m⁻² s⁻¹.

#### 3.3.2 Batch temperature experiments

Each PBR was inoculated with a concentration of 0.5x10⁶ cells.L⁻¹ of *C. vulgaris* to maintain the cells in their exponential growth phase. The experiments were conducted at temperatures of 1°C,
5°C, 7.5°C, 10°C, 12.5°C, 15°C, 20°C and 23°C in batch PBRs using BBM as the substrate, or the source of nutrients for the microalgae. Nutrient and CO₂ supplementation was not provided. Air, supplied from a Danner AP-20 Air pump with a capacity of 1.67 m³.min⁻¹, was bubbled to the system to provide turbulent mixing to the system and the PBRs were illuminated at a constant light intensity of 100 µmol photons m⁻² s⁻¹ for twenty four hours per day. Experiments were continued until microalgae growth rates began to decline.

3.3.3 Microalgal growth rate analysis

Growth rates were measured on a daily basis for the duration of the experiment. The microalgal concentration in each PBR was monitored via cell enumeration using a Bright-Line haemocytometer viewed under 200x magnification with a Nikon Eclipse E200 Microscope. Samples were diluted up to ten times to facilitate cell counting.

The daily specific growth rate of *C. Vulgaris* was calculated from the cell count using the following Equation 3.1 as per Barsanti and Paolo (2006)

\[
\mu = \frac{\ln(X_2) - \ln(X_1)}{t_2 - t_1}
\]  

(3.1)

where \( \mu \) was the specific growth rate (d⁻¹), and \( X_2 \) and \( X_1 \) were the microalgal cell concentrations (cells.L⁻¹) at times \( t_2 \) and \( t_1 \) (d).

Three microalgae growth models were compared using the collected data. The Eppley relationship, Arrhenius formula and a third model developed by (Peeters and Eilers 1978) are models that have been used to predict maximal microalgal growth rates at various temperatures (Beran and Kargi 2005; Bissinger et al. 2008; Bernard and Rémond 2012; Xin et al. 2011)

The Eppley relationship has been employed to represent the relationship between temperature and microalgal growth in marine and freshwater ecosystem models (Bissinger et al. 2008; Moisan et al. 2002). The empirical equation was originally derived from growth curve data for a variety of microalgal species by Eppley (1972) and can is expressed in Equation 3.2:

\[
\mu_T = 0.59e^{(0.0633T)}
\]  

(3.2)
where $\mu_T$ is the maximum predicted growth rate (d$^{-1}$) for a given temperature $T$ (°C).

The Arrhenius formula is used to predict various reaction rates at various temperatures, from wastewater treatment rates in WSPs (Beran and Kargi 2005; Sah et al. 2012) to microalgal growth (Xin et al. 2011). The method described by Xin et al. (2011) to describe the relationship between temperature and specific growth rates of the microalgal strain *Scenedesmus sp.* At different temperatures was also used in this study, as expressed in Equation 3.3:

$$\mu = A \exp\left(-\frac{E_a}{RT}\right)$$

(3.3)

where $\mu$ is the maximum predicted growth rate at a given thermodynamic temperature, $T$ (°K), $A$ is the Arrhenius constant (d$^{-1}$), $E_a$ is the growth activation energy (kJ.mol$^{-1}$), $R$ is the universal gas constant (8.314x10$^{-3}$ kJ.mol$^{-1}$.K$^{-1}$). The Arrhenius constant, $A$, and the growth activation energy, $E_a$, described in Equation 3 were obtained through a regression analysis of the linear relationship between $\ln \mu$ and $1/T$, as per Equation 3.4:

$$\ln \mu = \ln A - \frac{E_a}{R T}$$

(3.4)

Peeters and Eilers (1978) developed a mathematical equation to describe the relationship between light intensity and photosynthesis. This model can also be used to describe the relationship between microalgal growth and temperature (Bernard and Rémond 2012; Bouterfas et al. 2002) and is expressed in Equations 3.5.a and 3.5.b as follows:

$$\mu_T = 2\mu_{opt} (1 + \beta) \left( \frac{x}{x^2 + 2\beta x + 1} \right)$$

(3.5.a)

with

$$x = \frac{T - T_0}{T_{opt} - T_0}$$

(3.5.b)

where $\mu_T$ is the maximum predicted growth rate (d$^{-1}$) at a temperature $T$ (°C); $\mu_{opt}$ is the maximal growth rate (d$^{-1}$) occurring at the optimal temperature ($T_{opt}$, °C); $T_0$ is the temperature at which microalgae growth no longer occurs; and $\beta$ is the shape factor for growth limitation due to temperature and is determined using the least squares method (Dermoun et al. 1992).
3.4 Results and Discussion

The maximum growth rates computed for *C. vulgaris* at temperatures between 1°C and 23°C are illustrated in Table 3-1. Growth rates were found to increase with time to a maximum value before starting to decrease when the microalgal cells entered their decay phase, as described by Barsanti and Paolo (2006). Maximum growth rates were achieved after two days for temperatures ranging between 7.5°C and 23°C, and three days at temperatures of 1°C and 5°C. The highest $\mu_{max}$ value (2.1 d$^{-1}$) was recorded at 23°C, while the lowest $\mu_{max}$ (0.28 d$^{-1}$) occurred at 1°C.

Table 3-1 - Maximum growth rates of the microalgal strain *C. vulgaris* at various temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$\mu_{max}$ (d$^{-1}$)</th>
<th>Std dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.28</td>
<td>0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.53</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>1.07</td>
<td>0.16</td>
</tr>
<tr>
<td>10</td>
<td>1.61</td>
<td>0.20</td>
</tr>
<tr>
<td>12.5</td>
<td>1.53</td>
<td>0.05</td>
</tr>
<tr>
<td>15</td>
<td>1.65</td>
<td>0.43</td>
</tr>
<tr>
<td>20</td>
<td>1.99</td>
<td>0.08</td>
</tr>
<tr>
<td>23</td>
<td>2.1</td>
<td>0.10</td>
</tr>
</tbody>
</table>

3.4.1 Eppley Curve

The Eppley curve is a relationship that describes the maximal growth rate of microalgae at various temperatures, as stated in Equation 3.2. The curve, developed by Eppley (1972), was obtained from a data set comprising many species of marine microalgae. The constants used in the equation to describe the curve therefore remain the same regardless of the modelled species (Bissinger et al. 2008) and imply an exponential relationship between microalgal growth and temperature, as illustrated in Figure 3-1.
Figure 3-1- Experimentally determined maximal growth rates of C. vulgaris at various temperatures plotted against the Eppley Curve

The curve generated by the Eppley relationship had an $r^2$ value of 0.76, and, when compared to the collected $\mu_{\text{max}}$ data in this study, was found to overestimate maximum growth rates at temperatures below 5°C. The predicted $\mu_{\text{max}}$ value at 1°C was 0.63 d$^{-1}$, while the measured value at the corresponding temperature was 0.28 d$^{-1}$, resulting in a 44% overestimation. A study of more than 100 species of phytoplankton conducted by Moisan et al. (2002) noted that the Eppley curve could overestimate growth rates at colder temperatures by up to 80%, resulting in significant variability in the predicted versus observed microalgal growth patterns. Therefore, the experimentally-derived results from this study would suggest that the Eppley curve may not be suitable to model the relationship $C. vulgaris$ growth at different temperatures in Arctic WSPs.
3.4.2 Arrhenius Relationship

The collected experimental data was linearized as per Equation 3.4 and the relationship between $\ln \mu_{\text{max}}$ and $1/T$ was plotted in order to perform a regression analysis, shown in Figure 3-2.

![Figure 3-2: Linearized relationship between $\mu_{\text{max}}$ and temperature over three different data sets: 1-10°C, 20-23°C and 1-23°C.](image)

A regression analysis of the experimental data to the model yielded an Arrhenius constant, $A = e^{24.3}$ d$^{-1}$ and a growth activation energy of 57.2 kJ.mol$^{-1}$, with an $r^2$ value of 0.78. However, a study conducted by Xin et al. (2011) suggested that Arrhenius models were most accurate for specific temperature ranges. The data was therefore separated into two different subsets to determine Arrhenius constants for cold (1-10°C) and temperate (10-23°C) temperatures. Separating the data resulted in more accurate regression analyses, yielding $r^2$ values of 0.97 and 0.89 for the cold and
temperate temperature subsets, respectively. The resulting Arrhenius constants are summarized in Table 3-2.

