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A Thesis
entitled
Evaluating Opportunities to Improve Resource Efficiency of Conventional Wastewater Treatment Using the Alga *Cladophora glomerata*
by
Adam Szabo
Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Civil Engineering

Dr. Cyndee L. Gruden, Committee Chair

Dr. Defne Apul, Committee Member

Dr. Ashok Kumar, Committee Member

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College of Graduate Studies

The University of Toledo
May 2012
An Abstract of
Evaluating Opportunities to Improve Resource Efficiency of Conventional Wastewater Treatment Using the Alga *Cladophora glomerata*

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Adam Szabo

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Eutrophication of natural waters has become a problem throughout the world and locally in Lake Erie. In order to stop eutrophication, nitrogen and phosphorus levels entering natural waters must be decreased. One manageable source of nitrogen and phosphorus to the environment is wastewater treatment. Batch experiments were carried out using the alga *Cladophora glomerata* to determine its growth rate in wastewater, nutrient removing potential in wastewater, and biofuel potential. Trials were conducted using an optimized medium, a synthetic wastewater medium, and an effluent wastewater sample. Specific growth rates were highest in the effluent wastewater sample, 0.115 grams algae growth per grams algae per day (day$^{-1}$) and 0.159 day$^{-1}$. Nutrient concentrations in wastewater grown *Cladophora glomerata* were determined to be approximately 0.0136 g phosphorus per dry weight (DW) gram of algae and 0.0415 g nitrogen per DW gram of algae. Predicted methane yield from *Cladophora glomerata* was calculated at 0.289 – 0.578 L CH4/g DW algae. A proposed *Cladophora glomerata* wastewater nutrient removal system was designed based on these parameters and compared to a conventional nutrient removal system. It was concluded that the algal
system could be potentially viable for wastewater treatment plants with flows up to 1.0 million gallons a day (MGD). For larger facilities, the estimated initial capital costs of the algal treatment system and associated land requirements were far too significant.
Acknowledgements

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Chapter 1

Introduction

Eutrophication of natural waters has become a problem throughout the world and locally in Lake Erie. Eutrophic bodies of water are bodies of water that have high levels of nutrients present. These nutrients can lead to excess growth of algae and other aquatic plants, loss of component species, and loss of ecosystem function (Bennett, et al., 2001) (Aslan, 2006). Algae growths themselves can have a variety of negative impacts such as decreased water quality, beach fouling, clogging of waterworks, excessive swings in O$_2$, and even water toxicity (Dodds & Gudder, 1992; Lake Erie Millennium Network Synthesis Team, 2011). Nitrogen and phosphorus have been identified as main nutrients causing eutrophication (Ruiz-Marin, 2010). In order to stop eutrophication, nitrogen and phosphorus levels entering natural waters must be decreased. One manageable source of nitrogen and phosphorus to the environment is wastewater treatment. Wastewater treatment plant effluent often contains nitrogen and phosphorus levels much higher than in receiving waters (Hoffmann, 1998). For this reason, much research and effort has been focused on nitrogen and phosphorus removal systems for wastewater treatment plants. Using the natural process of algae growth to remove nutrients from wastewater has been an area of continual research (Hoffmann, 1998). Most of this research has focused on
using suspended microalgae. Algae species are often referred to as microalgae or macroalgae. Microalgae, as the name suggest, are typically small microscopic organisms. Macroalgae on the other hand, are larger species and many species are commonly referred to as “seaweed”.

The main objective of this study was to evaluate opportunities to improve resource efficiency of conventional wastewater treatment using the alga *Cladophora glomerata*, a macroalgae. Experiments were carried out using *Cladophora glomerata* to determine its growth rate and nutrient removing potential. The data collected from these experiments was used to investigate the feasibility of a wastewater treatment plant employing the use of *Cladophora glomerata* for nutrient removal. A conventional wastewater treatment plant (Figure 1-1) was used as a base to compare conventional nutrient removal (Option 1 in Figure 1-2) to a *Cladophora glomerata* algal system for nutrient removal (Option 2 in Figure 1-2) (Reynolds & Richards, 1996). The abbreviation key for these wastewater treatment process figures is below, Table 1-1.
Table 1-1: Abbreviation key for Figure 1-1 and Figure 1-2

<table>
<thead>
<tr>
<th></th>
<th>Abbreviation key for Figure 1-1 and Figure 1-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Algal Treatment System</td>
</tr>
<tr>
<td>AB</td>
<td>Algal Biomass</td>
</tr>
<tr>
<td>AD</td>
<td>Anaerobic Digestion</td>
</tr>
<tr>
<td>AN</td>
<td>Aerobic Nitrification</td>
</tr>
<tr>
<td>ANDN</td>
<td>Anaerobic Denitrification</td>
</tr>
<tr>
<td>AT</td>
<td>Aeration Tank</td>
</tr>
<tr>
<td>BS</td>
<td>Bar Screens</td>
</tr>
<tr>
<td>C</td>
<td>Chlorine Addition</td>
</tr>
<tr>
<td>CC</td>
<td>Chemical Coagulant</td>
</tr>
<tr>
<td>CM</td>
<td>Completely Mixed Reactor</td>
</tr>
<tr>
<td>DW</td>
<td>Dewatering</td>
</tr>
<tr>
<td>E</td>
<td>Effluent</td>
</tr>
<tr>
<td>FC</td>
<td>Final Clarifier</td>
</tr>
<tr>
<td>GR</td>
<td>Grit Removal</td>
</tr>
<tr>
<td>I</td>
<td>Influent</td>
</tr>
<tr>
<td>PC</td>
<td>Primary Clarifier</td>
</tr>
<tr>
<td>PS</td>
<td>Primary Sludge</td>
</tr>
<tr>
<td>RS</td>
<td>Return/Recycled Sludge</td>
</tr>
<tr>
<td>TH</td>
<td>Thickening</td>
</tr>
<tr>
<td>WS</td>
<td>Waste Sludge</td>
</tr>
</tbody>
</table>
Figure 1-1: Conventional wastewater treatment system process

Figure 1-2: Wastewater treatment nutrient removal options
Conventional wastewater treatment plants are composed of preliminary, primary, and secondary treatment processes. Preliminary and primary treatment processes, including screening, grit removal, and primary clarification, are designed to remove suspended solids from the influent water, while secondary treatment processes (e.g., activated sludge) are designed to biologically remove the remaining organic solids and organic dissolved solids. The conventional wastewater treatment plant used in this study (Figure 1-1) consists of screening, grit removal, primary clarification, activated sludge treatment, and chlorination. Bar screens remove any large solids and the grit removal system takes care of sand and silt. The wastewater then moves to primary clarification where suspended solids are settled out by gravity and oil/grease can be removed from the top. The water then enters activated sludge treatment, consisting of an aeration tank and final clarifier. This system bio-oxidizes the organic matter in the aeration tank, and then the biological solids are settled out and removed from the wastewater in the final clarifier. Finally, the wastewater is chlorinated to kill pathogenic organisms and discharged to a receiving stream. The remainder of the system is designed to manage sludge created from the primary and final clarifiers. A portion of activated sludge from the final clarifier is returned via recycle line to the aeration tank, while the rest is combined with primary clarifier sludge and sent for solids processing which usually begins with thickening. The thickener reduces water content in the sludge and the thickened sludge (typically 4% – 6% solids) is sent to the anaerobic digester. Anaerobic digestion stabilizes the sludge, increases solids content, allows water to be removed from the solids more easily, and destroys nearly 99.8% of coliforms. A byproduct of anaerobic digestion is bio-gas, consisting mostly of methane and carbon dioxide. After anaerobic
digestion the sludge is dewatered and removed from the wastewater treatment plant; normally it is land applied, incinerated, or sent to a landfill (Reynolds & Richards, 1996).

Along with primary and secondary treatment processes in wastewater treatment, there is also tertiary treatment. Tertiary treatment occurs after secondary treatment, and includes any treatment to further increase the effluent water quality (Reynolds & Richards, 1996). The nutrient removal options used in this study are considered tertiary treatment options. Option 1 accomplishes nutrient removal using chemical coagulants to remove phosphorus and biological methods to remove nitrogen. This system requires the addition of aerobic nitrification and anaerobic denitrification processes (Figure 1-2). A waste sludge is produced during this process and is sent to the thickener along with the other sludge residuals.

Option 2 uses the proposed *Cladophora glomerata* algal system to remove phosphorus and nitrogen from the wastewater. *Cladophora glomerata* will be grown in a shallow (1.0m) rectangular tank, with the wastewater flowing over the algae. The bottom of the tank will be made of some solid substrate that allows *Cladophora glomerata* to attach, concrete is one option. Mechanical aerators will be used to impart carbon dioxide into the wastewater if it is required and promote mixing. The system will operate as a continuous flow system, one end of the tank allowing in influent and the other letting out the treated effluent. This system produces an algal biomass that can be sent to the anaerobic digester and contribute to methane gas production (Figure 1-2). The purpose of this thesis is to determine the feasibility of the *Cladophora glomerata* algal system for nutrient removal in wastewater treatment plants.
To determine if the *Cladophora glomerata* algal system is a viable option, the effects of wastewater on the growth, nutrient removal rates and composition of the algae needed to be determined. This investigation was done in the laboratory with a *Cladophora glomerata (L.) Kütz* sample obtained from the Canadian Phycological Culture Center. Batch cultures were cultured in a growth chamber and then evaluated for growth rates by weight, nutrient levels by colorimetric tests, and algae composition by CHN analysis. An important aspect of these test were to determine if the algal biomass could be used for bio-energy products.

Biofuels grown from plants have the potential to provide a renewable energy source for the future, replacing a substantial portion of our fossil fuel needs (Perlack, et al., 2005). However, one concern being voiced over these new biofuels is that the use of food crops will increase food prices while having little impact on greenhouse gas emissions. Algae have been repeatedly investigated as a solution to this problem. Being paired as a biofuel source and wastewater treatment technique algae could potentially be a practical and economically feasible approach (Woertz, 2009).
Chapter 2

Literature Review

2.1 Algae

In order to evaluate an algal treatment system an understanding of what algae is, is required. Algae can range from single-celled species to large seaweeds over 50 meters long and they are found in nearly every ecosystem on earth. Algae are unique in that they do not fall within a single group linked by a common ancestor; they do not exclusively share a specific group of defining features. They are divided into 9 major groups or phyla and each phylum of algae share a set of characteristics. Table 2-1 below shows the 9 major groups or phyla of algae and the number or species in each group (Graham & Wilcox, 2000).
Table 2-1: The major groups (phyla) of algae and number of species in each

<table>
<thead>
<tr>
<th>Major Group (phyla)</th>
<th>Number of species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td>difficult to determine</td>
</tr>
<tr>
<td>(chloroxybacteria, blue-green algae)</td>
<td></td>
</tr>
<tr>
<td><strong>Glaucophyta</strong></td>
<td>NA</td>
</tr>
<tr>
<td>(glaucophytes)</td>
<td></td>
</tr>
<tr>
<td><strong>Euglenophyta</strong></td>
<td>900</td>
</tr>
<tr>
<td>(euglenoids)</td>
<td></td>
</tr>
<tr>
<td><strong>Cryptophyta</strong></td>
<td>200</td>
</tr>
<tr>
<td>(cryptomonads)</td>
<td></td>
</tr>
<tr>
<td><strong>Haptophyta</strong></td>
<td>300</td>
</tr>
<tr>
<td>(haptophytes or prymnesionphytes)</td>
<td></td>
</tr>
<tr>
<td><strong>Dinophyta</strong></td>
<td>2000-4000</td>
</tr>
<tr>
<td>(dinoflagellates)</td>
<td></td>
</tr>
<tr>
<td><strong>Ochrophyta</strong></td>
<td>&gt;10000</td>
</tr>
<tr>
<td>(chromophytes)</td>
<td></td>
</tr>
<tr>
<td><strong>Rhodophyta</strong></td>
<td>4000-6000</td>
</tr>
<tr>
<td>(red algae)</td>
<td></td>
</tr>
<tr>
<td><strong>Chlorophyta</strong></td>
<td>17000</td>
</tr>
<tr>
<td>(green algae or chlorophyceans)</td>
<td></td>
</tr>
</tbody>
</table>

(Graham & Wilcox, 2000)

The major groups of algae and their species almost all have three things in common. These similarities form the most common definition of algae. First, most are photosynthetic oxygenic producers of organic compounds. Secondly, most are aquatic. Lastly, most are smaller and less complex than land plants. The exceptions are heterotrophic and mixotrophic algae, algae that occur in non-aquatic habitats, and large species of algae. (Graham & Wilcox, 2000)

2.2 Cladophora Ecology

*Cladophora* is the genus of algae that will be used in the algal wastewater treatment system; it is a genus of filamentous green algae that belongs to the *chlorophyta*
phyllum. Algal members of this genus are found throughout the earth and often dominate the benthos in fresh and marine waters. It is generally an attached benthic alga, but can also be found in floating mats or as loose masses on soft substrate. This genus’s habitat ranges from ultraoligotrophic to eutrophic freshwaters and artic to temperate marine waters (Dodds & Gudder, 1992).

Of the *Cladophora* species, a variety fitting the description of *Cladophora glomerata* (L.) Kütz, typically dominates the benthic region of eutrophic freshwaters (Robinson & Hawkes, 1986). However it should be noted that taxonomic identification of *Cladophora* species is difficult. Almost all criteria used to differentiate them are of variable expressions that have overlaps between species. There is also a wide degree of morphological variation in response to environmental conditions and plant age. RNA and DNA testing may be used for species identification; although, efforts to validate species identification with nucleotide sequences revealed that freshwater *Cladophora* compromise few, possibly one, species (Dodds & Gudder, 1992). However, some note that the presence of two distinct freshwater species cannot be ruled out, *Cladophora glomerata* and *Cladophora fracta* (Higgens, et al., 2008). In spite of the difficulties identifying species, *Cladophora glomerata* (L.) Kütz is considered one of the most common freshwater species of macroalgae in the world. It has a presence on all continents except Antarctica (Higgens, et al., 2008). *Cladophora glomerata* (L.) Kütz is considered the most common species found in the Laurentian Great Lakes and is the algae used in the algal wastewater treatment system. Figure 2-1 below shows a view of laboratory grown *Cladophora glomerata* (L.) Kütz under a 20x microscope.
For an idea of growth, average shallow depth (0.5m) bed densities in eastern Lake Erie ranged from approximately 175 to 600 g dry weight/m² (gDW/m²) with values as high as 900 gDW/m² from 1979 through 2006 (Higgins, et al., 2008). Values for the more recent data years (2001, 2002, and 2006) are near 200 gDW/m². The median length of Cladophora filaments in beds along the northern shore of Lake Erie’s eastern basin ranged from 1.0 – 35 cm from the years 1995 -2002 (Higgins, et al., 2005). Filament lengths in the Great Lakes have been reported as long as 90 cm (Lorenz & Herdendorf, 1984).

The maximum net specific growth rate for Cladophora collected from Lake Huron was found to be 0.77day⁻¹ (Auer & Canale, 1982). In laboratory grown batch
cultures similar to what was used in this study, Gerloff & Fitzgerald (1976) routinely achieved specific growth rates of 0.20 - 0.21 day$^{-1}$. In a continuous flow culture system Robinson & Hawkes (1986) showed growth rates up to 0.23 day$^{-1}$.

2.2.1 Factors related to distribution, abundance and growth of *Cladophora*

2.2.1.1 Life Cycle:

In the Laurentian Great Lakes *Cladophora* generally follows a seasonal growth pattern; a midsummer biomass peak followed by a period of detachment and die-off, then a period of low growth, another biomass peak in the fall, and then a winter die-off (Dodds & Gudder, 1992). In Figure 2-2 below the seasonal growth pattern for eastern Lake Erie can be seen.