Table 3-2 - Arrhenius constants derived from a regression analysis of collected μ_max data at temperature ranges of 1-23°C, 1-10°C and 10-23°C

<table>
<thead>
<tr>
<th>Temperature range</th>
<th>1-23°C</th>
<th>1-10°C</th>
<th>10-23°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r^2 )</td>
<td>0.78</td>
<td>0.97</td>
<td>0.89</td>
</tr>
<tr>
<td>( A ) (d^{-1})</td>
<td>( \exp(24.3) )</td>
<td>( \exp(55.8) )</td>
<td>( \exp(7.7) )</td>
</tr>
<tr>
<td>( E_a ) (kJ.mol^{-1})</td>
<td>57.2</td>
<td>130.2</td>
<td>17.2</td>
</tr>
</tbody>
</table>

The derived constants were then used in Equation 3.3 to plot the Arrhenius relationship against the collected experimental data. As can be seen in Figure 3-3, the constants derived using the 1-23°C growth data generated a curve that did not fit the experimentally derived μ_max data well. However, the Arrhenius constants generated from the regression analysis of the cold temperature subset yielded a relationship, which was a good fit to the experimental μ_max values in the range 1-10°C. Similarly, the constants obtained from the regression analysis using the temperate data subset also yielded an Arrhenius relationship, which adequately fit the collected experimental data for a temperature range of 10-23°C.

Figure 3-3 - Arrhenius relationship for μ_max and temperature using constants derived through a linear regression of experimental data in the range of 1-23°C, 1-10°C and 10-23°C
The Arrhenius relationship was found to effectively predict maximum growth rates of \textit{C. vulgaris} within specific temperature ranges. This is, therefore, an adequate model to represent microalgal growth rates in Arctic WSPs, provided consideration be given to use the appropriate Arrhenius constants as a function of ambient temperature.

\subsection*{3.4.3 Peeters and Eilers}

The shape factor ($\beta$) in the Peeters and Eilers method was computed using Equation 3.5 for each temperature, as reported in Table 3-3.

\begin{table}[h]
\centering
\begin{tabular}{lcc}
\hline
Temperature (°C) & $\mu_{\text{max}}$ & $\beta$ \\
\hline
1 & 0.28 & -0.22 \\
5 & 0.53 & -0.62 \\
7.5 & 1.07 & -0.39 \\
10 & 1.61 & 0.05 \\
12.5 & 1.53 & -0.54 \\
15 & 1.65 & -0.69 \\
20 & 1.99 & -0.84 \\
23 & 2.1 & - \\
\hline
\end{tabular}
\caption{Shape factor ($\beta$) calculation for the Peeters and Eilers growth model equation at different temperatures}
\end{table}

Dermoun et al. (1992) demonstrated that this model was not sensitive to variations in $\beta$ and that an average of these values could provide an adequate representation of the microalgal system. A fixed average value of -0.46 (±0.1) was therefore used for Equation 3.5, along with a $\mu_{\text{opt}}$=2.1 d$^{-1}$, $T_{\text{opt}}$=23°C and $T_0$=0°C. The resulting relationship between the experimental and predicted $\mu_{\text{max}}$ values is illustrated in Figure 3-4, along with the upper and lower boundaries of the function obtained by taking into account the error in $\beta$. 

54
Figure 3-4 - Experimentally derived $\mu_{\text{max}}$ values plotted against the Peeters and Eilers growth model using $\beta = -0.46$, $\mu_{\text{opt}} = 2.1 \text{ d}^{-1}$, $T_{\text{opt}} = 23^\circ C$ and $T_0 = 0^\circ C$

The resulting equation generated a curve with an $r^2$ value of 0.95 which followed a trend similar to the experimentally derived $\mu_{\text{max}}$ data, rapidly increasing between 1$^\circ C$ and 15$^\circ C$ before gradually levelling off as it approached the maximum growth value at 23$^\circ C$. The Peeters and Eilers model was therefore considered to be a suitable tool in predicting growth rates of *C. vulgaris* in Arctic WSPs over a temperature range of 1-23$^\circ C$.

3.5 Conclusion

Experimentally derived average $\mu_{\text{max}}$ values for temperatures between 1$^\circ C$ and 23$^\circ C$ are summarized in Table 3-4, along with corresponding values predicted using the Eppley, Arrhenius and Peeters and Eilers models.
Table 3-4 - Summary comparison of the experimentally derived and predicted $\mu_{\text{max}}$ values generated by the Eppley, Arrhenius and Peeters and Eilers models for maximum microalgae growth rates at different temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Experimental Data (error)</th>
<th>Eppley Curve</th>
<th>Arrhenius General</th>
<th>Arrhenius Cold</th>
<th>Arrhenius Warm</th>
<th>Peeters &amp; Eilers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°C</td>
<td>0.28 (0.02)</td>
<td>0.63</td>
<td>0.45</td>
<td>0.27</td>
<td>-</td>
<td>0.20</td>
</tr>
<tr>
<td>5°C</td>
<td>0.53 (0.01)</td>
<td>0.81</td>
<td>0.64</td>
<td>0.62</td>
<td>-</td>
<td>0.68</td>
</tr>
<tr>
<td>7.5°C</td>
<td>1.07 (0.16)</td>
<td>0.92</td>
<td>0.76</td>
<td>0.92</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>10°C</td>
<td>1.61 (0.20)</td>
<td>1.11</td>
<td>0.99</td>
<td><strong>1.67</strong></td>
<td>1.51</td>
<td>1.31</td>
</tr>
<tr>
<td>12.5°C</td>
<td>1.53 (0.05)</td>
<td>1.30</td>
<td>1.22</td>
<td>-</td>
<td>1.61</td>
<td><strong>1.59</strong></td>
</tr>
<tr>
<td>15°C</td>
<td>1.65 (0.43)</td>
<td>1.52</td>
<td>1.51</td>
<td>-</td>
<td><strong>1.72</strong></td>
<td>1.82</td>
</tr>
<tr>
<td>20°C</td>
<td>1.99 (0.08)</td>
<td>2.09</td>
<td>2.27</td>
<td>-</td>
<td><strong>1.94</strong></td>
<td>2.07</td>
</tr>
<tr>
<td>23°C</td>
<td>2.10 (0.10)</td>
<td>2.53</td>
<td>2.87</td>
<td>-</td>
<td>2.08</td>
<td><strong>2.10</strong></td>
</tr>
</tbody>
</table>

The Arrhenius relationship divided into the cold and temperate subsets was found to be more accurate over each of the respective temperature ranges, with $r^2$ values of 0.97 and 0.89 at cold and warm temperatures, respectively. However, the curve generated by the Peeters and Eilers method had an $r^2$ value of 0.95 over a wider range of temperatures. Figure 3-5 presents a summary of all five curves compared to the experimental data.
Figure 3-5: Five mathematically derived growth functions compared to collected experimental growth data of *C. vulgaris* at temperatures between 1°C and 23°C

The growth rates of the microalgal strain *C. vulgaris* were found to be highly affected by cold temperatures. Maximum growth values increased from 0.26 d⁻¹ at 1°C and 2.1 d⁻¹ at 23°C. The Eppley curve was found to over-predict maximal growth rates by up to 44% at temperatures below 5°C and was therefore deemed inappropriate to model the growth rates of *C. vulgaris* in Arctic WSPs. The Arrhenius relationship was found to be an accurate model for the prediction of $\mu_{\text{max}}$ values, but required the use of different constants for temperature ranges of 1-10°C and 10-23°C. This method could therefore be suitable for modelling the growth of *C. vulgaris* in Arctic WSPs if the appropriate Arrhenius constants are used at temperatures between 1-10°C and 10-23°C. Finally, the
Peeters and Eilers model accurately predicted maximum growth rates for temperatures of 1-23°C, making it most suitable to model the effects of temperature of the growth rates of *C. vulgaris* in Arctic WSPs.