![Figure 2-2: Seasonal changes in Cladophora biomass in eastern Lake Erie over the years 1979-2006 (Higgens, et al., 2008)](image-url)
2.2.1.2 Substrate:

*Cladophora* grows on many different types of substrate, but solid substrates are usually preferred, the most common being rock. Freshwater *Cladophora* can be found attached to rock, plants, or to the surface of animals (Dodds & Gudder, 1992). Detachment most often happens when weakened filaments are broken or torn from the substrate by water turbulence. The weakening of filaments can be caused by temperature stress, nutrient deficiency, metabolic imbalances, or self-shading (Higgens, et al., 2008).

2.2.1.3 Light:

In the natural environment *Cladophora* is found in high and low light habitats (Dodds & Gudder, 1992). Its photosynthetic response follows a hyperbolic, or similar, response to irradiance. Maximum net photosynthesis occurred at a light intensity of 300 to 600 µmol m⁻² s⁻¹ for *Cladophora* samples from Lake Huron (Graham, et al., 1982). *Cladophora glomerata* samples collected from Lake Michigan showed light saturation at about 790 µmol m⁻² s⁻¹ (Lester, et al., 1988). It is well known that *Cladophora* beds often flourish at shallow depths. Although, studies have suggested that high intensities of light induce photo-inhibition in *Cladophora*. There are physiological and/or population level mechanisms that allow for growth in high light environments. One important population level response is related to the positive effect of self-shading. Self-shading lowers the intensity of light entering the bed of *Cladophora* after a few millimeters and limits the potential effect of photo-inhibition. Self-shading can also be a negative factor that controls the maximum potential standing crop, with more biomass growth much needed light may not reach the innermost thallus (body) (Higgens, et al., 2008). In the environment, turbulent waters result in a very uneven light field, the thallus movement
constantly changes the degree of shading (Dodds & Gudder, 1992). Growth rates of *Cladophora* have been shown to increase linearly as the photoperiod increases to 24 hours per day, suggesting that there is no necessity for a light/dark cycle (Robinson & Hawkes, 1986).

**2.2.1.4 Hydrodynamics:**

*Cladophora*’s ability to withstand shear stress is part of the reason for its success in benthic and intertidal regions. The thallus of the algae is tough but flexible. During low flow the thallus spreads out, but during higher flow the thallus become more streamlined. Relating hydrodynamic conditions to growth is not easy because such conditions vary considerable over time; but, it is important to look at these conditions because transport of materials by flow could play a significant role. *Cladophora* is mostly found in areas dominated by turbulent transport; it is suggested that the transport of materials to and from the thallus is not constrained by molecular rates of diffusion (Dodds & Gudder, 1992).

Photosynthetic rates in freshwater *Cladophora* have been linked to water velocity. The rate of photosynthesis was observed to increase as water velocity increased from 0 to 2.1cm/s (Pfeifer & McDiffett, 1975). In another experiment, photosynthetic rates in small tufts of algae doubled as water velocity increased from 0 to 8cm/s; a decrease in rates occurred as velocity increased over 8cm/s (Dodds, 1991). The decrease in photosynthetic rates associated with a current over 8cm/s may be related to the streamlining effect. When the current increases the algae tuft become more tightly packed, this may cause a hindrance in materials transport or an increase in self-shading (Dodds & Gudder, 1992).
2.2.1.5 Temperature:

The minimum temperature required to initiate vegetative growth of *Cladophora glomerata* is near 5°C (Higgens, et al., 2008). Temperatures reported for optimum growth and thresholds for growth vary greatly among studies. Optimal growth temperatures for *Cladophora glomerata* taken from the Great Lakes have been reported to range from 13°C to 31°C (Gerloff & Fitzgerald, 1976; Higgens, et al., 2008). The maximum threshold temperature (temperature that will sustain growth) has been reported at 30°C to 35°C (Higgens, et al., 2008).

2.2.1.6 Water Chemistry:

*Cladophora* growths are often associated with marine and freshwater eutrophication events. Examples of these include nutrient enrichments in the Great Lakes, in Western Australia, near marine fish farms, in pulp mill effluent in the Bothnian Sea, in wetlands, and in rivers or streams (Dodds & Gudder, 1992). During exponential growth in the population, *Cladophora* beds act as a nutrient sink, removing large amounts of carbon, nitrogen and phosphorus from the water (Higgens, et al., 2008).

Reports of nitrogen limited growth in freshwaters are few, most often phosphorus is considered the cause of excess *Cladophora* growths (Dodds & Gudder, 1992) (Higgens, et al., 2008). There is much evidence which suggest that phosphorus reductions limit the growth of *Cladophora*. From the 1970s to mid-1990s nutrient abatement programs on the Laurentian Great Lakes reduced total phosphorus (TP) concentrations. The reduction in TP resulted in 60-90% reductions in *Cladophora* biomass at locations throughout the Great Lakes (Higgins, et al., 2008).
Various forms of phosphorus (ortho-, pyro-, meta-, and tripoly-P) can be utilized by *Cladophora*. Gerloff & Fitzgerald (1976) illustrated that *Cladophora* yields using these forms of phosphorus were all similar after a 27 day growth period. They also showed that extractable phosphorus concentrations from P-limited algae exposed to these different forms of phosphorous were comparable. *Cladophora glomerata* can also utilize various forms of nitrogen (NH$_4$-N, NO$_3$-N, and NO$_2$-N) (Gerloff & Fitzgerald, 1976). Gerloff & Fitzgerald (1976) showed that yields using these forms of nitrogen were comparable after a 22 day growth period.

Robinson & Hawkes (1986) showed evidence that unionized ammonia (NH$_3$) is toxic to *Cladophora glomerata*. Other studies have shown negative regression coefficients for ammonium (NH$_4$) relating to *Cladophora* growth (Ensminger, et al., 2000). High levels of phosphate are also known to be toxic to *Cladophora*. In Robinson & Hawkes (1986) study, levels of phosphate over 2.0 mg/L (0.65 mg/L P) were detrimental to the alga’s specific growth rate.

Other nutrients required for growth of *Cladophora glomerata* include silicon, boron, thiamine, zinc, vitamin B$_1$ and vitamin B$_{12}$ (Dodds & Gudder, 1992). *Cladophora glomerata* must be supplied with vitamin B$_1$ and vitamin B$_{12}$, because it is unable to synthesize required amounts of these vitamins on its own. The need for an external vitamin source is unusual for a freshwater green alga (Gerloff & Fitzgerald, 1976).

Table 2-2 shows the recommended synthetic nutrient solution for *Cladophora glomerata* growth (Gerloff & Fitzgerald, 1976). B7 and C13 are both defined trace metal solutions. Table 2-3 shows the range of concentrations of essential nutrients in *Cladophora glomerata* as a percent of total weight, found by Gerloff & Fitzgerald, 1976.
by quantitative procedures for plant analysis. Other studies report dry weight tissue
phosphorus concentration to be 0.08 – 0.81% P, nitrogen tissue concentration to be 1.31 –
5.14 % N and carbon concentration to be 13.7 – 40.5 % C for wild grown Cladophora
(Lorenz & Herdendorf, 1984).

Table 2-2: Recommended synthetic nutrient solution for Cladophora glomerata growth
(Gerloff & Fitzgerald, 1976)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>16.4</td>
</tr>
<tr>
<td>P</td>
<td>2.8</td>
</tr>
<tr>
<td>K</td>
<td>18</td>
</tr>
<tr>
<td>Mg</td>
<td>7.5</td>
</tr>
<tr>
<td>S</td>
<td>9.7</td>
</tr>
<tr>
<td>Ca</td>
<td>23.4</td>
</tr>
<tr>
<td>Fe</td>
<td>1.12</td>
</tr>
<tr>
<td>Cl</td>
<td>0.35</td>
</tr>
<tr>
<td>B</td>
<td>0.054</td>
</tr>
<tr>
<td>Mn</td>
<td>0.054</td>
</tr>
<tr>
<td>Zn</td>
<td>0.026</td>
</tr>
<tr>
<td>Cu</td>
<td>0.006</td>
</tr>
<tr>
<td>Mo</td>
<td>0.002</td>
</tr>
<tr>
<td>B7</td>
<td>0.001</td>
</tr>
<tr>
<td>C13</td>
<td>0.0005</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0.0005</td>
</tr>
</tbody>
</table>
Table 2-3: Range of concentrations of essential nutrients in Cladophora glomerata (Gerloff & Fitzgerald, 1976)

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.83 - 4.89%</td>
</tr>
<tr>
<td>P</td>
<td>0.04 - 0.54%</td>
</tr>
<tr>
<td>K</td>
<td>0.99 - 4.74%</td>
</tr>
<tr>
<td>Mg</td>
<td>0.05 - 0.37%</td>
</tr>
<tr>
<td>S</td>
<td>1.22 - 2.32%</td>
</tr>
<tr>
<td>Ca</td>
<td>0.20 - 1.06%</td>
</tr>
<tr>
<td>Fe</td>
<td>0.0036% - 0.1110%</td>
</tr>
<tr>
<td>B</td>
<td>0.0110% - 0.0211%</td>
</tr>
<tr>
<td>Mn</td>
<td>0.0007% - 0.4690%</td>
</tr>
<tr>
<td>Zn</td>
<td>0.0005% - 0.1039%</td>
</tr>
<tr>
<td>Cu</td>
<td>0.0005% - 0.0015%</td>
</tr>
<tr>
<td>Mo</td>
<td>0.0007% - 0.1360%</td>
</tr>
</tbody>
</table>

2.3 The nitrogen and phosphorus cycles

2.3.1 The nitrogen cycle

Nitrogen is an essential element for life on earth. It plays a vital role in the biochemical processes of living things, including algae. Nitrogen exists in a number of forms and locations on earth. The largest pool of nitrogen on earth is the N₂ gaseous form that makes up approximately 80% of the atmosphere. This form of nitrogen is largely unavailable to most organisms; they require the nitrogen to be transformed to another state before they are able to utilize it. Most plants need nitrogen to be in the ammonium (NH₄⁺) or nitrate (NO₃⁻). This all happens as part of the natural nitrogen cycle. (Ricklefs & Miller, 2000; Campbell & Reece, 2002)

Nitrogen (N₂) from the atmosphere enters into biological ecosystems primarily through nitrogen fixation. This process is accomplished by bacteria in soils, in plant roots, and in aquatic ecosystems. The direct result of nitrogen fixation is ammonia, NH₃.
Much of this ammonia picks up a hydrogen ion and takes the form of ammonium (NH$_4^+$). Ammonia that is not converted to ammonium in the water/soil can evaporate back into the atmosphere where it will form ammonium. This atmospheric ammonium can re-enter the water/soil through rainfall or settling in dust particles. (Campbell & Reece, 2002)

Ammonium can be used directly by plants but most of it is utilized by aerobic bacteria. They oxidize ammonium to nitrite (NO$_2^-$) and then to nitrate (NO$_3^-$), a process called nitrification. Nitrate release by these bacteria can be assimilated by plants for their life processes, converting the nitrate to an organic form. These organic forms of nitrate are used by animals when they consume the plants. A portion of the nitrate created during nitrification undergoes denitrification; the nitrate is used by bacteria anaerobically resulting in N$_2$ gas, the original atmospheric nitrogen that the cycles began with. The organic nitrate that was consumed by animals eventually is converted back to ammonium in soil/water through ammonification. This is accomplished when animal matter is decomposed by bacteria and fungi (Campbell & Reece, 2002).

### 2.3.1.1 Algae and the nitrogen cycle

Algae play an important part in the nitrogen cycle. They do for aquatic ecosystems what land plants do for terrestrial ecosystems (along with other aquatic plants). They assimilate nitrogen, providing an organic source of nitrogen to aquatic food webs. Nitrogen is an important component of algal peptides, proteins, enzymes, chlorophylls, ATP, ADP, RNA, DNA, and other cellular parts (Barsanti & Gualtieri, 2006). Most algae use the inorganic sources of nitrogen; ammonium and nitrate. These algae will either be eaten by other organisms and enter the aquatic food web, or die and be decomposed. There are also other species of algae that are nitrogen fixers.
(cyanobacteria), taking nitrogen gas (N₂) from the atmosphere and converting it into ammonium. Along with being a source of food and decomposing, these algae also play another role in the nitrogen cycle; around 40-60% of the nitrogen they fix can be excreted into the open water providing a nitrogen source to other algae and aquatic plants.

### 2.3.2 The phosphorus cycle

Phosphorous is another element that living organisms require for life processes. It exist in the environment in several forms; particulate organic phosphate, inorganic orthophosphate (soluble), inorganic colloidal phosphate (soluble), and in other compounds, typically metal precipitates. Orthophosphate is the only form available for use by organisms, and is a small percentage of the total phosphorus in the soil and water. Unlike most other cycles, the atmosphere does not play a significant role in the phosphorous cycle (Ricklefs & Miller, 2000).

The weathering of rock is the primary source for soluble phosphate in developing terrestrial environments, but it can also be made available by the soil biota. This soluble phosphorus enters the soil and is either, assimilated by organisms into a land food web, run off into a body of water, or kept within the soil in a form unavailable to organisms. Only soluble orthophosphate can be utilized by organisms and make its way into the food web as particulate organic phosphorous. This particulate phosphorus will eventually be recycled, returning to the soil after decomposition or run off into a body of water. Phosphorus that finds its way into a body of water will be involved in a cycle much like that in soil. Phosphate can also enter aquatic systems through weathering of rock. The soluble orthophosphate will be utilized by organisms and enter the aquatic food web, eventually being recycled after decomposition, or becoming part of the sediment. Other
forms of phosphorus entering a body of water will be suspended in the water column unavailable to organisms, or settle in the sediments. Ultimately, these sediments will become the rock that releases phosphorus into soil/water (Ricklefs & Miller, 2000).

2.3.2.1 Algae and the phosphorus cycle

Algae is a key element in the phosphorus cycle, it provides a pathway for aquatic organism to obtain phosphate. Algae utilize soluble phosphorus in water converting it to organic phosphorus; it is an important part of the lipid portion of cell membranes, coenzymes, DNA, RNA, and ATP (Barsanti & Gualtieri, 2006). The algae can then either be eaten and enter the aquatic food web or die and be decomposed; decomposers will return the phosphorus to the water, where is can be again used by algae or other aquatic plants.

2.3.3 Human impact on the nitrogen and phosphorous cycles

Human activities have had a large impact on the natural nitrogen and phosphorus cycles. The nitrogen cycle is now influenced by the industrial creation of nitrogen fertilizer, the combustion of fossil fuels, increase cultivation of nitrogen-fixing crops, and other human activities that cause mobilization of nitrogen. These cause the fixation of 140 Tg of nitrogen to terrestrial ecosystems a year, double the amount that would naturally occur. Nitrogen is being transformed from biologically unavailable sources to biologically available sources (Vitousek, et al., 1997). The phosphorus cycle is being impacted primarily by the mining of phosphorus for fertilizers and other products. This phosphorus is accumulating in soils and in bodies of water around the world. As with nitrogen, the bioavailability of phosphorus is increasing (Bennett, Carpenter, & Caraco, 2001).
The growths in many unmanaged ecosystems are limited by the supply of nitrogen and phosphorus. Humans are increasing the amount of these nutrients available which is causing problems (Vitousek, et al., 1997; Bennett, et al., 2001; Ricklefs & Miller, 2000). One of the most prominent problems is the eutrophication of aquatic systems. Eutrophic bodies of water have high levels of nutrients, which in turn can cause excess growth of algae and other aquatic plants (Bennett, et al., 2001). These growths can have a variety of negative impacts such as decreased water quality, beach fouling, clogging of waterworks, excessive swings in $O_2$, and even water toxicity (Dodds & Gudder, 1992; Lake Erie Millennium Network Synthesis Team, 2011).