### 3.6 References


Chapter 4

Modelling a Wastewater Stabilization Pond in the Canadian High Arctic

4.1 Abstract

A mathematical model was modified to simulate the processes of a wastewater stabilization pond located in Pond Inlet, Nunavut using partial differential equations and matrix manipulation techniques. Historical weather data were used to compare the effects of climate on microalgae growth and other biological processes on an annual basis. Microalgae growth was found to be closely related to environmental factors such as temperature and available solar radiation, requiring a combination of both in order to proliferate in the WSP. The presence of extensive microalgae growth in the system were predicted, and surmised to promote biological treatment processes by increasing the concentration of dissolved oxygen (DO) in the system, thus improving the overall efficiency of the WSP. It was concluded that changes in climate from year to year could affect WSP treatment rates, suggesting that the performance of these systems may be inconsistent.

4.2 Introduction

Wastewater stabilization ponds (WSPs) are an appropriate method to treat municipal wastewater for small communities where the space is available, due to their relative operational simplicity and low costs (Heaven et al. 2003). Until February 2009, WSPs met regulatory discharge guidelines in the Northwest Territories and the northern part of other Canadian provinces and territories where extreme climatic cycles are common (Dawson et al. 1969). However, the development of a Canada-wide strategy for the management of municipal wastewater effluent required wastewater treatment facilities across the country to achieve minimum national performance standards. Provinces and Territories in Canada’s far north deemed these standards to be too stringent and requested that special considerations be given due to the challenges associated with treating wastewater in these locations. These challenges include the remoteness of communities, which limits
the access to chemical and mechanical wastewater treatment resources, as well as extreme climates which reduce the effectiveness of outdoor wastewater treatment systems compared to more temperate systems. Therefore, the governments of the Northwest Territories, Nunavut, Newfoundland, Labrador and Quebec, through consultation with the federal government, were tasked to assess the performance of existing wastewater treatment facilities and develop performance standards as well as timelines for their implementation (Canadian Council of Ministers of the Environment 2009). This created a need to develop a better understanding of WSP kinetics in cold climates in order to determine whether or not these systems could be considered sufficient as a method of wastewater treatment in smaller arctic and rural communities.

WSPs are one of the simplest forms of biological treatment from an operational point of view and are commonly used to store and treat municipal domestic wastewater, as well as to settle and remove suspended solids from a waste stream (Shammas et al. 2009). Two of the principal objectives of biological treatment processes are to transform and remove undesirable biodegradable constituents nutrients, such as nitrogen and phosphorous from a wastewater stream (Tchobanoglous et al. 2014b). These biologically-mediated processes occur when microorganisms, such as bacteria and microalgae, oxidize particulate and dissolved organic matter into simpler end products and additional biomass. Microorganisms can also actively participate in the removal of nitrogen and phosphorous from wastewater. Nitrogen removal occurs with the oxidation of ammonia to nitrite and nitrate (nitrification) by specific aerobic bacteria (Nitrosomonas, Nitrobacter), while other anaerobic microorganisms (Archomobacter, Bacillus, Methanomonas, Moraxella or Neisseria) are capable of reducing the oxidized nitrogen to its gaseous form through denitrification. Phosphorous removal from wastewater occurs in systems that can promote the growth of microorganisms capable of a large uptake of inorganic phosphorous (Tchobanoglous et al. 2014b). The removal of harmful bacteria can also occur in WSPs, with sunlight in the ultraviolet range having been shown to impact enteric pathogens (Curtis et al. 1992; Kohn and Nelson 2007).
A variety of internal and external factors influence the efficiency and efficacy of biological treatment processes. High nutrient concentrations and long wastewater retention times combined with the right temperature and solar radiation may lead to the establishment of microalgae within the system. Under these conditions, a bacterial-microalgae synergy can promote biological treatment. Abundant microalgae growth in the upper portion of the water column in the pond can lead to the supersaturation of the wastewater with dissolved oxygen via photosynthesis, which will provide aerobic microorganisms with a constant supply of dissolved oxygen to allow for the oxidation of organic matter or nitrogenous compounds such as ammonia. Furthermore, high dissolved oxygen concentrations have been linked to increased pathogen inactivation due to the formation of reactive oxygen species (ROS) which may damage cell constituents. These ROS are formed due to the excitation of light-absorbing compounds which transfer energy to other molecules, referred to as sensitizers, which can either be located inside the cell, leading to endogeneous photoinactivation, or outside the cell, leading to exogeneous photoinactivation (Kohn and Nelson 2007). Photosynthetic activity requires a large amount of CO₂ for microalgae synthesis, the demand for which is satisfied by the oxidation process which includes CO₂ as an end product (Shammas et al. 2009). Two of the main environmental factors that affect microalgae growth in WSPs are temperature and solar radiation. Temperature has been found to influence the metabolic activities of microbial populations, while variations in light intensity have been shown to affect microalgae growth, and therefore photosynthetic rates, in the system (Dermoun et al. 1992; Tchobanoglous et al. 2014b).

While the effects of microalgae in WSPs have not fully been characterized beyond their role in the photosynthetic process, their presence could enhance pathogen and nutrient removal due to their ability to produce oxygen and the high uptake capacity for nitrogen and phosphorous shown by several strains (Kohn and Nelson 2007; McGinn et al. 2012; Tang et al. 1997). Mathematical models that incorporate biological reactions, including microalgae growth, as well as photosynthetic and
nutrient uptake rates, present a useful tool to determine the effect of microalgae on wastewater
treatment in WSPs.

A number of numerical models have previously been developed to simulate WSP dynamics in a variety of temperate climates (Beran and Kargi 2005; Gehring et al. 2010; Heaven et al. 2011) but they do not include adequate parameters that would make them applicable to geographical locations above the Arctic Circle. Cold temperatures during the summer months in the Arctic would require that these models to be recalibrated to account for temperature corrections, and parameters simulating the amount of solar radiation affecting WSPs would also need to be modified. Several methods for the modelling of solar radiation have been developed (Gueymard 1995; Kumar et al. 1997; Woo and Young 1996; Wyser et al. 2007; Young et al. 1995), but they have not commonly been integrated into WSP models.

The purpose of this paper is to present a mathematical model that could be used to predict the efficiency of arctic WSPs under different climatic conditions. This was accomplished by integrating solar radiation models and historical arctic weather data into an existing WSP model which was then adapted for cold temperatures, specifically pertaining to microalgae growth.

4.3 Methodology

4.3.1 Site description

The modelled WSP is located in Pond Inlet, NU (72.7°N, 78.0°W), shown in Figure 4-1, a small arctic community on the north-eastern tip of Baffin Island with a population of approximately 1,500 inhabitants (Statistics Canada, 2011).
Figure 4-1. Geographical location of Pond Inlet, NU (A), relative to Iqaluit, NU (B) and Kingston, ON (C)

The geographical location of Pond Inlet poses several unique challenges to the collection and treatment of wastewater. Extreme weather conditions and year round permafrost make the installation of underground sewage pipe systems impractical, requiring the use of collection trucks to transport wastewater from residences to the WSP. Furthermore, temperatures are below freezing during eight months of the year, as seen in Figure 4-2(a), hindering biological treatment processes and thus only allowing only for a three month treatment period from June until the end of August. However, Pond Inlet’s position above the Arctic Circle also implies twenty-four hour photoperiods during this time frame, as illustrated in Figure 4-2(b), which creates a favourable environment for microalgae growth.
Figure 4-2 - (a) Average daily temperature for Pond Inlet, NU between 2009 and 2013 (Weatherspark.com 2015) (b) Calculated hours of daylight for Pond Inlet, NU as per Hebeler (2008)

The WSP consists of a basin with a capacity of over 40,000 m$^3$ that covers an area of 31,500 m$^2$ and has a depth between 0.8 m and 1.1 m. The system is continuously loaded with residential wastewater and discharged once per year, after the treatment season period and prior to freezing (typically in early September). The treated wastewater is emptied from the WSP into the Northwest Passages in the Arctic Ocean.

4.3.2 Historical meteorological data

The proposed mathematical model was developed to incorporate historical weather data from Pond Inlet into its equations in order to determine the effects of climatic environmental factors on the WSP from year to year. Annual weather data for the time period between 2009 and 2013 was provided by Weather Spark and included hourly values of temperature, wind speed and cloud cover, which were all used in the model (Weatherspark.com 2015).
4.3.3 Solar radiation modelling

The equations for the solar radiation portion of the model were based on the approach presented by Kumar et al (1997) and a MATLAB_2009 script developed by Hebeler (2008). Values for direct and diffuse shortwave solar radiation were calculated on an hourly basis based on latitude, slope, time of year and hour of the day (Hebeler 2008; Kumar et al. 1997). It should be noted that angles in these equations were computed in radians as per Kumar et al. (1997).

Solar radiation intensity was calculated as a function of the solar direction relative to the local plane of the Earth’s surface at a given point in time. Variables used in these calculations either remained constant each day, such as solar declination angle, and was therefore calculated once daily; or changed continuously throughout the day, requiring hourly computation of values for each day, such as solar altitude angle or solar azimuth angle.

The solar declination angle ($\delta_s$) was varied daily and was defined as the angular distance of the sun from the Earth’s equator. Values for $\delta_s$ varied between 23.45°S and 23.45°N based on the tilt of the Earth and could be expressed using the Equation 4.1:

$$\delta_s = 23.45 \times dr \times \sin \left( 2\pi \times \frac{284+i}{365} \right)$$

where $dr$ converts angles from degrees to radians ($dr=0.0174532925$), and $i$ is the Julian day number, January 1st being day 1 and December 31st being day 365.

Solar hour angle, $h_s$, is a variable that changes on an hourly basis and was used as a method of expressing time in angular measurements from solar noon, when $h_s$ is equal to 0 radians. Time before and after solar noon was expressed as negative and positive radians, respectively, and typically increases at a rate of $\pi/12$ radians per hour. Solar hour angle was calculated as follows:

$$h_s = -180 \times dr + \frac{\pi}{12} \times (j - 1)$$

where $j$ is the hour of day (0100 hours is equal to 1 and 2400 hours is equal to 24).

The solar altitude angle ($\alpha$) is defined as the angular elevation of the sun above the horizon and was calculated as a function of latitude (Lat), solar declination angle and solar hour angle. This
value is positive between sunrise and sunset, negative between sunset and sunrise and equal to 0 radians at $h_{sr}$ and $h_{ss}$.

$$\sin \alpha = \sin \text{Lat} \sin \varphi + \cos \text{Lat} \cos \varphi \cos h_s$$

(4.3)

The solar azimuth angle ($a_s$) defines the direction of the sun from the modelled site relative to a line due south and was also calculated as a function of solar declination angle, solar hour angle and solar elevation as can be seen in the Equation 4.4:

$$\sin a_s = \frac{\cos \varphi \sin h_s}{\cos \alpha}$$

(4.4)

Convention dictates that when solar azimuth values are positive, the sun is East of South and negative if the sun is West of South.

The solar radiation acting on a site will depend on the solar flux outside of the atmosphere as well as atmospheric properties such as optical air mass, water vapour and aerosol content. Solar flux outside the atmosphere ($I_{atm}$, expressed in W.m$^2$) was calculated as follows (Duffie and Beckman 2013; Kumar et al. 1997):

$$I_{atm} = S_o \left(1 + 0.0344 \cos \left(\frac{2\pi i}{365}\right)\right)$$

(4.5)

where $S_o$ is a solar constant which can be defined as the irradiance of an area perpendicular to the rays of the sun outside the atmosphere at the mean sun-Earth distance (Kumar et al. 1997). Several values for this constant have been reported (Jansen 1985; Monteith and Unsworth 1990) but a global value of 1367 W.m$^2$ has been adopted by the World Radiation Center (Duffie and Beckman 2013) and was used in this model.

The solar flux is attenuated as it passes through the atmosphere due to absorption by different gases in the atmosphere, molecular scattering by permanent gases and aerosol scattering due to particulates in the air. Furthermore, the air mass ratio ($M$), defined as the direct optical path length through the atmosphere, also contributes to the attenuation of solar radiation. Kumar et al. (1997) proposed the following equation to calculate $M$:

$$M = [1229 + (614 \sin \alpha)^2]^{\frac{1}{2}} - 614 \sin \alpha$$

(4.6)
The air mass ratio varies on an hourly basis and can be used to calculate atmospheric transmittance ($\tau_b$) for beam radiation using the following equation as described by Kumar et al. (1997).

$$\tau_b = 0.56(e^{-0.65M} + e^{-0.095M}) \quad (4.7)$$

Two types of radiation were accounted for in this model, direct and diffuse radiation. Direct radiation is defined as the solar radiation travelling in a straight line from the sun onto an incident surface. Diffuse radiation is the solar radiation that has been scattered by particles in the atmosphere but has still indirectly made it to the surface of the earth (Liu and Jordan 1960). The equation used to calculate the direct solar radiation ($I_{direct}$, expressed in W.m$^{-2}$) acting on a given surface while accounting for atmospheric attenuation can be expressed as follows:

$$I_{direct} = I_{atm} \tau_b \quad (4.8)$$

Kumar et al. (1997) go on to describe the calculation of direct solar radiation on a tilted surface, however, this step was omitted in this model since the WSP is assumed to be on an even plane.

Diffuse solar radiation ($I_{diffuse}$, expressed in W.m$^{-2}$) can be calculated as a function of the radiation diffusion, solar elevation angle and tilt angle of the modelled surface. The radiation diffusion coefficient for diffuse radiation ($\tau_d$) and can be calculated using the following equation

$$\tau_d = 0.271 - 0.294\tau_b \quad (4.9)$$

Having accounted for atmospheric diffusion, diffuse solar radiation can be calculated with the following equation:

$$I_{diffuse} = \frac{I_{atm}\tau_d(\cos^2\beta_{rad})}{2\sin\alpha} \quad (4.10)$$

where $\beta_{rad}$ is the tilt angle of the surface, the value for which is 0 radians due to the level nature of the modelled area.
As can be seen in Equations 4.9 and 4.10, the coefficient for diffuse radiation is directly related to the coefficient for direct radiation. This relationship shows that an increase in direct solar radiation corresponds to a decrease in diffuse solar radiation.

The total solar radiation acting on the modelled surface \( I_{tot} \) (expressed in W.m\(^{-2}\)) is the sum of direct and diffuse radiation as can be seen in the following equation.

\[
I_{tot} = I_{direct} + I_{diffuse} \tag{4.11}
\]

Decreases in solar radiation due to overcast conditions were accounted for by incorporating historical measured cloud cover values into the solar radiation model. Cloud cover ranged between 0 and 1, with values of 0 indicating clear skies and values of 1 indicating completely overcast conditions. Since, direct radiation is the solar radiation travelling in a straight line from the sun towards an incident surface, the effects of cloud cover on this value was accounted for by making the following change to Equation 4.8:

\[
I_{direct\_adj} = I_{direct}(1 - TC) \tag{4.12}
\]

where TC (unitless) is the fraction of total cloud cover observed. Equation 4.11 was therefore modified as follows to reflect this change.

\[
I_{tot} = I_{direct\_adj} + I_{diffuse} \tag{4.13}
\]

The model presented by Kumar et al. (1997) estimated total shortwave radiation at the earth’s surface. Shortwave radiation can be broken down into the ultraviolet (approximately 300 nm to 400 nm), visible (400 nm to 700 nm) and infrared (700 nm to 4000 nm) spectrums. However, microalgae derive their biologically usable energy from the visible light spectrum, also known as photosynthetic active radiation (PAR), which typically accounts for less than half of the total solar energy that reaches the surface of the Earth (Barsanti and Paolo 2006). The ratio of PAR to the whole spectrum of solar irradiance (SI) has been shown to vary between 0.34 and 0.499 (Papaioannou et al. 1993), with cloud cover further affecting these values. Papaioannou et al. (1993) stated that a mean annual PAR to SI ratio of 0.473 was comparable to values reported in literature and a WSP model developed
by Gehring et al. (2010) also assumed that PAR was 0.47 of total light radiation. As such, a ratio of PAR to SI of 0.47 was used to estimate the amount of available photosynthetic radiation at the surface of the WSP as seen in Equation 4.14:

$$I_0 = 0.47 I_{tot}$$  \hspace{1cm} (4.14)

### 4.3.4 Modelling WSP processes

The WSP processes modelled in this project were based on a model presented by Beran and Kargi (2005), as illustrated in Figure 4-3. As such, only the modifications made to this work are presented in this section.