2.4 Nitrogen and phosphorus removal in wastewater treatment

Wastewater treatment plant effluent often contains nutrient levels much higher than in receiving waters (Hoffmann, 1998). Nitrogen and phosphorus are the most common nutrients released by wastewater treatment. Elevated levels of nitrogen and phosphorus have been identified as the main causes leading to eutrophication in natural waters (Ruiz-Marin, 2010). Therefore, in order to prevent eutrophication, wastewater treatment plants employ nutrient removal processes. The typical levels of inorganic nitrogen and soluble phosphorus entering a 120 gpcd (gallons per capita per day) wastewater treatment plant are listed in Table 2-4 below. Typical nutrient removal goals are to have effluent phosphorus concentration below 1.0 mg/L and nitrogen concentration below 8 - 10 mg/L (Hartman & Cleland, 2007; CH2MHILL, 2010; USEPA Office of Water, 2007). There are several processes capable of removing nitrogen and phosphorus from wastewater, but most of them are costly and produce a large amount of sludge.
Nitrogen is typically removed from wastewater to keep effluent ammonia and ammonium concentrations low. This can be accomplished through physical, chemical, or biological processes. A summary of these nitrogen removal options is listed in Table 2-5. Physical methods that are used to remove nitrogen from wastewater are induced-draft stripping towers, and spray ponds. These processes require the wastewater to be at a high pH, typically 10.8 or greater, to effectively remove ammonia. The ammonia is released in the form of ammonia gas ($NH_3$). Chemical methods that are used to remove nitrogen from wastewater are breakpoint chlorination and ion exchange. Breakpoint chlorination involves adding chlorine gas or hypochlorite salts to oxidize ammonia and form nitrogen gas. It is an expensive process that increases dissolved solids in the water and may produce dichloramine, trichloramine, nitrate ions, and trihalomethanes. Ion exchange uses a natural mineral in a fixed bed column to remove ammonia. After a usage period the mineral column is regenerated by a brine solution. The brine solution then undergoes breakpoint chlorination to remove the nitrogen as N$_2$ gas. There are many biological methods to remove nitrogen; they include oxidation ditches, the three stage nitrification-denitrification process, four-stage Bardenpho process, and five-stage Bardenpho process. These nitrogen removal options employ microorganisms to convert the nitrogen present in the wastewater to nitrogen gas, much like what takes place in the natural nitrogen cycle. A waste sludge is produced by these biological processes and that sludge needs to
be disposed of afterward; nitrogen removal efficiency is not 100 percent, therefore nitrogen is still present in the resulting sludge (Reynolds & Richards, 1996).

Table 2-5: Nitrogen removal processes for wastewater treatment

<table>
<thead>
<tr>
<th>Method</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>Induced-draft stripping towers</td>
</tr>
<tr>
<td></td>
<td>Spray ponds</td>
</tr>
<tr>
<td>Chemical</td>
<td>Breakpoint chlorination</td>
</tr>
<tr>
<td></td>
<td>Ion exchange</td>
</tr>
<tr>
<td>Biological</td>
<td>Oxidation ditches</td>
</tr>
<tr>
<td></td>
<td>Three stage nitrification-denitrification process</td>
</tr>
<tr>
<td></td>
<td>Four-stage Bardenpho process</td>
</tr>
<tr>
<td></td>
<td>Five-stage Bardenpho process</td>
</tr>
</tbody>
</table>

Phosphorus removal is often accomplished by either chemical or biological methods. The chemical method of phosphorus removal involves precipitating the phosphorus and settling it out; the result is a sludge that must be disposed of. Most often this process is accomplished by adding calcium (in the form of lime), aluminum as a metal salt, or iron as a metal salt. The biological removal of phosphorus is done by suspended growth systems that include steps of an anaerobic zone followed by an aerobic zone. There are many different set ups for this but all result in the phosphorus being removed in a waste sludge. Biological phosphorus removal can be combined with biological nitrogen removal in the same system, such as the five-stage Bardenpho. (Metcalf & Eddy, Inc., 2003)

Wastewater sludge obtained from nutrient removal practices and other wastewater processes can be disposed of or utilized in multiple ways. The most recent data for Ohio shows that in 2009, 46.8% of sludge was land applied/distributed for sale, 33.4% was
incinerated, and 19.7% was landfilled (Ohio EPA Biosolids Program). Only sludge that has been treated and meets EPA guidelines contained in 40 CFR Part 503 can be land applied/distributed for sale. These biosolids (treated sludge) are used as a fertilizer for raising crops; they contain nutrients such as nitrogen, phosphorus, potassium, and trace elements such as calcium, copper, iron, magnesium, manganese, sulfur and zinc (United States Environmental Protection Agency, 2012). They typically contain a concentration of 1.77 - 6.5% nitrogen and 1.34 – 3.6% phosphorus per unit dry weight (Aulrajah, Disfani, Suthagaran, & Imteaz, 2011; Ontario Ministry of Agriculture, Food, and Rural Affairs, 2011; Stehouwer, 2000). These nutrients re-used from wastewater help reduce the human impact on the nitrogen and phosphorus cycles. Any nitrogen and phosphorus obtained from wastewater and land applied does not enter a body of water; it also offsets the use of other fertilizers that would have been used for agriculture.

One alternative treatment method is to use algae as a nutrient removal tool. This is not a new idea; the use of algae to treat wastewater has been investigated for over 50 years (Hoffmann, 1998). Most of this research has focused on using suspended and nonsuspended microalgae in shallow ponds, and high rate algal ponds (HRAPs); although, there has also been research done on attached algal turf scrubber designs. It has been such a popular topic because algal treatment of wastewater provides an ecologically safe, less expensive, and more efficient way to remove nutrients and metals than conventional tertiary treatment (Hoffmann, 1998). The algae also produces a biomass, this algal biomass can be used for protein complements and food additives, energy, pharmaceuticals, cosmetics, or other valuable chemicals (Mallick, 2002). The algae can also be combined with other wastewater sludge and used for agricultural land application.
Algal treatment also offers the benefits of using low-grade technology that can save energy (Mallick, 2002).

Research has shown that algae have the potential to remove nutrients and release effluent with low nitrogen and phosphorus levels. Removal percentage can vary with changes in influent nutrient concentration and residence time. Table 2 displays the total ammonia-N removal and phosphate-P removal efficiencies of several studies.

Table 2-6: Algal nutrient removal efficiencies in municipal wastewater

<table>
<thead>
<tr>
<th>Study</th>
<th>% total ammonia-N removal</th>
<th>% phosphate-P removal</th>
<th>Algae species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li, et al. 2011</td>
<td>93</td>
<td>81</td>
<td>Chlorella sp.</td>
</tr>
<tr>
<td>Green, et al. 1995</td>
<td>99</td>
<td>92</td>
<td>Polyculture</td>
</tr>
<tr>
<td>Martinez, et al. 2000</td>
<td>80-99</td>
<td>54-98</td>
<td><em>Scenedesmus obliquus</em></td>
</tr>
<tr>
<td>Lua, et al. 1995</td>
<td>74-99</td>
<td>69-93</td>
<td><em>chlorella vulgaris</em></td>
</tr>
<tr>
<td>Aslan, et al. 2006</td>
<td>24-99</td>
<td>&lt;30-78%</td>
<td><em>chlorella vulgaris</em></td>
</tr>
</tbody>
</table>

2.4.1 Algal Systems

2.4.1.1 Ponds

Wastewater treatment ponds or lagoons are the simplest way in which algae is used to treat wastewater. They are typically shallow earthen basins ranging from 2-5m in depth (Metcalf & Eddy, Inc., 2003). The lagoons are known for their effectiveness in removing biological oxygen demand, suspended solids, and pathogens (Green, et al., 1995). They can also provide nitrogen removal at times. Algae play a less prominent role in these systems; other biological solids associated with activated sludge are used for much of the treatment. Algal growth that occurs naturally aids in the oxygen requirements in the water.
2.4.1.2 High Rate Algal Ponds (suspended algae):

HRAPs are shallow oxidations ponds used for the growth of suspended microalgae, most often constructed as paddlewheel-mixed raceway ponds. They are considered one of the most well established technologies for algal production (Craggs, et al., 2011). Optimum design criteria such as size, depth, hydraulic loading, and flow rates have been thoroughly researched, reviewed, and updated (Hoffmann, 1998). HRAPs can be used to remove nutrients from many types of wastewaters, such as anaerobic pond effluents, domestic wastewater pre-treated to the primary or secondary level, and agricultural wastewaters (Craggs, et al., 2011). It has been shown that HRAPs can attain high levels of treatment in both domestic and agricultural wastewater. They provide reductions in BOD, TSS, nitrogen, phosphorus, and metals (Hoffmann, 1998). Nutrient removal rates can reach 24kg N/ha.d and 3kg P/ha.d with a maximum productivity of 30g/m²d dry algae biomass (Craggs, et al., 2011). Wastewater treatment HRAPs are normally part of an Advanced Pond System (APS), which consist of multiple different ponds in series.

2.4.1.3 Advanced pond systems

Advanced pond systems are typically earthwork systems that can replace conventional wastewater primary, secondary and tertiary treatment and. The most commonly known of these design is the advanced integrated wastewater pond system (AIWPS), developed at the University of California Berkeley (Oswald, 1990). It consists of a minimum of four ponds in a series and relies highly upon algal treatment. The system incorporates primary sedimentation, flotation, fermentation, aeration, secondary sedimentation, nutrient removal, effluent storage, and final liquid disposal. The first pond
in the system is an advanced facultative pond, followed by secondary facultative pond or HRAP. The third pond in the system is an algal settling pond, and then last is a maturation pond (Green, et al., 1996).

2.4.1.4 Problems for algae Systems:

One of the major problems with suspended microalgae systems is harvesting the algae (Mallick, 2002; Hoffmann, 1998). The microalgae are difficult to separate from the water because they typically occur in low concentrations, their cell densities are comparable to water, and their cell size is very small (Craggs, et al., 2011). Many methods of separation have been investigated, such as flocculation, filtration, flotation, sedimentation, centrifugation, and microstraining (Laliberte, et al., 1994). None of these methods have proven to be efficient, cost effective, and scalable to a large design (Hoffmann, 1998; Craggs, et al., 2011).

Nonsuspended or immobilized algae systems are another option for wastewater treatment. The algae are prevented from moving throughout the liquid, most often by being placed in a gel matrix. These systems can provide more flexibility in design, accelerated reaction rates, low to no algal washout, and better system stability compared to suspended systems (Mallick, 2002).

Efforts to grow larger species of algae in wastewater for easier harvesting have had mixed results. Often, the larger species are unable to maintain dominance over microalgae that contaminate the system (Hoffmann, 1998).

2.4.1.5 Algal Turf Scrubbers

Algal turf scrubbers (ATS) are bioengineered ecosystems that use attached algae, microalgae, and bacteria to treat water. They were invented at the Smithsonian Institution
by Walter Adey during the 1980s to control water quality in model ecosystems and since that time they have been investigated for use in treating wastewater (Adey & Bannon, 2008). This system is the most similar to the proposed system being used in this study. A community of algae and bacteria (a periphyton) are grown on a screen or rough surface on an inclined flow-way. A thin layer of water (1-4cm depth) moves down the flow-way in a series of pulses, being treated by the periphyton as it goes (Craggs, et al., 1996; Adey & Bannon, 2008). The periphyton that develops in these systems is naturally seeded by the algae/bacteria in the wastewater itself and/or with the aid of harvested local periphyton communities. A Pilot scale system of this nature was constructed in the mid-1990s to provide tertiary treatment of wastewater in Patterson, California (Craggs, et al., 1996). The wastewater treatment plant received a flow between 436 and 889 m$^3$ per day (0.12 MGD – 0.23 MGD). Species composition in the algal turf varied over the study period, but consisted mostly of Cyanophyta, green filamentous periphyton, and diatomaceous epiphytes. Yearly mean production was 35 g dry mass per m$^2$ per day. This system achieved nutrient removal rates of approximately 1.1 g nitrogen per m$^2$ per day and 0.7 g phosphorus per m$^2$ per day. Table 2-7 shows influent and effluent concentrations of nutrients.

Table 2-7: Yearly means of nutrient parameters of pilot scale ATS system (Craggs, et al., 1996)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium (mg/l)</td>
<td>3.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Nitrate/nitrite (mg/l)</td>
<td>5.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Soluble Reactive Phosphorus</td>
<td>2.7</td>
<td>1.2</td>
</tr>
</tbody>
</table>
2.5 Algae used as a biomass source and why to consider *Cladophora glomerata*

Being paired as a biofuel source and wastewater treatment technique algae could potentially be a practical and economically feasible approach to biofuel creation (Woertz, 2009). Macroalgae have properties that make them an attractive biomass source compared with microalgae. The first is that they can be much more easily harvested than microalgae. One of the major problems with suspended microalgae systems is harvesting the algae (Mallick, 2002; Hoffmann, 1998). As stated in the previous section, the microalgae are difficult to separate from the water because they typically occur in low concentrations, their cell densities are comparable to water, and their cell size is very small (Craggs, at al., 2011). This is not the case for macroalgae. For example, *Cladophora glomerata* has a filamentous branching structure that is more plant like, making separation from water easy. *Cladophora glomerata* can also be grown attached to a hard substrate; water could potentially flow freely though the biomass system without any algal washout.

Algae can be used as a feedstock for three types of biofuels; biodiesel, bioethanol, and biogas. Figure 2-3 summarizes what parts of algae are used in each fuel’s creation. Different portions of the algae contribute to biodiesel and bioethanol. Biodiesel is made from the lipid (oil) portion of the algae and bioethanol is made from the carbohydrate. Biogas can be made from the entire algae, including the lipid and carbohydrate portions.
To date, the majority of algae-to-energy research has focused on microalgae as a biomass source because microalgae’s ability to produce natural oils (lipids) (Sheehan, et al., 1998). These oils are extracted from algae and used to produce biodiesel. Microalgae can contain a high oil content, up to 20-50% of their dry mass (Xin, et al., 2010; Craggs, et al., 2011). The U.S. Department of Energy has also suggested that algae have productivity 20-40 times that of current oilseed crops such as soy and canola. Macroalgae on the other hand are known to have low oil contents; *Cladophora glomerata*’s lipid content is only about 5% of its dry mass (Gottumukala, 2010). This makes macroalgae a poor choice for biodiesel. Since Biodiesel has been the fuel of choice for algae, microalga research has flourished.

### 2.5.1 Bioethanol

Macroalgae also can contain a large amount of carbohydrates and cellulose (cellulose is a part of the carbohydrate), as is the case with *Cladophora glomerata* (Mihranyan, 2011) (Gottumukala, 2010). *Cladophora glomerata* has carbohydrate...
content near 33% and a cellulose content of approximately 15% (Gottumukala, 2010). Microalgae typically have a lower carbohydrate content of around 20% (Craggs R., 2011) or 5-23% (Brown, et al., 1997). The carbohydrate portion of algae biomass can be used to produce bioethanol. This makes a dry mass of Cladophora glomerata a better bioethanol feedstock than the same dry mass of microalgae. Other studies showed that Cladophora fascicularis contained close to 40% carbohydrates (Kumar, et al. 2010). Although this is not the same species, Cladophora glomerata belongs to the same genus. This suggests the possibility of increasing the carbohydrate content of Cladophora glomerata through growth of controlled cultures under optimal conditions (Gottumukala, 2010).

Not only is Cladophora glomerata better suited for bio-ethanol production compared to microalgae, it has several advantages over grain based ethanol. Currently, 95% of ethanol production in the U.S. is from corn, a grain ethanol. The environmental impact of corn based ethanol production is debatable; while some report a 10-15% reduction in CO₂ emissions over fossil fuels, others say it doubles the amount of greenhouse gas emissions released over 30 years (Solomon, et al., 2007; Searchinger, et al., 2008). Corn based ethanol also negatively impacts the environment through soil erosion and loss of biodiversity. Cladophora glomerata has the advantage of not requiring arable land to be grown, not interfering with food supply, and the possibility of simultaneously treating wastewater while being produced (Solomon, et al., 2007).

2.5.2 Biogas/Methane

Some say methane biogas production from algae would be the simplest and most cost effective option to convert algae to biofuel (Park, et al., 2011). This is done through
the process of anaerobic digestion. A feedstock (algae in this case) is placed into a reactor with the microorganisms needed for methane production. It is kept at a temperature around 35°C with a hydraulic retention time between 20-30 days. Biogas is the result, which is typically around 60-75% methane (CH₄) and 40-25% carbon dioxide (Chynoweth, et al., 2001; Sialve, et al., 2009).

The composition of algae plays an important role in their methane yield potential. Microalgae typically have proportions of 6-52% proteins, 7-23% lipids, and 5-23% carbohydrates according to Brown, Jeffrey, Volkman, & Dunstan (1997). Others report lipid contents that can be much higher, commonly 20-50% (Chisti, 2007; Xin, et al., 2010) (Craggs, et al., 2011). Using values given in Angelidaki & Sanders (2004) and a revised protein value given in Heaven, et al., (2011) it is possible to estimate methane production based upon these proportions. These values are given in Table 2-8 below.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Composition</th>
<th>L CH₄ g VS⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>C₆H₁₃.₁O₁N₀.₆</td>
<td>0.446</td>
</tr>
<tr>
<td>Lipids</td>
<td>C₅₇H₁₀₄O₆</td>
<td>1.014</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>(C₆H₁₀O₅)ₙ</td>
<td>0.415</td>
</tr>
</tbody>
</table>

From Table 2-8 and the composition of *Cladophora glomerata* (Gottumukala, 2010), the theoretical methane yield of *Cladophora glomerata* is 0.278 liters CH₄ per gram total solids (TS). Compared to microalgae species *Cladophora glomerata’s* L CH₄ g⁻¹TS is low. The reason for *Cladophora glomerata’s* low yield as TS is only about 58% of its dry weight is composed of carbohydrates, lipids, and proteins. An analysis of 19
microalgae species resulted in an average of 0.488 L CH$_4$ g$^{-1}$TS (Heaven, et al., 2011). Table 2-10 below summarizes these methane yields.

Table 2-9: Composition of *Cladophora glomerta* (Gottumukala, 2010)

<table>
<thead>
<tr>
<th>Portion</th>
<th>Percent of total dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>32.3%</td>
</tr>
<tr>
<td>Lipids</td>
<td>5.0%</td>
</tr>
<tr>
<td>Protein</td>
<td>20.8%</td>
</tr>
</tbody>
</table>

Table 2-10: Theoretical Methane yields for *Cladophora glomerata* and microalgae based on carbohydrate, protein, and lipid content

<table>
<thead>
<tr>
<th>Algal species</th>
<th>Theoretical methane yield (liters CH$_4$ g$^{-1}$ TS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cladophora glomerata</em></td>
<td>0.278</td>
</tr>
<tr>
<td>19 microalgae, average (Heaven, et al., 2011)</td>
<td>0.488</td>
</tr>
</tbody>
</table>

Gunaseelan (1997) measured methane yields for marine *Cladophora* mixed with *Ulva* and *Chaetomorpha*. The methane yield with this mixture was between 0.25 – 0.48 L CH$_4$ g$^{-1}$ (Gunaseelan, 1997).

Although the methane production per unit total solid weight for *Cladophora glomerata* is theoretically variable, it could also require a lower hydraulic retention time than other algal biomass. Lipid hydrolysis is slower than protein and carbohydrate hydrolysis, and microalgae are higher in lipid content. The minimum values of limiting generation time for anaerobic treatment are 0.18, 0.43, and 3.2 days for carbohydrates, proteins, and lipids respectfully (Pavlostathis & Giraldo-Gomez, 1991).
A problem facing all methane production from algal biomass is the low C/N ratio of the biomass (Yen & Brune, 2007). The optimum C/N ratio for anaerobic digestion is around 20/1 – 30/1, while the C/N ratio in algal sludge is about 6/1 (Yen & Brune, 2007; Parkin & Owen, 1986). *Cladophora glomerata* samples have shown to have a C/N ratio of near 10.7/1, better than the stated 6/1 for current algal sludge. Co-digesting the algal sludge with other high carbon content waste, such as waste paper or solids removed by primary treatment, would help improve methane production (Yen & Brune, 2007; Huebeck & Craggs, 2007).

Another factor that could affect methane yields of a *Cladophora glomerata* biomass is the makeup of its lignocellulosic material. Lignocellulosic material consists mainly of cellulose, hemicellulose and lignin in the algal cells. High crystallinity of cellulose and the high amounts of lignin are both known to negatively affect biodegradability (Hendriks & Zeeman, 2009). *Cladophora* species are known to have highly crystalline cellulose and a low lignin content (Mihranyan, 2011; Gottumukala, 2010), but the impact these factors would have on methane yield is unknown.

In order to achieve higher methane yields with anaerobic digestion, a pretreatment step could be implemented. Pretreatments alter the feedstock in some way that makes it more digestible (Appels, et al., 2011). For example, *Cladophora glomerata* biomass pretreatment could improve microbial digestion of the alga’s highly crystalline cellulose, potentially increasing methane yield. In spite of this, pretreatments are not commonly used to treat materials that will be anaerobically digested (Hendriks & Zeeman, 2009). More often they are used before ethanol fermentation.
Methane production through anaerobic digestion can be combined with other biofuel production techniques. For example, after lipids have been extracted from microalgae for biodiesel production the left over algal residue can be digested to produce methane (Chisti, 2007; Ehimen, et al., 2011). Even with microalgae’s high oil content, research has shown that with less than 40% lipid content it is more efficient to digest the algae for biogas production that to extract the lipids for biodiesel (Sialve, et al., 2009). It may also be an option to pair ethanol production with methane production for algae such as *Cladophora glomerata.*
Chapter 3

Methods

To effectively evaluate the feasibility of an algal (*Cladophora glomerata*) nutrient removal process bench-scale growth studies were carried out to obtain three main pieces of information. First of all, a relevant *Cladophora glomerata* specific growth rate was needed in grams algae growth per grams algae per day; $g \cdot g^{-1} \cdot \text{day}^{-1}$ (simplifies to day$^{-1}$); to approximate algae growth rates and biomass yield from an algal treatment system. In addition, nutrient tests were needed to calculate the amount of nitrogen and phosphorus being harvested with the algae and, consequently, being removed from the wastewater. It was also important to know the efficiency of nutrient removal using *Cladophora glomerata*. Lastly, the amount of methane production from the anaerobic digestion of *Cladophora glomerata* grown as a result of nutrient removal from the wastewater was needed. A CHN analysis was used to predict this, and this estimate was used to compare the costs and benefits associated with the algal treatment system as compared to conventional nutrient removal approaches.
3.1 General methods

A sample of *Cladophora glomerata* (L.) Kütz was obtained from the Canadian Phycological Culture Center. This sample was used as the base culture for the experiment. The experiments were conducted in batch, using 500 ml Erlenmeyer flask. The flasks were filled with 200 ml of culture medium, and in each flask gentle aeration was provided by a tube extending down through a rubber stopper.

To inoculate a batch, samples were taken from a *Cladophora glomerata* (L.) Kütz culture being grown in D11 medium. Each sample was extracted using an inoculating loop, rinsed with DI water and then placed on filter paper. The filter paper was folded over and paper towels were placed on the top and the bottom. An empty beaker weighing 128 grams was placed on top for a time of 5 seconds. After 5 seconds the *Cladophora* sample was taken off the filter paper with an inoculation loop and placed on a digital scale to obtain a wet weight. These samples were then placed in a flask with 200 ml of medium. Each flask was inoculated with a $3.8 \pm 0.2$ mg (dry weight) sample. Before inoculation the flasks were washed and autoclaved. The flasks containing the algae samples were then placed in a growth chamber; the growth chamber was set to a constant temperature of 22° Celsius and constant light of 115 µmol m$^{-2}$s$^{-1}$. Growth periods between 6 and 25 days were used. A temperature of 22° Celsius was used as an average of the reported optimum growth temperatures of 13° to 31°C (Higgens, et al., 2008). Also, Gerloff & Fitzgerald 1976, were able to produce high yields (over 600 g dry weight per liter) at a temperature of 23°C. Yields were calculated as the ending weight of *Cladophora glomerata* after a given growth period, not subtracting out the initial weight.
Three mediums were used in this study: D11, a synthetic wastewater, and effluent wastewater obtained from the city of Toledo’s Bay View Wastewater Treatment Plant. D11 medium was used as the optimized medium. This medium was developed specifically for culturing freshwater *Cladophora*. Included in this medium are a large number of trace elements and it is suggested that many are possibly unnecessary (Anderson, 2005). All trace elements listed in the D11 medium are not used in this study. The C13 and B7 trace element solutions were eliminated with the exception of the compounds containing Se, V, Co, and Ni; as per recommendation (Anderson, 2005). The recipe calls for selenic acid but as recommended selenious acid was used in its place (Anderson, 2005). Biotin (vitamin H) was also excluded. The chemical composition of the D11 medium can be seen in Table 3-1.
Table 3-1: D11 medium composition

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO$_4$ * 7H$_2$O</td>
<td>100</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$ * 4H$_2$O</td>
<td>150</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>100</td>
</tr>
<tr>
<td>KCl</td>
<td>30</td>
</tr>
<tr>
<td>Na$_2$SiO$_3$ * 9H$_2$O</td>
<td>60</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>15</td>
</tr>
<tr>
<td>Na$_2$EDTA * 2H$_2$O</td>
<td>12.5</td>
</tr>
<tr>
<td>FeSO$_4$ * 7H$_2$O</td>
<td>5</td>
</tr>
<tr>
<td>H$_3$BO$_3$</td>
<td>1</td>
</tr>
<tr>
<td>ZnSO$_4$ * 7H$_2$O</td>
<td>0.1</td>
</tr>
<tr>
<td>MnSO$_4$ * H$_2$O</td>
<td>0.02</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$ * 2H$_2$O</td>
<td>0.025</td>
</tr>
<tr>
<td>CuSO$_4$ * 5H$_2$O</td>
<td>0.015</td>
</tr>
<tr>
<td>Na$_2$SeO$_3$</td>
<td>0.0012</td>
</tr>
<tr>
<td>NH$_4$VO$_3$</td>
<td>0.0023</td>
</tr>
<tr>
<td>NiSO$_4$ * 6H$_2$O</td>
<td>0.0045</td>
</tr>
<tr>
<td>CoCl$_2$ * 6H$_2$O</td>
<td>0.004</td>
</tr>
<tr>
<td>Thiamine * HCl (vitamin B1)</td>
<td>0.1</td>
</tr>
<tr>
<td>Cyanocobalamin (B12)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The synthetic wastewater (SWW) composition was based upon a synthetic wastewater medium in Aslan, 2006. The composition of the synthetic wastewater can be seen in Table 3-2.
Table 3-2: Synthetic wastewater (SWW) medium composition

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO₄ * 7H₂O</td>
<td>1000</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>25</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>111</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>14-57</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>35-140</td>
</tr>
<tr>
<td>Na₂EDTA * 2H₂O</td>
<td>12.5</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>5</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>1</td>
</tr>
<tr>
<td>ZnSO₄ * 7H₂O</td>
<td>0.1</td>
</tr>
<tr>
<td>MnSO₄ * H₂O</td>
<td>0.2</td>
</tr>
<tr>
<td>Na₂MoO₄ * 2H₂O</td>
<td>0.025</td>
</tr>
<tr>
<td>CuSO₄ * 5H₂O</td>
<td>0.015</td>
</tr>
<tr>
<td>Thiamine * HCl (vitamin B1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Cyanocobalamin (B12)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Effluent wastewater from the city of Toledo’s Bay View Wastewater Treatment Plant was obtained on February 28, 2012. The water was tested for nutrients and pH prior to use, Table 3-3 shows these values. Nutrient tests were performed using colorimetric HACH kits and a HACH DR 2800 Spectrophotometer. Phosphorus levels were measured as reactive orthophosphate using Method 8048 PhosVer 3 (Ascorbic Acid) Method, Powder Pillows. Ammonia nitrogen was measured using Method 8038 Nessler Method (mineral stabilizer and polyvinyl alcohol solutions were not used). Nitrate nitrogen was measured using Method 8171 Cadmium Reduction Method, Powder Pillows. Nitrite nitrogen levels were measured using Method 8507 Diazotization Method, Powder Pillows (Hach Company, 2007).
Table 3-3: Bay View Wastewater Treatment Plant effluent medium characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthophosphate</td>
<td>0.19 mg/L PO$_4$-P</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.29 mg/L NH$_3$-N</td>
</tr>
<tr>
<td>Nitrate</td>
<td>6.0 mg/L NO$_3$-N</td>
</tr>
<tr>
<td>Nitrite</td>
<td>0.010 mg/L NO$_2$-N</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
</tbody>
</table>

After the appointed growth period each algae sample was separated from the medium by filtration; some of the samples were weighed to obtain a final wet weight, using the same procedure listed above to find the initial wet weight. Algae was then either directly lyophilized, or stored frozen and lyophilized at a later date. The lyophilized samples were stored frozen until analyses were run.

3.2 Individual growth trials

*Cladophora glomerata* growth was conducted in a series of trials, each having its own significance to the study. Table 3-4 gives a summary of each trial. Trial 1 was conducted using D11 media and a growth period of 25 days. Trial 2 was conducted with D11 media and low nutrient synthetic wastewater (SWW-L) over a 2 day growth period. The low nutrient synthetic wastewater contained 14 mg/L KH$_2$PO$_4$ and 35 mg/L NH$_4$Cl. Table 3-5 shows the nutrient concentrations used for low nutrient synthetic wastewater (SWW-L), medium nutrient synthetic wastewater (SWW-M), and high nutrient synthetic wastewater (SWW-H). Trial 3 was conducted the same as trial 2. Trial 4 was conducted using low, medium, and high nutrient synthetic wastewaters with a growth period of 19 days. Trial 5 used low nutrient synthetic wastewater, *Cladophora* and water samples were taken every 6 days for a period of 18 days. Trial 6 used Toledo’s Bay View Wastewater Treatment Plant effluent as a medium, *Cladophora* and water samples were taken every 6
days for a period of 18 days. Water samples taken from trials 5 and 6 were subjected to nutrient tests; the amount of nitrogen and phosphorus present in the samples was found.

Table 3-4: Summary of *Cladophora glomerata* growth trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Medium</th>
<th>Growth period</th>
<th>Periodic testing</th>
<th>Nutrient testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D11</td>
<td>25 days</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>D11</td>
<td>25 days</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>SWW L</td>
<td>25 days</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>D11</td>
<td>25 days</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>SWW L</td>
<td>25 days</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>SWW L</td>
<td>19 days</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>SWW M</td>
<td>19 days</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>SWW H</td>
<td>19 days</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>SWW L</td>
<td>18 days</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>BVWW</td>
<td>18 days</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 3-5: Synthetic wastewater nutrient concentrations

<table>
<thead>
<tr>
<th>Synthetic Wastewater</th>
<th>Nutrient Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KH$_2$PO$_4$</td>
</tr>
<tr>
<td>SWW-L</td>
<td>14 mg/L</td>
</tr>
<tr>
<td>SWW-M</td>
<td>28 mg/L</td>
</tr>
<tr>
<td>SWW-H</td>
<td>57 mg/L</td>
</tr>
</tbody>
</table>

3.3 Specific growth rate

Specific growth rates were calculated using equation 3-1: Algal specific growth rate. The specific growth rate is in units of grams algae growth per grams algae per day; simplifies to day$^{-1}$. 
3.4 Nutrient removal

Colorimetric HACH kits and a HACH DR 2800 Spectrophotometer were used to test water samples before and after *Cladophora glomerata* growth. Phosphorus levels were measured as reactive orthophosphate using Method 8048 PhosVer 3 (Ascorbic Acid) Method, Powder Pillows. Ammonia nitrogen was measured using Method 8038 Nessler Method (mineral stabilizer and polyvinyl alcohol solutions were not used). Nitrate nitrogen was measured using Method 8171 Cadmium Reduction Method, Powder Pillows. Nitrite nitrogen levels were measured using Method 8507 Diazotization Method, Powder Pillows (Hach Company, 2007). After obtaining before growth and after growth values for nutrient concentrations in the medium, the amount of nutrients taken in by *Cladophora glomerata* was calculated. The concentration on nitrogen and phosphorus in *Cladophora glomerata* per unit dry weight was found using two methods. The first was an “average” method; the concentration of nutrients was found for each sample using equations 3-2: Percent nitrogen or phosphorus in algae per unit dry weight, and colorimetric test results. An average plus or minus standard deviation was found combining the data from all the samples. The second method was a “linear regression method.” *Cladophora glomerata* dry weight was plotted against nutrients removed from the sample (found by colorimetric test) on a scatterplot. A linear regression line was added, the slope of this line had a unit of mg nutrient/mg algae; multiply this by 100 and it becomes a percent nutrient per weight of algae.
\[
\% \text{N or P in algae} = \frac{\text{initial mg/L N or P in medium} - \text{measured mg/L N or P in medium}}{\text{algae dry weight (mg)}} \times 100
\] (3-2)

CHN Results were also used to find nitrogen concentrations per unit dry weight of *Cladophora glomerata*. Samples of *Cladophora glomerata* were taken after growth periods of 6-25 days and a CHN analysis was performed on these samples. CHN analyses were performed using a PerkinElmer 2400 Series II CHNS/O analyzer.