![Graphical representation of the modelled WSP processes](image)

**Figure 4-3 - Graphical representation of the modelled WSP processes**

#### 4.3.4.1 WSP Geometry

The WSP was assumed to be a cylinder with a constant radius and an increasing volume and depth due to the continuous loading of wastewater and a one time annual discharge as illustrated in Figure 4-4.
The volume of wastewater at a given point in time, \( V_t \) in \( m^3 \), could be calculated as follows:

\[
V_t = V_{t-1} + (Q_{in} - Q_{out}) \Delta t
\]  
(4.15)

where \( V_{t-1} \) is the volume at the previous time step (\( m^3 \)), \( Q_{in} \) is the wastewater inflow (\( m^3/hr \)), \( Q_{out} \) is the wastewater outflow (\( m^3/hr \)) and \( \Delta t \) is the time step (hour). The lack of continuous discharge of wastewater from the WSP was accounted for by setting \( Q_{out} \) to 0 \( m^3/hr \). Changes in volume due to evaporation and precipitation were deemed to be outside of the scope of this study due to a lack of available data and should be considered in future work. It was also assumed that the permafrost layer underneath the WSP would prevent any leakage from the system.

The increase in volume over time will to an increase in wastewater depth (\( d_t \)) in the WSP and the assumed cylindrical shape of the WSP will dictate the rate at which the depth of the wastewater will increase in the system. This term is computed as a function of volume and WSP radius as seen in Equation 4.16:

\[
d_t = \frac{V_t}{\pi r^2}
\]  
(4.16)

where \( r \) is the WSP radius (100 m).
Due to its extended detention times and shallow nature, the WSP was assumed to be a continuously stirred tank reactor, meaning that microalgae, bacteria and nutrient concentrations were uniform within the system, a common assumption when modelling wastewater processes inside reactors (Sah et al. 2012; Yi et al. 2009).

4.3.4.2 Modelling microalgae kinetics

The dynamic equation that accounts for the change in microalgae concentration in the WSP over time depends on the microalgae growth rate, basal metabolism, settling velocity, predation rate and concentration. Beran and Kargi (2005) assumed that microalgae settling resulted primarily from mortality and therefore considered the two terms together. The authors also specified a constant rate for microalgae predation assuming that the fraction of protozoa in the microalgae-bacterial biomass ratio was constant. Finally, the depth of the WSP as well as the concentration of microalgae in the influent and effluent also affect changes in microalgae concentration with time. All of these variables were accounted for as follows (Beran and Kargi 2005):

$$\frac{\Delta c_a}{\Delta t} = (G_a - BM_a)C_a - \left(\frac{V_{Sa}}{d_t} + PR_a\right)C_a + \frac{(Q_{in}c_{a}^{in} - Q_{out}c_{a}^{eff})}{V_t}$$

(4.17)

where $C_a$ is the microalgae concentration (gC.m$^{-3}$), $G_a$ is the microalgae growth rate (day$^{-1}$), $BM_a$ is the microalgae basal metabolism (day$^{-1}$), $V_{Sa}$ is the microalgae settling velocity (m.day$^{-1}$), $d_t$ is the WSP depth (m), and $PR_a$ is the microalgae predation rate (day$^{-1}$) and in and eff are the superscripts denoting influent and effluent, respectively.

Three microalgal strains were identified in the Pond Inlet WSP during the 2011 field season: *Chlorella vulgaris*, *Chlamydomonas angulosa* and *Monoraphidium skujae* at concentrations of 47%, 36% and 17%, respectively. *C. vulgaris* was selected as the representative species for this model since it was the dominant species in the pond and is often considered to be representative species WSPs (Bartosh and Banks 2007; Shammas et al. 2009). Furthermore, *C. vulgaris* has been shown to be able to survive at extreme temperatures, with a study by Bartosh and Banks (2007) showing cell
survival under dark conditions at temperatures below -20°F. This single-specie assumption was made since modelling the competition of multiple microalgal species within the same system was deemed to be outside the scope of this project, Characteristics of *C. vulgaris* were therefore used in Equations 4.17 through 4.23 when applicable.

Microalgae growth depends on several factors that affect growth rates; including nutrient availability, solar radiation intensity, as well as the temperature and pH of the wastewater. These are related in a multiplicative fashion (Beran and Kargi 2005).

\[ G_a = \mu_a \min[f(N), f(I)] f(pH) f(T) \]  

(4.18)

where \( \mu_a \) is the maximum specific growth rate for the modelled microalgae species (day\(^{-1}\)), \( f(N) \) is the effect of nutrient concentration, \( f(I) \) is the effect of solar radiation, \( f(pH) \) is the effect of pH and \( f(T) \) is the effect of temperature. These terms are unitless and range between 0 and 1.

The nutrient limitation term specifies that the extent of growth is limited by the nutrient in least supply, which in the case of microalgae is either nitrogen or phosphorous (Barsanti and Paolo 2006; Beran and Kargi 2005).

\[ f(N) = \min\left(\frac{(NH_4+NO_3)}{KHN(NH_4+NO_3)}, \frac{PO_4}{KHP+PO_4}\right) \]  

(4.19)

where NH\(_4\) is the ammonium nitrogen concentration (gN.m\(^{-3}\)), NO\(_3\) is the nitrate nitrogen concentration (gN.m\(^{-3}\)), KHN is the half-saturation constant for nitrogen uptake by microalgae (gN.m\(^{-3}\)), PO\(_4\) is the dissolved phosphate phosphorous concentration (gP.m\(^{-3}\)) and KHP is the half-saturation constant for phosphorous uptake by microalgae (gP.m\(^{-3}\)).

The equation used to quantify the effects of light intensity on microalgae growth in the WSP was modified to account for changes in light attenuation due to variations in microalgae concentrations (Yun and Park 2001). It also takes into account the light attenuation coefficient of the wastewater, the WSP depth, actual light intensity, as well as the optimal light intensity for microalgae growth.

\[ f(I) = \frac{2.718}{(K_e+K_a C_a) d_t} \left[ \exp\left(-\frac{l_s}{l_e}\right) \exp\left(-(K_e + K_a C_a) d_t\right) - \exp\left(-\frac{l_s}{l_e}\right) \right] \]  

(4.20)
where \( K_e \) is the wastewater light attenuation coefficient (m\(^{-1}\)), \( K_a \) is the specific light attenuation coefficient of microalgae (m\(^2\).g\(^{-1}\)), \( C_a \) is the microalgae concentration (g.m\(^{-3}\)), \( d \) is the WSP depth (m), \( I_0 \) is the solar radiation acting on the WSP (W.m\(^{-2}\)) and \( I_s \) is the optimal light intensity for microalgae (W.m\(^{-2}\)).

The effects of pH on microalgae growth were assumed to be represented by the Monod equation (Beran and Kargi 2005)

\[
f(pH) = \frac{K_{pH}}{K_{pH} + y} \quad (4.21)
\]

\[
y = 10^{[Opt_{pH} - pH]} - 1 \quad (4.22)
\]

where \( K_{pH} \) is the half-velocity constant, \( Opt_{pH} \) is the optimal pH for microalgae growth and pH is the pH of the wastewater. Microalgae has been shown to affect the pH of wastewater due to the utilization of CO\(_2\) in the photosynthesis process and subsequent formation of OH\(^-\) ions, as demonstrated in Equation 4.23 (UUsitalo 1996). However, modelling variations in the pH of the wastewater was deemed to be outside the scope of this model and a constant pH value was used, as per the model proposed by (Beran and Kargi 2005). The following represents the carbonate pH buffer system:

\[
CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \quad (4.23)
\]

The change in one carbon species in Equation 4.23 results in a compensation by the other carbon species, meaning that if CO\(_2\) is removed, HCO\(_3^-\) will form CO\(_2\) and OH\(^-\) ions in order to maintain equilibrium, increasing the pH of the medium in the process.

Finally, the equation describing the effects of temperature on microalgae growth was modified from the Arrhenius relationship used by Beran and Kargi (2005) to a model developed by Peeters and Eilers (1978). Previous experimental work found that the Arrhenius relationship was only accurate within increments of 10\(^\circ\)C before requiring the use of different constants. However, the model developed by (Peeters and Eilers 1978) more accurately reflected the growth behaviour of the
microalgae strain \textit{C. vulgaris} within a temperature range of 1°C and 20°C. The modified relationship is expressed in Equations 4.24.a and 4.24.b:

\[
\begin{align*}
    f(T) &= (1 + \beta) \left( \frac{x}{x^2 + 2\beta x + 1} \right) \\
    \chi &= \frac{T - T_0}{T_{opt} - T_0}
\end{align*}
\] (4.24.a) (4.24.b)

where \( T \) is the temperature of the wastewater in the WSP, \( T_{opt} \) is the optimal temperature for the growth of \textit{C. vulgaris} (°C), \( \beta \) is the shape factor for limitation by irradiance and \( T_0 \) is the temperature at which growth no longer occurs (°C). Due to the shallow nature of the WSP and the low hourly temperature variation on a daily basis, the temperature of the wastewater was assumed to be the same as the measured historical ambient air temperatures provided by Weather Spark. A similar assumption was made in a model used by Heaven et al. (2007) in the investigation of climate variability on WSP design and operation in continental climate.

\textbf{4.3.5 Model assembly}

The equations were assembled in MATLAB and each variable was assigned a matrix of size \((i,j)\) in order to compute their values at each time step. In each matrix, \( i \) was equal to 24 and represented the hours of each day and \( j \) represented the number of simulated days (\( j=90 \) in a 3 month treatment season).

With this set up, initial conditions could be specified into the first cell of each matrix and used to calculate the rates of change of various parameters (such as \( \partial C_a / \partial t \)), allowing for the computation of these parameters at the next time step. For example, the concentration of microalgae at \( t_{i+1} \) is calculated by adding the microalgae concentration at \( t \) to the rate of change at that time, as illustrated in Equations 4.25 and 4.26, as well as in Table 4-1.

\[
\begin{align*}
    C_{a_{t+1}} &= C_{a_t} + \frac{\Delta C_a}{\Delta t} \\
    C_{a_{t+2}} &= C_{a_{t+1}} + \frac{\Delta C_a}{\partial t_{i+1}} \Delta t
\end{align*}
\] (4.25) (4.26)
Table 4-1 – A 24 x n matrix used to represent values of modelled parameters at each time step.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>...</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>$C_{a_1}$</td>
<td>$C_{a_1+1}$</td>
<td>$C_{a_1+2}$</td>
<td>...</td>
<td>$C_{a_1+23}$</td>
</tr>
<tr>
<td>Day 2</td>
<td>$C_{a_1+24}$</td>
<td>$C_{a_1+25}$</td>
<td>$C_{a_1+26}$</td>
<td>...</td>
<td>$C_{a_1+47}$</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Day n</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>$C_{a_1+24n-1}$</td>
</tr>
</tbody>
</table>

4.3.6 Other model parameters

Other parameters such as variations in dissolved oxygen (DO) concentrations, the behaviour of heterotrophic microorganisms and nutrient removal rates were also accounted for in the model presented by Beran and Kargi (2005). DO concentrations were calculated as a function of microalgae photosynthesis and respiration as well as nitrification, bacterial heterotrophic respiration and surface re-aeration caused by wind. Aerobic and anaerobic bacteria concentrations were modelled in a similar fashion as microalgae, taking into account the effects of dissolved chemical oxygen demand (COD), nutrient and oxygen availability, the pH of the wastewater as well as the temperature of the system (Beran and Kargi 2005).

The equations presented by Beran and Kargi (2005) for these relationships were not modified in this model and will, therefore, not be presented in detail. It should however be noted that further work could be undertaken to calibrate these parameters to reflect the Arctic environmental conditions. The script for the model can be found in Appendix A, along with the values for the constants and initial WSP conditions.

4.4 Results and Discussion

4.4.1 Solar radiation

Daily and hourly solar irradiance values for Pond Inlet, NU were calculated for a period of 365 days and can be seen in Figures 4-5 and 4-6, respectively. Daily average PAR values do not begin to exceed 1 W.m$^{-2}$ until 33 days into the year, when total daylight hours begin to rise from 0
hrs.d\(^1\) to 1 hrs.d\(^1\). From this point, average daily irradiance values gradually increased to the yearly maximum of 325 W.m\(^2\) on the 172\(^{nd}\) day of the year, towards the end of June, after which point they once again began to decrease, eventually reaching values under 1 W.m\(^2\) on the 313\(^{th}\) day of the year in early November. The results calculated in the model match values presented by Mundy et al. (2011) and follow the same trend as radiation values reported for various locations in the Canadian Arctic (Woo and Young 1996) as well as at the Ny-Ålesund weather station (78.9250° N, 11.9222° E), located in Svalbard, Norway (Maturilli et al. 2014).

![Graph](image)

**Figure 4-5 – Calculated average daily clear-sky PAR irradiance (W.m\(^2\)) for Pond Inlet, NU.**

Figure 4-5 illustrates the computed hourly PAR values for four different days during the three month treatment season. Irradiance on June 1\(^{st}\), the first day of the simulated treatment season or the 152\(^{nd}\) day of the year, varied between 129 and 439 W.m\(^2\). Solar radiation values remained well above 0 W.m\(^2\) due to the 24 hours of daylight experienced at this latitude during this time of year.
The same trends can be observed on the 180th and 210th days of the year, one and two months into the treatment season, with PAR values ranging from 154 W.m\(^{-2}\) to 442 W.m\(^{-2}\) and 39 W.m\(^{-2}\) to 426 W.m\(^{-2}\), respectively. Daylight durations begin to decrease towards the end of the treatment season, reaching a minimum value of 16 hours on the 240th day of the treatment season, when PAR irradiance values vary between 0 W.m\(^{-2}\) and 382 W.m\(^{-2}\).

![Graphs showing hourly PAR irradiance for Pond Inlet, NU](image)

**Figure 4-6** – Calculated hourly clear-sky PAR irradiance (W.m\(^{-2}\)) for Pond Inlet, NU at: (a) Day 1 of the treatment season; (b) Day 30 of the treatment season; (c) Day 60 of the treatment season; (d) Day 90 of the treatment season

### 4.4.2 WSP geometry

Data provided by the Hamlet of Pond Inlet estimates that 3.8x10\(^4\) m\(^3\) of wastewater is generated each year, or approximately 100 m\(^3\) per day. The continued loading of the WSP at a rate of 100 m\(^3\).d\(^{-1}\) when the system was considered to be inactive between September and June led to the
accumulation of approximately $2.5 \times 10^4$ m$^3$ of wastewater prior to the start of the treatment season. This value was therefore used as the initial volume of the WSP.

The continuous loading of the WSP with wastewater, combined with the lack of continuous discharge from the WSP throughout the season, led to a linear increase of wastewater volume in the system. After the three month treatment season, a maximum wastewater volume of $3.4 \times 10^4$ m$^3$ was attained the end of August, as illustrated in Figure 4-7.

![Graph](image)

**Figure 4-7 - Calculated wastewater volume in the WSP between days 1 and 90 of the treatment season**

Based on the assumed WSP radius and initial wastewater volume, a depth of approximately 0.8 m was calculated for the start of the treatment season. The depth increased in a linear fashion as the treatment season progressed, reaching a maximum value of just under 1.1 m at the end of August as seen in Figure 4-8. The depth parameter is of special importance while modelling microalgae.
growth in a WSP due to the attenuation of light in the water column, as per Equation 4.20, and could also affect disinfection rates within the system. A WSP with geometric properties that allow for a greater exposed surface area, and therefore reduced depth, would present more favorable conditions for microalgae growth when compared to a narrower, deeper system which would have comparatively less available light for photosynthetic processes.