### 3.5 Methane production

Methane production was calculated using Equation 3-3: Estimation of methane, carbon dioxide, ammonia, and hydrogen sulfide that will be produced under anaerobic conditions (Metcalf & Eddy, Inc., 2003). This equation estimates methane gas production from an anearobic process based on a biomass’s carbon, hydrogen, oxygen, nitrogen, and sulfur content. The CHN analysis done for this study only provides the carbon, hydrogen, and nitrogen content of *Cladophora glomertata*; any oxygen and sulfur content in the algae would increase the methane yield. This formula also assumes 100% conversion, which is typically not the case. More often in a single stage anaerobic digestion of algae, efficiencies are close to 50% (Yang, et al., 2011). A variable biogas payback will be used for costs evaluations of the *Cladophora glomertata* system, using 100% of the value found using Equation 3-2 and 50%.

\[
\begin{align*}
C_vH_wO_xN_yS_z+ & \quad v \cdot \frac{w}{4} + \frac{x}{2} + \frac{3y}{4} + \frac{z}{2} \quad \text{H}_2\text{O} \quad \text{yields} \quad v \cdot \frac{w}{8} + \frac{x}{4} + \frac{3y}{8} + \frac{z}{4} \quad \text{CH}_4 \\
& \quad + \quad v \cdot \frac{w}{2} \cdot \frac{x}{8} + \frac{3y}{4} + \frac{z}{4} \quad \text{CO}_2 + y\text{NH}_3 + z\text{H}_2\text{S}
\end{align*}
\] (3-3)
3.6 Design of the proposed *Cladophora glomerata* treatment system

The proposed *Cladophora glomerata* algal treatment system consists of a rectangular tank the wastewater flows through at a depth of 1.0m, to allow for algal filament lengths of 90 cm. The size of the tank will depend on treated flow and the algae’s characteristics; specific growth rate and the nutrient concentration in the algae. The bottom of the tank will be made of concrete, a solid substrate, which will allow *Cladophora glomerata* to attach. Mechanical aerators will be used to incorporate carbon dioxide from the air into the wastewater. The growth tank will be split into sections. The harvesting of each section will be staggered according to growth period. This will be done to keep nutrient removal rates consistent due to a consistent amount of algal biomass being present in the system. The time it will take to harvest a section of the system is unknown, but it may be feasible to harvest the algae without shutting down portions of the system. A proposed harvesting method may look something like a moving bridge with some sort of mechanical cutting and capturing contraption attached. The system will operate as a continuous flow system, one end of the tank allowing in influent and the other letting out the treated effluent. The algae will be mechanically harvested. Results from growth trials will predict the solid content of the algae. These solids will be added to the anaerobic digester where they will contribute to methane production.

The influent concentrations of nitrogen and phosphorus were taken from Table 2-4, the composition of a typical municipal wastewater (Hammer & Hammer, 2008). Nitrogen will be removed to a concentration of 10 mg/L, and phosphorus to a target concentration of 1.0 mg/L. Total nitrogen effluent values of 8 mg/L – 10 mg/L are common goals to achieve with nitrogen removal processes, along with 1.0 mg/L

An algal density maximum of 600 g DW/m$^2$ was used to design the system. This value was the maximum average value of bed densities of in eastern Lake Erie from the years 1979 through 2006 (see section 2.1) (Higgens, et al., 2008). Growth periods for the algal treatment system were calculated from specific growth rates found during growth trials. The growth periods were found by assuming that when a section of algae was harvested it would be cut back to 100 g DW/m$^2$; the amount of time this 100 g DW/m$^2$ would take to grow back to 600 g DW/m$^2$ was the growth period. All Specific growth rates used to design the algal treatment system were experimentally found specific growth rates that had to be scaled down to account for photoperiod. In a real world implementation a 24 hour growth period is not applicable; approximately 12 hours of sunlight a day would reach the system a day. According to data given by Robinson & Hawkes (1986) a decrease in photoperiod from 24 hours to 12 hours would result in a 0 – 45% decrease in specific growth rate, approximately 23% on average. Other studies suggest that photoperiod affects growth rate more severely, decreasing the growth period from 24 hours to 12 hours would decrease the specific growth rate by 50% (Canale & Auer, 1982; Higgins, Hecky, & Guildford, 2005). Specific growth rates used in the system design were experimentally found growth rates decreased by 37%, an average of the two values found in literature.

The *Cladophora glomerata* treatment system tank area was calculated from influent nutrient concentration, the desired effluent nutrient concentration, nutrient concentrations in the algae, specific growth rate of the algae, and daily flow of the
wastewater treatment plant. An example calculation done below, assuming 4.15% percent nitrogen by weight in the *Cladophora glomerata*, a growth rate of 0.115 day\(^{-1}\), and that nitrogen will determine the size of the tank. The first step was to determine how much nitrogen needed to be removed from the wastewater each day. The influent concentration of 24 mg/L nitrogen will have to be reduced by 14 mg/L nitrogen in order to meet 10 mg/L nitrogen effluent.

\[
\frac{24}{L} \text{mg N Influent} - \frac{10}{L} \text{mg N Effluent} = \frac{14}{L} \text{mg N removed}
\]

Next, the influent flow was multiplied by 14 mg/L to obtain the total weight of nitrogen that needed to be removed a day to achieve a 10 mg/L effluent.

\[
378500 \frac{L}{day} \text{influent flow} \times \frac{14}{L} \text{mg N} \times \frac{1 \text{ kg}}{1000000 \text{ mg}} \approx 5.3 \frac{\text{kg N}}{\text{day}} \text{ removed}
\]

From this number it was calculated how much algae growth would be needed to remove this amount of nitrogen from the wastewater; the amount of nitrogen multiplied by the concentration of nitrogen present in one kilogram of *Cladophora glomerata*.

\[
5.30 \frac{\text{kg N}}{\text{day}} \times \frac{1}{0.0415} \frac{\text{kg algae}}{\text{kg N}} \approx 128 \frac{\text{kg algae}}{\text{day}}
\]

Lastly, this weight of algae was divided by the average weight of algae harvested each day, resulting in the area of the tank. The average weight of algae harvested each day was calculated using Table A-1 in Appendix A; taking the maximum biomass before harvest minus the amount left behind to continue growth and dividing this difference by the growth period.
Hydraulic retention times were calculated by taking the volume of a tank divided by the flow. The volume was found by multiplying the tank area by given depth of the tank, 1.0m. An example can be seen below using a 4340 m² tank area and 378.5 m³/day flow.

\[
\frac{128 \text{ kg algae day}^{-1} \times 1000 \text{ g kg}^{-1}}{571 \text{ g m}^{-2} - 100 \text{ g m}^{-2} \text{ harvested biomass}} \approx 4340 \text{ m}^2 \text{ Tank area}
\]

\[
\frac{16 \text{ day growth period}}{378.5 \text{ m}^3 \text{ day}^{-1}} \approx 11.47 \text{ days hydraulic retention time}
\]

3.7 **Cost of a Cladophora glomerata nutrient removal system**

The costs associated with the construction of a *Cladophora glomerata* nutrient removal system were calculated using RSmeans online software. These costs included a reinforced concrete tank with a six inch thick base and six inch thick walls; site grading; a six inch thick base of compacted stone; mechanical aerators; and harvesting equipment. The reinforced concrete tank was sized to have a length to width ratio of 2:1. A variable cost was determined for aerators, it was unknown how many would be needed for each size system. This would depend on influent CO₂ concentrations, natural CO₂ transfer rates to the water from the atmosphere, and algal CO₂ usage. Using data from mechanical aerators technical specifications it was found how many aerators would be needed for gas dispersion throughout the entire tank (Siemens, 2009); this number of aerators was used as a high end cost. The low end cost was priced for no aerators being used in the system. Harvesting equipment costs were derived from the cost of sedimentation tank mechanical
equipment; typically mechanical scrapers for rectangular tanks. These costs were taken from US EPA (1979) updated to 2012 cost with ENR’s construction cost index and adjusted for each tank size (US EPA, 1979; McGraw-Hill, 2012). The cost of the equipment was taken as the cost per linear yard of equipment with a width of 20ft.

Operations and maintenance costs for the proposed *Cladophora glomerata* nutrient removal system were estimated from the operation and maintenance costs associated with sedimentations basins, and the power consumption of proposed aerators. Sedimentation basin operation and maintenance cost were considered to be a reasonable estimate because the similarities of equipment and of how the tanks are used; the values were derived from US EPA (1979).

The value of methane payback was calculated using the average commercial price for natural gas in 2011, $8.86 per thousand ft$^3$ of gas (eia, 2012). The average weight of algae harvested each day was calculated using Table A-1 in Appendix A; taking the maximum biomass before harvest minus the amount left behind to continue growth and dividing this difference by the growth period. This algal weight was multiplied by the theoretical methane yield per unit weight of *Cladophora glomerata*, to obtain a methane yield per day. This methane yield was multiplied by 365 to get the total methane volume produced each year. This volume was then valued using the $8.86 per thousand ft$^3$ of gas (eia, 2012).
Chapter 4

Experimental Results

4.1 Growth Results

*Cladophora glomerata* growth trial 1 was conducted during summer 2011. An alga sample received from the Canadian Phycological Culture Center was divided into 3 approximately equal masses and cultured in D11 media for a period of 25 days. Table 4-1 shows the results from trial 1. A small portion of *Cladophora glomerata* from sample 1 was removed before a final dry weight was taken, this adversely affected sample 1’s yield. This sample was placed in D11 medium and used as a base for the next growth trial. Since an initial wet weight was not taken, growth rates could not be calculated for trial 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Medium</th>
<th>Growth period, days</th>
<th>Initial dry weight, mg</th>
<th>Final dry weight, mg</th>
<th>Yield in mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D11</td>
<td>25</td>
<td>NA</td>
<td>54.2</td>
<td>271</td>
</tr>
<tr>
<td>2</td>
<td>D11</td>
<td>25</td>
<td>NA</td>
<td>48.1</td>
<td>241</td>
</tr>
<tr>
<td>3</td>
<td>D11</td>
<td>25</td>
<td>NA</td>
<td>65.3</td>
<td>327</td>
</tr>
</tbody>
</table>

Table 4-1: *Cladophora glomerata* growth trial 1 results
Yields from this trial were less than to be expected. Gerloff & Fitzgerald (1976) routinely obtained 21 day yields of 400-600 mg/L dry weight from their synthetic culture medium, on which the D11 medium is based (Gerloff & Fitzgerald, 1976). Omission of several trace elements from this study’s D11 media could have negatively affected yields; this was discussed in the general methods portion of this paper.

*Cladophora glomerata* growth trial 2 was started on November 1, 2011 and growth trial 3 was started on November 23, 2011. For both trials four alga samples were cultured in D11 medium and four samples were cultured in a synthetic wastewater medium. Results from trials 2 and 3 can be seen below in Table 4-2. The samples from trial 3 cultured in synthetic wastewater (SWW L) did not grow well this trial, much of the algae died; results from these samples were omitted from the study. Initial dry weights were calculated by using a conversion factor found by comparing final alga dry weights to final wet weights. The synthetic wastewater produced algal yields much lower than yields in D11 medium. The average yield in D11 medium for trials 2 and 3 was 274 ± 73 mg/L while the average yield in synthetic wastewater was 84 ± 10 mg/L. These proved to be statistically different; a t-test returned p < 0.05. D11 grown *Cladophora glomerata* produced yields an average of 3.26 times greater than synthetic wastewater grown *Cladophora glomerata*.

Table 4-2: *Cladophora glomerata* growth trials 2 and 3 results

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth period, days</th>
<th>Initial dry weight, mg</th>
<th>Final dry weight, mg</th>
<th>Yield in mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>D11</td>
<td>25</td>
<td>3.7 ± 0.2</td>
<td>54.8 ± 14.6</td>
<td>274 ± 73</td>
</tr>
<tr>
<td>SWW L</td>
<td>25</td>
<td>3.6 ± 0.2</td>
<td>16.8 ± 2.0</td>
<td>84 ± 10</td>
</tr>
</tbody>
</table>
*Cladophora glomerata* growth trial 4 was started on January 27, 2012. Nine samples of alga were grown in synthetic wastewater of varying nutrient levels. These levels can be seen in Table 4-3. The trial 4 growth period was planned to be 25 days, but periodic inspection of the samples revealed that some were not doing well after 17 days; some of the algal samples were turning brown in color, a sign that the those samples were not surviving. The decision was made to harvest the samples early, after a growth period of 19 days, to prevent algal death as in trial 3. Results from trial 4 can be seen in Table 4-3. The yields of algae appeared to decrease as the nutrients in the synthetic medium increased. The average SWW L yield was $79 \pm 13$ mg/L, the average SWW M yield $68 \pm 4.8$ mg/L, and the average SWW H yield was $39 \pm 2.7$ mg/L. It was found there was no statistical difference between the yields for SWW L and SWW M, a t-test returned $p = 0.19$. There was a significant difference between the yields of algae grown in SWW M and SWW H (t-test, $p < 0.05$). There was also a significant difference in yields of algae grown in SWW L and SWW H (t-test, $p < 0.05$). *Cladophora glomerata* yields were shown to decrease with levels of nutrients at or above $12.9$ mg/L PO$_4$-P and $36.7$ mg/L NH$_4$-N, Table 4-3 (see Table 3-5 for nutrient concentrations in medium).

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth period, days</th>
<th>Initial dry weight, mg</th>
<th>Final dry weight, mg</th>
<th>Yield in mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWW L</td>
<td>19</td>
<td>$4.2 \pm 0.0$</td>
<td>$15.7 \pm 2.6$</td>
<td>$79 \pm 13$</td>
</tr>
<tr>
<td>SWW M</td>
<td>19</td>
<td>$4.2 \pm 0.1$</td>
<td>$13.6 \pm 1.0$</td>
<td>$68 \pm 5$</td>
</tr>
<tr>
<td>SWW H</td>
<td>19</td>
<td>$4.2 \pm 0.0$</td>
<td>$7.8 \pm 0.5$</td>
<td>$39 \pm 3$</td>
</tr>
</tbody>
</table>

Growth trial 5 was started on February 21, 2012. Six samples of *Cladophora glomerata* were grown in low nutrient synthetic wastewater. Two samples were
harvested every six days for eighteen days. Samples 1 and 2 were harvested at six days, samples 3 and 4 at twelve days, and samples 5 and 6 at eighteen days. The results from trial 5 can be seen in Table 4-4 below. The average yield of algae after 6 days was $59 \pm 2.3\text{ mg/L}$, the average yield after 12 days was $98 \pm 5.5\text{ mg/L}$, and the average yield after 18 days was $113 \pm 0.75\text{ mg/L}$. It was determined there was a significant different between algal yields after 6 days and 12 days ($t$-test $p < 0.05$). For the growth between 12 days and 18 days there was no significant difference in algal yields ($t$-test $p = 0.11$). For algae growth in low nutrient synthetic wastewater (SWW L) there was significant yield increase from zero to 12 days, but after 12 days there was no significant yield increase. A summary of the algal yields from trial 5 can be seen in Table 4-4.

Table 4-4: *Cladophora glomerata* growth trial 5 results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Medium</th>
<th>Growth period, days</th>
<th>Initial dry weight, mg</th>
<th>Final dry weight, mg</th>
<th>Yield in mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SWW L</td>
<td>6</td>
<td>3.7</td>
<td>12.3</td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>SWW L</td>
<td>6</td>
<td>3.8</td>
<td>11.4</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>SWW L</td>
<td>12</td>
<td>3.7</td>
<td>20.7</td>
<td>104</td>
</tr>
<tr>
<td>4</td>
<td>SWW L</td>
<td>12</td>
<td>3.9</td>
<td>18.5</td>
<td>93</td>
</tr>
<tr>
<td>5</td>
<td>SWW L</td>
<td>18</td>
<td>3.8</td>
<td>22.5</td>
<td>113</td>
</tr>
<tr>
<td>6</td>
<td>SWW L</td>
<td>18</td>
<td>3.8</td>
<td>22.8</td>
<td>114</td>
</tr>
</tbody>
</table>
Figure 4-1: Growth trial 5, *Cladophora glomerata* yields over time

*Cladophora glomerata* growth trial 6 was started on February 28, 2012. Seven samples of *Cladophora glomerata* were growth in Toledo’s Bay View Wastewater Treatment Plant effluent. Samples were harvested every six days for eighteen days. Samples 1 and 2 were harvested at six days, samples 3 and 4 at twelve days, and samples 5, 6, and 7 at eighteen days. The results from trial 6 can be seen in Table 4-5 below. The average yield of algae after 6 days was $207 \pm 8$ mg/L, the average yield after 12 days was $386 \pm 51$ mg/L, and the average yield after 18 days was $494 \pm 24$ mg/L. After statistical analysis it was determined that there was no significant difference between day 6 yields and day 12 yields, and between day 12 yields and day 18 yields (t-test $p = 0.085$ and $0.13$, respectively). This can be attributed to the high variability in yields for the day 12
samples. It was found that there was a difference between algal yields after 6 days and after 18 days (t-test p < 0.05). Yields with BVWW medium were higher than in any other trial. A summary of the algal yields from trial 5 can be seen in Figure 4-2.

Table 4-5: *Cladophora glomerata* growth trial 6 results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Medium</th>
<th>Growth period, days</th>
<th>Initial dry weight, mg</th>
<th>Final dry weight, mg</th>
<th>Yield in mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BVWW</td>
<td>6</td>
<td>3.9</td>
<td>39.8</td>
<td>199</td>
</tr>
<tr>
<td>2</td>
<td>BVWW</td>
<td>6</td>
<td>3.6</td>
<td>43.0</td>
<td>215</td>
</tr>
<tr>
<td>3</td>
<td>BVWW</td>
<td>12</td>
<td>3.9</td>
<td>67.0</td>
<td>335</td>
</tr>
<tr>
<td>4</td>
<td>BVWW</td>
<td>12</td>
<td>3.6</td>
<td>87.5</td>
<td>438</td>
</tr>
<tr>
<td>5</td>
<td>BVWW</td>
<td>18</td>
<td>3.7</td>
<td>101.5</td>
<td>508</td>
</tr>
<tr>
<td>6</td>
<td>BVWW</td>
<td>18</td>
<td>3.9</td>
<td>102.7</td>
<td>514</td>
</tr>
<tr>
<td>7</td>
<td>BVWW</td>
<td>18</td>
<td>3.7</td>
<td>92.1</td>
<td>461</td>
</tr>
</tbody>
</table>

Figure 4-2: Growth trial 6, *Cladophora glomerata* yields over time
Figure 4-3 gives a summary of algae yields for each type of medium. Yields produced in real wastewater treatment plant effluent (BVWW) were much higher than any of the other yields. There are unknown factors associated with real wastewater effluent that may promote growth over a synthetic medium as it is impossible to identify and quantify all constituents in the wastewater. One objective of the growth trials was to see if Cladophora glomerata growth in wastewater compared to growth in an optimum medium. The results show that the Toledo Bay View Wastewater Treatment Plant medium produces a statistically higher algal yield than an “optimum” synthetic medium (t-test p <0.05), even when the growth period of D11 was 25 days and BVWW 18 days. The average yield from all trials using D11 medium was 275 ± 65 while the average yield obtained from the trial using BVWW medium was 494 ± 24.

![Average Cladophora glomerata yields for full growth periods (18-25 days)](image)

Figure 4-3: Average Cladophora glomerata yields for full growth periods (18-25 days)
A summary of the results for specific growth rates can be seen in Figure 4-4. The values were found using equation 3-1 and the longest growth periods in each trial (varied from 18 to 25 days). Specific growth is related to the algal yield, but takes into consideration the growth period and starting algal weight. Specific growth rates for algae growth in BVWW were statistically higher than for algae grown in D11 medium and any synthetic wastewater (SWW L, SWW M, SWW H) (t-test p < 0.05).

![Average Cladophora glomerata specific growth rates for full growth periods (18-25 days)](image)

Figure 4-4: Average Cladophora glomerata specific growth rates for full growth periods (18-25 days)

Results from trials 5 and 6 show that the average specific growth rate of Cladophora glomerata varied over time. Figure 4-5 displays the specific growth rates of Cladophora glomerata over time intervals in growth trials 5 and 6. In both trials, the specific growth rate starts high during the first six days of growth, and then the specific growth rate after 12 days declines (both trials t-test p < 0.05). For trial 5 it starts at 0.190
± 0.010 day⁻¹ and drops to 0.137 ± 0.006 day⁻¹ after 12 days; on average a 28% decrease.

For trial 6 it starts at 0.40 ± 0.013 day⁻¹ and drops to 0.252 ± 0.015 day⁻¹ after 12 days; on average a 37% decrease.

It appears that there is another decline from 12 to 18 days but the difference is not statistically significant between the two (t-test p = 0.052 and p = 0.065, for trials 5 and 6 respectively).

Figure 4-5: Growth trials 5 and 6, *Cladophora glomerata* specific growth rates over time intervals

### 4.2 Nutrient Results

For growth trials 5 and 6, each sample was tested for nutrient concentrations. These tests were conducted to find the levels of nitrogen and phosphorus present in the medium before and after *Cladophora glomerata* growth. It was important to know
whether *Cladophora glomerata* was removing enough nutrients from the wastewater to make it an effective tool for wastewater treatment.

### 4.2.1 Trial 5

The SWW L medium used in trial 5 contained only nitrogen in the form of ammonium (9.2 mg/L NH$_3$ – N), and only phosphorus in the form of orthophosphate (3.08 mg/L PO$_4$ – P). Figure 4-1 and Figure 4-2 show the results from nutrient tests conducted on trial 5 samples. A linear regression line was added to each figure and the R$^2$ value is displayed. Both regression lines have a R$^2 > 0.90$ indicating a strong linear correlation between growth period and nutrient removal. The regression line in Figure 4-6 has a slope of 0.078 relative to the primary y-axis, mg/L PO$_4$ – P removed. This means that *Cladophora glomerata* removed approximately 0.078 mg/L PO$_4$ – P each day of growth. Relative to the secondary y-axis the line has a slope of 2.5; 2.5 percent of the total PO$_4$ – P was removed each day. The regression line in Figure 4-7 has a slope of 0.39 relative to the primary y-axis, indicating that 0.39 mg/L NH$_3$ – N was removed each day of the growth period. The slope of the line relative to the secondary axis is 4.2; 4.2 percent of the NH$_3$ – N was removed each day over the 18 day growth period.

Over a period of 18 days *Cladophora glomerata* was able to remove 1.55 ± 0.015 mg/L PO$_4$ - P, which was 50.3 ± 0.49% of the initial concentration (3.08 mg/L) and 7.54 ± 0.55 mg/L NH$_3$ – N, which was 82.2 ± 6.0% of the initial concentration (9.17 mg/L) from the SWW L medium.
Figure 4-6: Trial 5 phosphorus removal over time

Figure 4-7: Trial 6 nitrogen removal over time
4.2.2 Trial 6

Trial 6 was conducted with BVWW medium and unlike the SWW L used in trial 5, it contained multiple forms of nitrogen and phosphorus. The nutrient makeup of BVWW can be seen in Table 3-3, Figure 4-8 and Figure 4-9 display the results for the nutrient test conducted on Trial 6 samples. After a growth period of six days, PO$_4$ – P levels were below detectable limits for 3 samples, these samples are shown near 100% removal but the lowest detectable limit was 0.007 mg/L PO$_4$ – P. PO$_4$ – P removal percentages over 96.6% were undetectable. A linear regression line was added to each figure and the R$^2$ value is displayed. Figure 4-8’s linear regression line has a R$^2$ value equal to 0.48. This indicates a low linear correlation between the amount of phosphorus removed from the medium and the growth period. The regression line in Figure 4-9 has a R$^2$ value of 0.97, which indicates a strong linear relationship between the amount of nitrogen that was removed from the medium and the growth period. The regression line has slopes of 0.2884 and 4.5 relative to the primary and secondary axis respectively. This means that Cladophora glomerata removed approximately 0.2884 mg/L N each day of growth or 4.5% of the total N each day.

Over a period of 18 days Cladophora glomerata was able to remove 0.178 ± 0.001 mg/L PO$_4$ - P, which was 96.0 ± 0.88% of the initial concentration (0.19 mg/L) (2 measurements at a growth period of 18 days were below detectable limits) and 4.9± 0.4 mg/L NH$_3$ – N, which was 77.7 ± 6.1% of the initial concentration (6.3 mg/L) from the SWW L medium.
Figure 4-8: Trial 6 phosphorus removal over time

Figure 4-9: Trial 6 nitrogen removal over time
4.3 Growth and nutrient combined results

For trials 5 and 6 growth data and nutrient data can be combined to analyze any relationships that exist between the two parameters. This information will be used in determining the viability of an algal wastewater treatment system. This data will show the amount of nutrients being removed per unit weight of algae; i.e., the concentration of nutrients in the algae. This data is needed to calculate the amount of nitrogen and phosphorus being harvested with the algae and consequently, being removed from the wastewater.

4.3.1 Trial 5

For trial 5, assuming no phosphorus removal other than through Cladophora glomerata, there was on average 0.0136 ± 0.0010 mg of PO₄-P consumed for every 1 mg of algal growth; the algae grown on SWW L consisted of approximately 1.36 ± 0.10 % phosphorus by dry weight. This percentage was found by taking the mg phosphorus removed from the medium of each sample divided by the final weight of each sample, equation 3-2; the results were then averaged ± standard deviation. The nitrogen concentration in the algae was found using the same method, but substituting in NH₃-N values in place of phosphorus values. Cladophora glomerata grown in SWW L consisted of 6.56 ± 0.38% nitrogen by dry weight.

As another way to compare the data it is worth graphing each samples nutrient removal compared to the algal weight. Figure 4-10 is a scatterplot with the amount of PO₄ – P removed from the medium (the amount of phosphorus used by Cladophora glomerata) on the y-axis and the dry weight (relates directly to yield) on the x-axis. It was expected that there would be a linear relationship between the two and Figure 4-10
shows this is true for Trial 5. A linear regression line was calculated and the $R^2$ value was 0.95, indicating a strong linear relationship between phosphorus removal and algal weight; there was a consistent amount of phosphorus used for 1 mg of algal growth. The slope of the line, 0.0126, reveals that 0.0126 mg of phosphorus was used for every mg of algal growth (it estimates the algae contained 1.26% P by dry weight). Figure 4-11 displays the relationship between nitrogen removed from the wastewater (nitrogen used by the alga) and *Cladophora glomerata* dry weight. As with phosphorus, a linear relationship was predicted; this proved to be correct when a linear regression line was added with an $R^2$ value of 0.93, indicating a strong linear relationship. The slope of the line, 0.0644, indicates that 0.0644 mg of nitrogen was consumed for every 1 mg of algal growth; the algae contained 6.44% N by dry weight.

Figure 4-10: Trial 5 phosphorus removal and *Cladophora glomerata* weight
Figure 4-11: Trial 5 nitrogen removal and *Cladophora glomerata* weight

4.3.2 Trial 6

Table 4-6 summarizes phosphorus concentrations in trial 6 *Cladophora glomerata* by percent dry weight using equation 3-2. Tests on days 6 and 12 were conducted on two samples; the average of the two samples is given for those days. There is a decline in the concentration because the alga biomass was increasing in weight while most of the phosphorus in the BVWW medium had already been used up.
The nitrogen concentration was calculated using $3-2 \pm$ standard deviation. Tests on days 6 and 12 were conducted on two samples; the average of the two samples is given for those days. According to these results the nitrogen concentration was on average $4.26 \pm 0.99\%$ of the algae’s dry weight, more detailed information for each testing period can be seen in Table 4-7 below.

Table 4-7: Trial 6 nitrogen concentration in *Cladophora glomerata* by percent dry weight

<table>
<thead>
<tr>
<th>Day tested</th>
<th>Nitrogen concentration in % DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>2.83</td>
</tr>
<tr>
<td>12</td>
<td>4.65</td>
</tr>
<tr>
<td>18</td>
<td>$4.96 \pm 0.33%$</td>
</tr>
<tr>
<td>Average of 6-18</td>
<td>$4.26 \pm 0.99%$</td>
</tr>
</tbody>
</table>

The above concentrations of phosphorus and nitrogen are averages of each samples theoretical concentration based on equation 3-2. As another way to compare the data it is worth graphing each samples nutrient removal compared to the algal weight. Figure 4-12 shows the results for phosphorus removal and algal weight for each sample. As in Trial 5, there was expected to be a linear relationship between the two parameters, but this prediction was wrong. Figure 4-12 show that the data points to not fit well into a linear regression line, it results in a $R^2$ value of 0.05, signifying a weak linear
relationship. For Trial 6 it was determined that phosphorus did not directly affect the algal growth. Figure 4-13 shows the relationship between nitrogen removal and algal weight. Again, a linear relationship was expected and this time it was found. The linear regression line has an $R^2$ value of 0.93. The slope of this line, 0.0615, indicates that 0.0615 mg of nitrogen was consumed for every 1 mg of algal growth; the algae contained 6.15% N by dry weight.

![Trial 6 phosphorus removal and algal weight](image)

Figure 4-12: Trial 6 phosphorus removal and *Cladophora glomerata* weight
4.3.3 Results Summary

A summary of growth and nutrient results from trials 5 and 6 can be seen below in Table 4-8. These values will be the basis to evaluate the Cladophora glomerata treatment system since they were obtained using municipal wastewater.

Table 4-8: Summary of nutrients concentrations in Cladophora glomerata for trials 5 and 6

<table>
<thead>
<tr>
<th>Trial</th>
<th>Method</th>
<th>P concentration per unit dry weight</th>
<th>N concentration per unit dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Averages</td>
<td>1.36 ± 0.10%</td>
<td>6.56 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>Linear regression</td>
<td>1.26% (R²=0.95)</td>
<td>6.44% (R²=0.93)</td>
</tr>
<tr>
<td>6</td>
<td>Averages</td>
<td>0.27 ± 0.11</td>
<td>4.26 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>Linear regression</td>
<td>NA (R²=0.05)</td>
<td>6.15% (R²=0.93)</td>
</tr>
</tbody>
</table>
4.4 CHN Results

A CHN analysis was performed on samples grown in D11 medium, SWW L medium, and BVWW medium. The CHN results give the percent of each element (carbon, hydrogen, nitrogen) as a part of total dry weight. The results for the CHN analyses can be seen in Table 4-9. BVWW was tested for samples taken at 6 days, 12 days and 18 days.