Figure 4-8- Calculated wastewater depth in the WSP between treatment day 1 and 90 of the treatment season

4.4.3 Microalgae growth

Microalgae concentrations in the WSP were computed for two separate treatment seasons, 2011 and 2013. As can be seen in Figure 4-9, microalgae concentrations in the WSP were predicted to be higher in 2011 than in 2013, with peak values reaching 91 gC.m\(^{-3}\) and 15.4 gC.m\(^{-3}\), respectively, towards the end of the treatment season. In the 2011 simulation, microalgae growth began 32 days
into the treatment season, after which point microalgae concentrations increased at an average rate of 1.7 gC.m\(^{-3}\) before reaching their maximal concentration on the 86\(^{th}\) day of treatment. In the 2013 simulation, growth was not predicted to begin until 50 days into the treatment season, and microalgae concentrations only increased at a rate of 0.8 gC.m\(^{-3}\) until peak concentrations were achieved on the 77\(^{th}\) day of the season.

![Graph showing daily average microalgae concentrations](image)

**Figure 4-9- Calculated daily average microalgae concentrations in the WSP for the 2011 and 2013 simulations**

The four parameters that directly affected microalgae growth in this model were nutrient availability, light intensity, pH and temperature as per Equation 4.18. Since the nutrient loadings and pH values were the same in both simulations, the difference in microalgae growth from year to year was attributed to variations in climate, specifically light availability and ambient temperature.
The daily average values for the temperature function of microalgae growth, Equation 4.24, were plotted for both simulated treatment seasons, along with their respective seasonal averages, and can be seen in Figure 4-10.

![Diagram showing temperature function values for microalgae growth over time](image)

**Figure 4-10- Calculated temperature function for microalgae growth in the Pond Inlet, NU WSP for the 2011 and 2013 simulations. The horizontal lines express the average values for this function for each treatment season.**

In the 2011 simulation, shortly after 30 days of treatment, the values of the microalgae temperature function were at their highest for the duration of the treatment season, with f(T) ranging between 0.6 and 0.8. This peak coincided with the appearance of microalgae in the WSP, indicating that favourable temperatures during this time frame contributed to microalgae growth in the system.

A similar trend was observed in the 2013 simulation, when f(T) values also peaked at around 0.8 at after 50 days, coinciding with the appearance of microalgae in the system. The f(T) values...
during the 2011 simulation were higher than they were for the 2013 simulation, averaging 0.44 and 0.34, respectively, suggesting that temperatures in 2011 were more favourable to the promotion of microalgae growth.

The daily average values for the light function of microalgae growth, Equation 4.20, were also plotted for both simulations, along with their respective seasonal averages, and can be seen in Figure 4-11.

Figure 4-11- Calculated light function for microalgae growth in the Pond Inlet, NU WSP for the 2011 and 2013 simulations. The horizontal lines express the average values for this function for each treatment season.

Once again, the peak values of $f(I)$ during each simulated treatment season coincided with the appearance of microalgae in the WSP. When microalgae concentrations began to increase after
30 days in the 2011 simulation, f(I) values were noted to be at their highest for the year, just above 0.65. The gradual decrease observed after this point was not necessarily due to a decrease in available PAR irradiance. Rather, it was attributed to the increase in microalgae concentrations in the water column, which would decrease the amount of available light due to the increased light attenuation by the large number of microalgae cells.

A high f(I) value of 0.5 also coincided with the beginning of the appearance of microalgae in the 2013 simulation. However, there were three other instances prior to the 52nd day where f(I) values were found to be even higher, ranging between 0.7 and 0.75 at the 10, 15 and 30 day marks. The f(T) values corresponding to these points were less than the threshold value of 0.6, indicating that while the lighting conditions were favorable for microalgae growth, the ambient temperature was not. After appearance of microalgae after the 52nd day, f(I) values also began to decline, likely due to the increase in light attenuating microalgae cells in the system. The average f(I) values for both the 2011 and 2013 simulations were similar, at 0.21 and 0.22, respectively. However, this does not imply that available irradiance was similar in the WSP during each of these treatment seasons since the equation for the term f(I) also takes into account light attenuation caused by the presence of microalgae cells in the system.

4.4.4 Dissolved oxygen

Dissolved oxygen (DO) concentrations in the system resulting from microalgae growth and other biological processes like bacterial respiration and nitrification were calculated in both simulations and can be seen in Figure 4-12.
Figure 4-12 - Dissolved oxygen concentration in the Pond Inlet, NU WSP over the course of a three month treatment season for the 2011 and 2013 simulations.

Higher DO concentrations were computed for the 2011 simulation, with the model predicting oxygen saturation within the system during the last few weeks of the treatment season. In contrast, predicted DO concentrations in the system were much lower for 2013, with values rarely exceeding 2 mgO₂.L⁻¹ throughout the three month treatment season. The differences in microalgae concentrations in the WSP observed in each of the simulations were deemed to be the main contributing factor to the variations in DO within the system between the two treatment seasons. The high DO values coincided with the appearance of microalgae in the 2011 simulation, whereas near-anoxic conditions were predicted for 2013, which could have been a result of the relatively lower microalgae
concentrations in the system for that treatment season. It should be noted that the spikes in DO concentration that occur throughout each simulation can be attributed to wind re-aeration.

However, predicted DO concentrations in the 2011 simulation were only noted to rapidly increase after 64 days of the treatment season, over ten days after the appearance of microalgae. The microalgae concentration at this time was approximately 30 gC.m\(^{-3}\), suggesting a threshold concentration for microalgae to significantly contribute to the DO concentration of the WSP through photosynthetic processes. This was further supported by the fact that microalgae concentrations in the 2013 simulation never reached this threshold concentration, peaking at 15.4 gC.m\(^{-3}\), therefore contributing little to the production of oxygen in the system.

These results agree with a study conducted by Rockne and Brezonik (2006) which concluded that microalgae growth was a significant source of DO in cold weather WSPs.

4.4.5 Heterotrophic microorganisms

Heterotrophic microorganism concentrations for both simulations were computed to demonstrate their responses to saturated and anoxic DO concentrations. The results presented in Figure 4-13 account for the total microbial concentration in the system, calculated as the sum of aerobic and anaerobic species.
The maximum heterotrophic microorganism concentrations in the system for the 2011 and 2013 simulations were 300 g.m$^{-3}$ and 188 g.m$^{-3}$, respectively. The higher concentrations in 2011 could be attributed to the predicted saturated DO conditions of the WSP promoting the growth of aerobic microorganisms. However, despite the predicted lower concentrations of DO in the WSP, bacterial concentrations still increased in the 2013 simulation, although at a lower rate. These results support the hypothesis that microalgae and heterotrophic microorganism have a synergistic relationship in WSPs, with microalgae producing DO which can be used in the respiration process of aerobic microorganisms (Shammas et al. 2009).
4.4.6 Nutrient removal

Predicted ammonium nitrogen and phosphorous concentrations for the 2011 and 2013 simulations can be found in Figures 4-14 and 4-15, respectively. These two parameters were selected to illustrate the effects of different climatic factors of WSP treatment efficiencies. It should however be noted that the model used the same constants as Beran and Kargi (2005), which modelled a temperate climate WSP, and was not calibrated to account for nutrient uptake rates by microalgae and bacterial colonies at cold temperatures.

![Graph showing predicted ammonium nitrogen concentrations](image)

**Figure 4-14 - Predicted ammonium nitrogen concentrations in the Pond Inlet, NU WSP over the course of a three month treatment season for the 2011 and 2013 simulations.**

Ammonium nitrogen treatment was predicted to be more efficient in 2011, with concentrations reaching less than 5 mgN.m\(^{-3}\) at the end of the treatment season. This was attributed to
the increase in microalgae and heterotrophic microorganism concentrations in the system which contributed to ammonium nitrogen removal from wastewater through nitrogen uptake, by both microalgae and bacteria, as well as through nitrification by aerobic bacteria (Beran and Kargi 2005). It should be noted that when ammonia concentrations began to approach zero in the 2011 simulation, microalgae growth rates began to decline, as can be seen in Figure 4-9.