Table 4-9: CHN results for *Cladophora glomerata*

<table>
<thead>
<tr>
<th>Medium and sample day</th>
<th>Carbon %</th>
<th>Hydrogen %</th>
<th>Nitrogen %</th>
</tr>
</thead>
<tbody>
<tr>
<td>D11 day 25</td>
<td>38.22 ± 1.34</td>
<td>6.40 ± 0.36</td>
<td>4.13 ± 0.19</td>
</tr>
<tr>
<td>SWW L day 18</td>
<td>39.83 ± 0.54</td>
<td>6.37 ± 0.08</td>
<td>6.58 ± 0.28</td>
</tr>
<tr>
<td>BVWW day 6</td>
<td>25.40 ± 0.60</td>
<td>2.86 ± 0.21</td>
<td>1.76 ± 0.10</td>
</tr>
<tr>
<td>BVWW day 12</td>
<td>29.48 ± 1.43</td>
<td>4.02 ± 0.41</td>
<td>1.61 ± 0.10</td>
</tr>
<tr>
<td>BVWW day 18</td>
<td>32.07 ± 0.33</td>
<td>4.60 ± 0.11</td>
<td>1.72 ± 0.11</td>
</tr>
</tbody>
</table>

The carbon results for the CHN analysis varied between the synthetic mediums and BVWW medium. Carbon percentage for *Cladophora glomerata* grown in D11 was 38.22 ± 1.34%, and for *Cladophora glomerata* grown in SWW L it was 39.83 ± 0.54. There was no significant difference between the carbon percentages, t-test p > 0.05. The carbon percentage for algae grown in BVWW medium was 32.07 ± 0.33% for samples tested on day 18. This sample had a carbon percentage between 4.5% and 8% less than the synthetic mediums. BVWW was tested after 6 days, 12 days and 18 days; the carbon percentages rise from day 6 to day 12. There was no statistical difference between the day 12 and day 18 samples, a t-test returned a p > 0.05.

The hydrogen results for the CHN analysis again varied between the synthetic mediums and BVWW medium. Hydrogen percentage for *Cladophora glomerata* grown
in D11 was 6.40 ± 0.36% and for *Cladophora glomerata* grown in SWW L it was 6.37 ± 0.08%. There was no significant difference between these two percentages, t-test p > 0.05. The hydrogen percentage for 18 day samples grown in BVWW was 4.60 ± 0.11%. This was between 1.33% and 2.27% less than algae from the synthetic mediums; it also proved to statistically differ from the synthetic samples, t-test p < 0.05. BVWW was tested after 6 days, 12 days and 18 days; the hydrogen percentages rise from day 6 to day 12. There was no statistical difference between the day 12 and day 18 samples, a t-test returned a p > 0.05.

The CHN results give a direct percent of nitrogen contained in *Cladophora glomerata*. These values varied greatly between the algae grown in different mediums. *Cladophora glomerata* grown in D11 contained 4.13 ± 0.19% nitrogen, grown in SWW L it contained 6.58 ± 0.28% nitrogen and *Cladophora glomerata* grown in BVWW contained 1.72 ± 0.11% nitrogen at day 18. A t-test showed that all these values were statistically different, p < 0.05. T-tests also proved that there was no significant statistical difference between any of the BVWW nitrogen percentages for day 6-18.

Results obtained from section 4.3 can be directly compared to these CHN to check accuracy; CHN analysis is considered more accurate than the nutrient test findings because of the error associated with the methods used (this is a third method to find the percent nitrogen concentration in *Cladophora glomerata*). From nutrient tests it was found that algae grown in SWW L contained 6.56 ± 0.19 %, this value was very close to the CHN result of 6.58 ± 0.28%. A t-test comparing the two data sets returned a value of p = 0.37, indicating that the two results were not significantly statistically different. Results from nutrient tests found that algae grown in BVWW contained 4.26 ± 0.99%
nitrogen, this value was far from the 1.69 ± 0.12 average found by CHN analysis. A t-test proved that the two data sets were statistically different with a p < 0.05.

The CHN results can be used to predict methane production using equation 3-3. These values can be seen in Table 4-10. *Cladophora glomerata* biomass grown in D11 and SWW L appears to have a higher potential methane yield than *Cladophora glomerata* biomass grown in BVWW. The theoretical yields for D11 and SWW L are 0.631 ± 0.024 and 0.663 ± 0.008 L CH₄/g algae. There is no significant difference between the two yields, t-test returns a p > 0.05 when comparing the two. Predicted methane yield from BVWW is lower, 0.493 ± 0.008 L CH₄/g algae. This is 18 -28% lower than the D11 and SWW L yields. It is important to remember that these predictions do not take into account other elements necessary to predict entire methane yield using equation 3-3. The amount of oxygen and sulfur present in the samples in unknown.

<table>
<thead>
<tr>
<th>Growth medium</th>
<th>Methane yield, liters per gram algal biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>D11</td>
<td>0.631 ± 0.024</td>
</tr>
<tr>
<td>SWW L</td>
<td>0.663 ± 0.008</td>
</tr>
<tr>
<td>BVWW 18 days</td>
<td>0.493 ± 0.008</td>
</tr>
</tbody>
</table>

CHN tests were run on trial 6 samples from day 6, day 12, and day 18. These results can be seen in Table 4-11. The methane yield increases from day 6 to day 12, from 0.369 ± 0.013 to 0.447 ± 0.028 L CH₄/g algae respectively. There was no significant statistical difference between the predicted methane yields at day 12 and day 18, t-test returns a p > 0.05 when comparing the two data sets.
Table 4-11: Methane production using equation 3-3 from *Cladophora glomerata* grown in BVWW

<table>
<thead>
<tr>
<th>Growth medium</th>
<th>Methane yield, liters per gram algal biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVWW 6</td>
<td>0.369 ± 0.013</td>
</tr>
<tr>
<td>BVWW 12</td>
<td>0.447 ± 0.028</td>
</tr>
<tr>
<td>BVWW 18</td>
<td>0.493 ± 0.008</td>
</tr>
</tbody>
</table>
Chapter 5

Discussion

As mentioned in the methods sections, there were three main pieces of information that needed to be found in order to evaluate a *Cladophora glomerata* wastewater treatment system; a relevant *Cladophora glomerata* specific growth rate, the nitrogen and phosphorus removal efficiencies from wastewater *Cladophora glomerata*, growth, and the amount of methane that could be produced from anaerobic digestion of *Cladophora glomerata*. The following sections will focus on these data.

5.1 Specific growth rates

The first piece of information that was needed was a relevant specific growth rate for *Cladophora glomerata* grown in wastewater. The growth results obtained in this experiment were used to obtain this information. Figure 4-4 summarizes the algal specific growth rates obtained from each type of medium. As discussed in section 2.2.1.6 increased levels of nitrogen and phosphorus can be detrimental to *Cladophora* growth. The levels of phosphorus in SWW H were above what studies have shown to be detrimental (Robinson & Hawkes, 1986; Gerloff & Fitzgerald, 1976; Ensminger, Hagen, & Braune, 2000). Typical levels of nitrogen and phosphorus present in wastewater can be seen in Table 2-4 (4 mg/L P and 24 mg/L N). Levels of nutrients in the SWW H medium
were above what is typical for wastewater (see Table 3-5 for nutrient concentrations in SWW H); therefore, the nutrient concentrations in wastewater should not negatively affect growth rate. In the growth trails it was also predicted that the SWW mediums would not perform as well as the D11 medium, which held true; although, the D11 did not produced yields as high as have been seen in the literature (600 mg/L) (Gerloff & Fitzgerald, 1976). Gerloff & Fitzgerald (1976) showed that *Cladophora glomerata* benefits from many trace metals that were not present in the SWW mediums, this was one reason their yields and specific growth rates were not as high as D11. Therefore, for synthetic mediums the results were as expected, the D11 performed best, followed by SWW L and SWW M, and finally SWW H.

*Cladophora glomerata* grown in BVWW medium showed the highest specific growth rates out of all five media types; over an 18 day period the average specific growth rate was $0.182 \pm 0.002 \text{ day}^{-1}$. This growth rate was similar to what was found by Gerloff & Fitzgerald (1976) in their batch trials, they routinely achieved specific growth rates of $0.20 - 0.21 \text{ day}^{-1}$. There were likely unknown factors that are difficult to characterize associated with real wastewater effluent that promoted growth over a synthetic medium; it was impossible to identify and quantify all constituents in the BVWW medium. The BVWW 18 day specific growth rate will be used to evaluate the *Cladophora glomerata* wastewater treatment system because it represents a growth rate in real wastewater. In order to use the BVWW 18 day average specific growth rate of $0.182 \text{ day}^{-1}$ to the value must be scaled to account for the 24 hour growth period used in the trial. The $0.182 \text{ day}^{-1}$ specific growth rate will decreased by 37%, an average between the two values found in literature; therefore, a specific growth rate of $0.115 \text{ day}^{-1}$.
Figure 4-5 shows how the average specific growth rate of *Cladophora glomerata* in BVWW medium falls over time; this may be due to the batch culture system used for this experiment. In a continuous culture system, like those employed in wastewater treatment systems, cultures are better able to maintain specific growth rates close to the maximum growth rate (Barsanti & Gualtieri, 2006). For this reason, the higher specific growth rate found for days 0 – 12 (0.252 day\(^{-1}\)) will be used as an upper limit for the system. When this value is decreased by 37% to account for the photoperiod changed, a value of 0.159 day\(^{-1}\) is obtained, this will be the number used.

5.2  **Nutrients**

The second essential piece of information needed to evaluate a *Cladophora glomerata* wastewater treatment system is the removal efficiency of the process. To find the removal efficiency of the real world system a mass balance is set up. A mass of nutrients will enter the system (based on flow and concentration) and a mass of nutrient will be taken out of the wastewater by *Cladophora glomerata*. If the mass of nutrients taken out of the wastewater is known, the removal efficiency of the system can also be found. To calculate the mass of nutrients removed from the wastewater, the tissue concentration of nitrogen and phosphorus present in an algal biomass is needed. These concentrations were found by mass balance through colorimetric testing and directly through CHN analysis. A higher nutrient tissue concentration would indicate that less growth would be needed to remove nutrients, resulting in a smaller system. The colorimetric tests measured the initial concentration of nitrogen and phosphorus in a sample and then the concentrations after algal growth, the difference between the two numbers was considered the amount removed by *Cladophora glomerata*; this value
divided by the algae weight gives projected tissue nitrogen and phosphorus concentrations. A summary of these results can be seen in Table 5-1.

Table 5-1: Summary of projected nutrient concentrations in *Cladophora glomerata* wastewater growth trials, all methods

<table>
<thead>
<tr>
<th>Medium</th>
<th>Method</th>
<th>P concentration per unit dry weight</th>
<th>N concentration per unit dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Averages</td>
<td>1.36 ± 0.10 %</td>
<td>6.56 ± 0.38 %</td>
</tr>
<tr>
<td></td>
<td>Linear regression</td>
<td>1.26 % (R2=0.95)</td>
<td>6.44 % (R2=0.93)</td>
</tr>
<tr>
<td></td>
<td>CHN</td>
<td>NA</td>
<td>6.58 ± 0.28 %</td>
</tr>
<tr>
<td>SWW L</td>
<td>Averages</td>
<td>0.27 ± 0.11 %</td>
<td>4.26 ± 0.99 %</td>
</tr>
<tr>
<td></td>
<td>Linear regression</td>
<td>NA</td>
<td>6.15 % (R2=0.93)</td>
</tr>
<tr>
<td></td>
<td>CHN</td>
<td>NA</td>
<td>1.72 ± 0.11%</td>
</tr>
<tr>
<td>BVWW</td>
<td>Averages</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Linear regression</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHN</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The nitrogen concentrations in *Cladophora glomerata* grown in SWW L were higher than what by Gerloff & Fitzgerald, 1976 and Lorenz & Herdendorf, 1984 found. They reported nitrogen concentrations in *Cladophora* to be 0.83 – 5.15% (see section 2.2.1.6); concentrations found in this study were 6.18 – 6.94% for algae grown in SWW L. All three methods used in this study gave similar results for SWW L; but as stated previously, the CHN analysis is considered to be a more accurate method, a value of 6.58 ± 0.28%. The nitrogen concentrations from BVWW grown *Cladophora glomerata* vary between the CHN analysis and the other methods used. The CHN analysis indicated that the *Cladophora glomerata* samples contained 1.72 ± 0.11% nitrogen, while results from colorimetric testing showed that the samples contained 4.26 ± 0.99%, these results did not agree. Since the CHN analysis results were directly obtained from a reliable method, there could have been an error in calculating the nitrogen concentration from colorimetric testing. Errors associated with the nitrogen colorimetric testing could include many
factors. Some of the methods are technique sensitive, shaking time and vigor affect results. Other chemicals such as chlorine, ferric iron, nitrite (in nitrate test) can interfere with results (Hach Company, 2007). Also, nitrogen in the samples may have been converted to a chemical form that was not accounted for or measured in this study. Also, all the nitrogen being removed from the medium might not have been integrated into *Cladophora glomerata*. The BVWW medium was not filtered or disinfected before use; bacteria and/or other organisms present in the sample may have used or converted some of the nitrogen to other forms that were not measured. They could have incorporated nitrogen into their own cellular structure or converted it into nitrogen gas.

The nitrogen concentration per unit dry weight value used to evaluate the *Cladophora glomerata* treatment system will be 4.15%, an average of the SWW L samples CHN results and the BVWW samples CHN results. The BVWW results were meant to representative *Cladophora glomerata* grown in real wastewater effluent; but effluent levels (6.3 mg/L N) were lower than what is considered normal for a municipal wastewater treatment plant. The SWW L had higher levels of nitrogen, and therefore might produce *Cladophora glomerata* with nitrogen levels close what might be found in a real wastewater treatment plant; that is shy the value used will be an average of the two values. The average algal tissue nitrogen concentration of 4.15% also agrees with what Gerloff & Fitzgerald, 1976 and Lorenz & Herdendorf, 1984 found, 0.83 – 5.15%.

The phosphorus concentrations in SWW L grown *Cladophora glomerata* samples were higher than values found by Gerloff & Fitzgerald, 1976 and Lorenz & Herdendorf, 1984. Concentration values in this study were 1.26 – 1.52% P while Gerloff & Fitzgerald reported phosphorus concentrations in *Cladophora* to be 0.04 - 0.54% P and Lorenz &
Herdendorf, 1984 reported values of 0.08 – 0.81% P. The BVWW medium produced algae with much lower concentrations of phosphorus than the SWW L. It was found that the algae contained 0.27 ± 0.11 % phosphorus. These low concentrations may be attributed to the fact that phosphorus was nearly depleted after 6 days. After these 6 days, the algae still continued to grow, lowering the amount of phosphorus per unit algal weight. Even though the values are much lower than what was found in SWW L grown algae, the values agree with the literature range range of 0.04% - 0.81 % phosphorus (Gerloff & Fitzgerald, 1976; Lorenz & Herdendorf, 1984).

The phosphorus concentration per unit dry weight value used to evaluate the Cladophora glomerata treatment system will be 1.36%, the concentration found in the SWW L samples by the “averages” method. This value was chosen for a couple reasons. First of all, the SWW L trials represent growth where orthophosphate was in abundant supply, the BVWW samples had a depleted orthophosphate supply after 6 days. The proposed system will be designed in a way that orthophosphate levels will stay above 0.0 for the vast majority of the growth period. The averages value for nitrogen in SWW L almost identically matched the results CHN nitrogen results. Since the “averages” method results (6.56 ± 0.38%) more closely resembled the CHN results (6.58 ± 0.28%) than the “linear regression” nitrogen value (6.44%), the averages method is considered more accurate. Since the averages method is more accurate than the linear regression method for nitrogen; then it may be assumed that the average method is more accurate than the linear regression method for phosphorus concentration also, 1.36 ± 0.10% vs. 1.26% respectively.
5.3 Methane yield

The third and final piece of information needed to evaluate a *Cladophora glomerata* wastewater treatment system was the methane production resulting from the algal biomass. The algae grown will be processed through anaerobic digestion, and the methane yield will provide an additional benefit to the wastewater treatment system. The CHN results provide a theoretical methane yield from each medium using equation 3-3. These yields can be seen in Table 4-10. There was no significant difference between the D11 and SWW L yields (0.631 ± 0.024 and 0.663 ± 0.008 respectively), but the BVWW was lower (0.493 ± 0.008). The CHN results in Table 4-9 showed that the BVWW day 18 samples had lower carbon, hydrogen and nitrogen content than the D11 and SWW L samples, this is the reason for the lower methane yield; equation 3-3 calculates the yield directly from those percentages. Although the CHN values for BVWW were lower than the D11 and SWW L (Table 4-9) they were not far off from values found in literature. The carbon percentage of 32.07 ± 0.33% C falls within the values given by Gottumukala, 2010 (32.11 – 37.72% C) and Lorenz & Herdendorf, 1984 (13.7 – 40.5% C). The hydrogen percentage 4.60 ± 0.11% H is slightly higher than what Gottumukala, 2010 found, 4.66 – 5.63% H. The Nitrogen percentage agrees with Lorenz & Herdendorf, 1984 (1.13 – 5.14% N) but is lower than what Gottumukala, 2010 found, 2.65 – 4.04% N. Carbon hydrogen and nitrogen values for D11 and SWW L *Cladophora glomerata* samples were all higher than what Gottumukala, 2010 found, but the carbon percentage agreed with Lorenz & Herdendorf, 1984. Table 4-11 shows that methane yield from BVWW grown algae increases from day 6 to day 12 by approximately 21%. The increase from day 12 to 18 is not statistically significant. This data suggest that a shorter growth
period produces algae with a lower methane yield. From this data is it concluded that the
growth period for the *Cladophora glomerata* treatment system should not be less than 12
days.