![Figure 4-15 – Predicted phosphorous concentrations in the Pond Inlet, NU WSP over the course of a three month treatment season for the 2011 and 2013 simulations.](image)

A similar trend was observed for total phosphorous removal, with predicted final total phosphorous concentrations being lower in the 2011 simulation. Once again, this was attributed to the higher microalgae and heterotrophic microorganism concentrations in the 2011 simulation, thus contributing to higher total phosphorous removal rates.
4.5 Conclusion

The model presented in this study illustrates the effects of climate on WSP processes by taking into account variations in temperature and available solar radiation from year to year. Environmental conditions favourable for microalgae growth were associated with predicted increases in DO concentrations within the WSP. The presence of DO was found to be the most important factor in promoting bioremediation processes such as nitrification as well as nitrogen and phosphorous removals.

It can therefore be concluded that the treatment efficiency of WSPs is closely related to ambient environmental factors, which could lead to the observation of inconsistent treatment performances from year to year. It could also lead to an inadequate primary wastewater treatment approach if these lower performance treatment seasons, occur over a period of multiple years. Combining WSPs with other treatment approaches or introducing simple mechanical components, such as aeration to promote aerobic processes, would assist in making the systems more reliable and consistent over a longer period of time.

A summary of the plots generated for 2011 and 2013 can be found in Appendices C and D, respectively.

4.6 References


Chapter 5

Summarizing Conclusions

5.1 Conclusions

The research presented in this thesis examined the effects of varying climate on microalgal growth in Arctic wastewater stabilization ponds (WSPs) by (1) analyzing the effects of temperature on the growth rates of the microalgal strain *Chlorella vulgaris* and fitting the resulting experimental data to three different growth models; and (2) modifying an existing numerical model to quantify the effects of climatic factors, such as temperature and solar radiation, on WSP processes, such as microalgal growth, dissolved oxygen concentrations and treatment efficiency. The purpose of this work was to develop evidence based recommendations supporting the use of WSPs as a primary method of wastewater treatment in communities located in the Canadian High Arctic.

The first study involved monitoring the growth rate of *C. vulgaris* for a temperature range of 1℃ to 23℃ in aerated photobioreactors under a continuous irradiance of 100 µmol photons m⁻²s⁻¹. The results of the study demonstrated that temperature had a significant effect on maximum microalgal growth rates, which increased from 0.26 d⁻¹ at 1℃ to 2.1 d⁻¹ at 23℃. The experimental data was then compared to three different models developed to predict maximum microalgal growth rates at various temperatures: the Eppley Curve, the Arrhenius relationship, and the Peeters and Eilers model. The study showed that the Eppley Curve overestimated maximum microalgal growth rates by up to 44% at temperatures below 5℃, hence, was likely not applicable for the prediction of maximum microalgal growth rates in Arctic WSPs which are commonly exposed to cold temperatures. The Arrhenius relationship was found to be more accurate at predicting maximum growth rates, but required the determination and use of different constants for temperature ranges of 1-10℃ and 10-23℃. Finally, the model developed by Peeters and Eilers was generally found to be a
good fit for the experimental data, accurately predicting maximum microalgal growth rates for temperatures ranging between 1°C and 23°C.

The Peeters and Eilers model applied in the first study was then incorporated into a numerical model developed by Beran and Kargi (2005) to account for the effects of temperature ranges that would be typical during an arctic treatment season on microalgae growth. These types of numerical models, which incorporate biological reactions such as microalgal growth, photosynthetic activity and nutrient uptake rates, present a useful tool to determine the contributions of microalgal to wastewater treatment in WSPs. The numerical model presented in this thesis was a modification of the work presented by Beran and Kargi (2005) in order to account for the growth of microalgae in WSPs under common summer arctic treatment temperatures and the subsequent effect of microalgae on treatment efficiencies. The model also incorporated the use of historical weather data to compare the effects of climate on microalgae growth and other biological processes on an annual basis. From the study, it was noted that microalgae growth was closely related to temperature and the available incident solar radiation, requiring the appropriate combination of both factors for microalgae to proliferate within the system. High concentrations of microalgae within WSPs were surmised to enhance biological treatment processes by increasing the amount of available dissolved oxygen within the system, thus improving the overall treatment efficiency of the WSP. It was therefore concluded that the performance of these systems was closely related to climatic conditions and that variations in climate could affect treatment efficiency, suggesting that the performance of these systems may be inconsistent year to year.

5.2 Recommendations and Future Work

The use of WSPs in communities located in the Canadian High Arctic is highly prevalent, either as a sole method of wastewater treatment or in combination with natural tundra wetlands (Chouinard et al. 2014; Hayward et al. 2014; Krkosek et al. 2012). The results presented in this work suggest that the treatment efficiency of WSPs is highly dependent on climatic conditions, which
could lead to systems inconsistencies and reliable issues, where WSPs are used as the primary form of wastewater treatment in Arctic Communities. The numerical model developed in Chapter 4 indicated that wastewater treatment efficiencies improved at higher dissolved oxygen concentrations, which were noted when microalgae were present at high concentrations in the system. However, having microalgae as the sole source of dissolved oxygen in Arctic WSPs creates a dependence on climate, requiring the appropriate combination of temperature and solar radiation to promote microalgal growth. One recommendation would be that WSPs not be used as the sole wastewater treatment process in Arctic communities, and that these systems be combined with other processes, such as incorporation or enhancement of tundra wetlands to treat WSP effluent. It is also recommended that the feasibility of introducing some form of mechanical aeration to supplement oxygen production in WSPs during the summer treatment seasons also be investigated. While this method may not be necessarily be feasible,

The work in presented in Chapter 3 focused exclusively on the effects of temperature on maximal microalgal growth rates. However, variations in light intensity have also been shown to affect growth patterns. Future work could include the investigation of varying light intensity on maximal growth rates of *Chlorella vulgaris* in order to determine the effects of solar radiation patterns common in areas above the 54\(^{th}\) parallel.

The model presented in Chapter 4 could be enhanced in several ways. First, the inclusion of evaporation and precipitation rates could allow for a more accurate representation of the effects of varying climate on the biological processes occurring within arctic WSPs. Further experimental work could also be conducted to determine the effects of summer arctic temperatures on nutrient uptake rates by microalgae and heterotrophic bacteria. In the current version of the model, the amount of oxygen produced through photosynthesis is calculated based on the form of nitrogen utilized in microalgae growth. The inclusion of photosynthetic rates as a function of light availability (Yun and Park 2003) would provide a more conservative approach since light is typically more limiting than
available nitrogen in WSPs. Finally, a knowledge gap currently exists in the field of disinfection and pathogen removal in WSPs located in both temperate and arctic climates. Further experimental research could be conducted to determine the effects of UV-radiation and wastewater composition on pathogen removal rates, specifically pertaining to microalgae and humic substances.

5.3 Engineering Contributions

The work presented in this thesis introduces two contributions to the field of environmental engineering through the investigation of microalgal growth at colder temperatures and the modification of a numerical model that could be used to predict the performance of WSPs under varying climatic conditions. The first study builds on existing knowledge of the effects of temperature on microalgal growth by investigating the maximum growth rates of *C. vulgaris* at cold temperatures, specifically at temperatures below 10°C. The results of this study were then incorporated into a numerical model in order to more accurately represent the growth patterns of microalgae in Arctic WSPs and their subsequent effects on nutrient removal efficiencies. Another novel feature of the numerical model was the incorporation of historical weather data to demonstrate and characterize the effects of changes in climatic conditions on the treatment performance of Arctic WSPs.

5.4 References


Appendix A
Numerical Model Code

The MATLAB code used for the numerical model can be found on the attached USB key or emailed upon request as a .mat file.
## Appendix B

### Numerical model constants

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Appendix C
Plot Summary 2011
Appendix D
Plot Summary 2013