A theoretical methane yield per gram of dried *Cladophora glomerata* value that
can be taken from CHN results and equation 3-3 and used for comparison is 0.578 L
CH$_4$/g DW algae. This is an average between the SWW L grown algae and the BVWW
grown algae theoretical methane yields from CHN analysis. The BVWW value was not
used by itself because it is not known what kind of affect the limited phosphorus had on
the composition, and the SWW L value was not used by itself because it was a synthetic
medium. This yield agrees with the theoretical methane yield obtained from the CHN
analysis on *Cladophora glomerata* done by Gottumukala, 2010.

Equation 3-3 assumes a full conversion of biomass to methane and this is
typically not true. A range of methane yields will be used to evaluate the *Cladophora
glomerata* algal treatment system, 0.289 – 0.578 L CH$_4$/g DW algae. The value 0.289 L
CH$_4$/g DW algae is a 50% efficiency conversion of the 0.578 L CH$_4$/g DW algae yield.
Chapter 6

Design of the Proposed *Cladophora glomerata* Treatment System

The relevant parameters needed to design the *Cladophora glomerata* wastewater treatment system are listed in Table 6-1.

Table 6-1: Design parameters for proposed *Cladophora glomerata* treatment system

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent orthophosphate</td>
<td>4.0 mg/L</td>
</tr>
<tr>
<td>Effluent orthophosphate</td>
<td>&lt; 0.01 mg/L</td>
</tr>
<tr>
<td>Influent inorganic nitrogen</td>
<td>24 mg/L</td>
</tr>
<tr>
<td>Effluent inorganic nitrogen</td>
<td>10 mg/L</td>
</tr>
<tr>
<td>Algal Density</td>
<td>600 g DW/m² max</td>
</tr>
<tr>
<td>Growth period</td>
<td>12 – 16 days</td>
</tr>
<tr>
<td><em>Cladophora glomerata</em> specific growth rate</td>
<td>0.115 - 0.159 day⁻¹</td>
</tr>
<tr>
<td><em>Cladophora glomerata</em> %N</td>
<td>4.15%</td>
</tr>
<tr>
<td><em>Cladophora glomerata</em> %P</td>
<td>1.36%</td>
</tr>
<tr>
<td><em>Cladophora glomerata</em> methane yield</td>
<td>0.0289 - 0.578 L CH₄/g DW algae</td>
</tr>
<tr>
<td>Tank depth</td>
<td>1.0 m</td>
</tr>
</tbody>
</table>

The influent concentrations of nitrogen and phosphorus were taken from Table 2-4, the composition of a typical municipal wastewater (Hammer & Hammer, 2008). The high influent of 24 mg/L inorganic nitrogen will require more algal growth to remove than the 4.0 mg/L orthophosphate; this is because of the *Cladophora glomerata*’s
composition, 4.15% nitrogen and 1.36% phosphorus. Phosphorus will be depleted before the nitrogen; approximately 100% removal of phosphorus by algal growth would only account for 50% of the nitrogen. Nitrogen will be removed to a concentration of 10 mg/L; with this amount of nitrogen removal virtually all the phosphorus in the system should be removed.

An algal density maximum of 600 g DW/m² was used to design the system. This value was the maximum average value of bed densities of in eastern Lake Erie from the years 1979 through 2006 (see section 2.1) (Higgens, et al., 2008). Growth periods of 16 and 25 days were used for the growth rates of 0.18 and 0.25 respectively. These values were found by assuming that when a section of algae was harvested it would be cut back to 100 g DW/m²; the amount of time this 100 g DW/m² would take to grow back to 600 g DW/m² was the growth period. Reasoning behind the *Cladophora glomerata* specific growth rates, %N, %P, and methane yield can be seen in Chapter 4. Results from growth trials also show that the biomass will likely consist of 15 -25% solids.

Table 6-2 shows the tank design for the *Cladophora glomerata* treatment system with a specific growth rate of 0.115 day⁻¹.

<table>
<thead>
<tr>
<th>Flow MGD</th>
<th>Area Required</th>
<th>Hydraulic retention time, days</th>
<th>Growth period, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m²</td>
<td>Acres</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>4340</td>
<td>1.07</td>
<td>11.47</td>
</tr>
<tr>
<td>1</td>
<td>43404</td>
<td>10.73</td>
<td>11.47</td>
</tr>
<tr>
<td>10</td>
<td>434044</td>
<td>107.25</td>
<td>11.47</td>
</tr>
<tr>
<td>20</td>
<td>868088</td>
<td>214.50</td>
<td>11.47</td>
</tr>
<tr>
<td>50</td>
<td>2170220</td>
<td>536.26</td>
<td>11.47</td>
</tr>
</tbody>
</table>
The tank dimensions were designed as follows. Since nitrogen values determine the amount of algal growth needed, nitrogen values were used to design the tank. With the given influent concentrations of nitrogen and phosphorus, *Cladophora glomerata* will deplete phosphorus before nitrogen levels are below the desired 10 mg/L; therefore, the tank must be sized according to nitrogen removal. A larger tank will be required to achieve effluent of 10mg/L than to achieve 100% phosphorus removal. An influent concentration of 24 mg/L nitrogen will have to be reduced by 14 mg/L nitrogen in order to meet 10 mg/L nitrogen effluent. The average weight of algae harvested each day was calculated using Table A-1 and A-2 in Appendix A.

Table 6-3 shows the tank design for the *Cladophora glomerata* treatment system with a specific growth rate 0.159 day\(^{-1}\). The increase in specific growth rate resulted in a smaller tank area required, and a shorter hydraulic retention time. An increased specific growth rate also meant that the algae would be harvested more frequently; the growth period was 12 days.

<table>
<thead>
<tr>
<th>Flow MGD</th>
<th>Area Required</th>
<th>Hydraulic retention time, days</th>
<th>Growth period, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m(^2)</td>
<td>acres</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>3143</td>
<td>0.78</td>
<td>8.30</td>
</tr>
<tr>
<td>1</td>
<td>31431</td>
<td>7.77</td>
<td>8.30</td>
</tr>
<tr>
<td>10</td>
<td>314312</td>
<td>77.67</td>
<td>8.30</td>
</tr>
<tr>
<td>20</td>
<td>628623</td>
<td>155.33</td>
<td>8.30</td>
</tr>
<tr>
<td>50</td>
<td>1571558</td>
<td>388.33</td>
<td>8.30</td>
</tr>
</tbody>
</table>

Both tank designs produce the same average weight of dry algae a day. Table 6-4 below shows the average for each flow examined. This dry weight will be the algae sent
to anaerobic digestion. The volume of the harvested biomass was calculated based on an average 15% solids concentration *Cladophora glomerata* that was found after algal harvesting in growth trials. A typical wastewater treatment plant anaerobic digester is sized to accommodate and average of 5 cubic feet of sludge per capita served and an average person will use 120 gallons of water a day. This results in *Cladophora glomerata* taking up less than 1% of an anaerobic digesters design volume; meaning no additional digester space would be required to treat the algae.

Table 6-4: Average *Cladophora glomerata* dry weight harvested per day

<table>
<thead>
<tr>
<th>Flow MGD</th>
<th>kg DW algae produced per day</th>
<th>Volume of algae produced per day, L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>128</td>
<td>851</td>
</tr>
<tr>
<td>1</td>
<td>1277</td>
<td>8512</td>
</tr>
<tr>
<td>10</td>
<td>12769</td>
<td>85124</td>
</tr>
<tr>
<td>20</td>
<td>25537</td>
<td>170249</td>
</tr>
<tr>
<td>50</td>
<td>63843</td>
<td>425622</td>
</tr>
</tbody>
</table>

The mean daily productions of each system design are 29.5 g DW/m² and 40.7 g DW/m² for the systems with a grow rate of 0.115 day⁻¹ and 0.159 day⁻¹ respectively. The 0.115 day⁻¹ specific growth rate systems would achieve removal rates of 1.22 g nitrogen per m² per day and 0.40 g phosphorus per m² per day. The 0.159 day⁻¹ specific growth rate systems would achieve removal rates of 1.59 g nitrogen per m² per day and 0.55 g phosphorus per m² per day.
Chapter 7

Evaluation of Nutrient Removal Options

7.1 Costs

Cost comparisons between nutrient removal options can be difficult because each case should be considered individually. Many factors can affect the cost, such as local conditions, local costs, characteristics of the wastewater, temperature, and many other conditions (Linden, et al., 2001). Capital cost to add nutrient removal to an existing plant can vary widely and depend very much on the level of removal desired. Costs are estimated below for option 1: conventional nutrient removal, and option 2: *Cladophora glomerata* nutrient removal.

7.1.1 Option 1: Conventional nutrient removal

Table 7-1 below summarizes costs of adding nutrient removal to different capacity wastewater treatment plants, similar to the conventional nutrient removal option used in this study. These values were estimated using data from five different sources and updated to 2012 costs using the ENR Construction Cost Index (McGraw-Hill, 2012). Table 7-2 is another cost evaluation that can be used, showing the unit cost for total phosphorus (TP) removal.
Table 7-3 identifies the sources used in Table 7-1 and Table 7-2. The information obtained from source 1 (Jiang, et al., 2005) most represents a nutrient removal option similar to the one used in this study (option 1, conventional nutrient removal); therefore, the costs associated with this option will be used for evaluations. The influent nutrient concentrations associated with this option are 2.97 mg/L orthophosphate (6.34 mg/L total P) and 16.1 mg/L nitrogen. The target effluent concentration of phosphorus was 1.0 mg/L total P. Evaluations for 0.1 MGD treatment plants will use data from source 2, 3 and 5; source 1 did not have estimates for flows this low.

Table 7-1: Cost estimates for the addition of conventional nutrient removal to several different capacity wastewater treatment plants

<table>
<thead>
<tr>
<th>Flow, MGD</th>
<th>Cost</th>
<th>Source 1</th>
<th>Source 2</th>
<th>Source 3</th>
<th>Source 4</th>
<th>Source 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Capital O&amp;M/yr</td>
<td>$932,479</td>
<td>$1,293,524</td>
<td>$3,297</td>
<td>$1,429,339</td>
<td>$207,121</td>
</tr>
<tr>
<td></td>
<td>O&amp;M/yr</td>
<td>$172,231</td>
<td>$3,900,000</td>
<td>$32,970</td>
<td>$290,000</td>
<td>$10,800</td>
</tr>
<tr>
<td>1</td>
<td>Capital O&amp;M/yr</td>
<td>$3,011,407</td>
<td>$14,500,000</td>
<td>$329,700</td>
<td>$2,900,000</td>
<td>$108,000</td>
</tr>
<tr>
<td></td>
<td>O&amp;M/yr</td>
<td>$835,797</td>
<td>$16,000,000</td>
<td>$659,400</td>
<td>$5,800,000</td>
<td>$216,000</td>
</tr>
<tr>
<td>10</td>
<td>Capital O&amp;M/yr</td>
<td>$8,307,784</td>
<td>$34,000,000</td>
<td>$1,649,000</td>
<td>$14,500,000</td>
<td>$540,000</td>
</tr>
<tr>
<td></td>
<td>O&amp;M/yr</td>
<td>$3,513,308</td>
<td>$1,520,232</td>
<td>$43</td>
<td>$7</td>
<td>$38</td>
</tr>
</tbody>
</table>

Table 7-2: Unit cost estimates for the removal of total phosphorus (TP) from wastewater

<table>
<thead>
<tr>
<th>Flow, MGD</th>
<th>Unit cost for TP removal $/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source 1</td>
<td>Source 4</td>
</tr>
<tr>
<td>1</td>
<td>115</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>43</td>
</tr>
<tr>
<td>50</td>
<td>38</td>
</tr>
</tbody>
</table>
Table 7-3: Sources used in Table 7-1 and Table 7-2

<table>
<thead>
<tr>
<th>Identification</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source 1</td>
<td>Jiang, et al., 2005</td>
</tr>
<tr>
<td>Source 2</td>
<td>USEPA Office of Water, 2007</td>
</tr>
<tr>
<td>Source 3</td>
<td>Linden, et al., 2001</td>
</tr>
<tr>
<td>Source 4</td>
<td>CH2MHILL, 2010</td>
</tr>
<tr>
<td>Source 5</td>
<td>Foess, et al., 1998</td>
</tr>
</tbody>
</table>

7.1.2 Option 2: *Cladophora glomerata* nutrient removal

The average commercial price for natural gas in 2011 was $8.86 per thousand ft$^3$ of gas (eia, 2012). This number was used to value the *Cladophora glomerata* methane production from anaerobic digestion, the results can be seen in Table 7-4. The system was assessed for a low end methane yield and high end methane yield, the actual annual value of methane is predicted to fall somewhere in between.

Table 7-4: Annual value of methane gas production from *Cladophora glomerata*

<table>
<thead>
<tr>
<th>Flow MGD</th>
<th>Annual Value of methane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.289 L CH$_4$/g algae yield</td>
</tr>
<tr>
<td>0.1</td>
<td>$4,214</td>
</tr>
<tr>
<td>1</td>
<td>$42,143</td>
</tr>
<tr>
<td>10</td>
<td>$421,428</td>
</tr>
<tr>
<td>20</td>
<td>$842,855</td>
</tr>
<tr>
<td>50</td>
<td>$2,107,138</td>
</tr>
</tbody>
</table>

A summary of construction costs for the algal treatment system concrete tanks can be seen in Table 7-5 and Table 7-6. These cost estimates were made using RS Means online estimating books with a tank length to width ratio of 2:1 (Reed Construction Data.
Inc. , 2012). It includes the estimated construction and materials cost of site grading, a stone aggregate base, and reinforced concrete tank.

Table 7-5: *Cladophora glomerata* concrete treatment tank costs for a specific growth rate of 0.115 day^{-1}

<table>
<thead>
<tr>
<th>Flow MGD</th>
<th>Tank construction cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>$668,274</td>
</tr>
<tr>
<td>1</td>
<td>$3,626,516</td>
</tr>
<tr>
<td>10</td>
<td>$26,600,519</td>
</tr>
<tr>
<td>20</td>
<td>$50,582,773</td>
</tr>
<tr>
<td>50</td>
<td>$120,648,764</td>
</tr>
</tbody>
</table>

Table 7-6: *Cladophora glomerata* concrete treatment tank costs for a specific growth rate of 0.159 day^{-1}

<table>
<thead>
<tr>
<th>Flow MGD</th>
<th>Tank construction cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>$540,614</td>
</tr>
<tr>
<td>1</td>
<td>$2,805,384</td>
</tr>
<tr>
<td>10</td>
<td>$19,829,534</td>
</tr>
<tr>
<td>20</td>
<td>$37,431,006</td>
</tr>
<tr>
<td>50</td>
<td>$88,634,948</td>
</tr>
</tbody>
</table>

Along with the tanks, other costs to consider for the *Cladophora glomerata* were harvesting equipment costs, mechanical aerator costs, and operation and maintenance costs. Table 7-7 below summarizes these costs along with the concrete tank cost to give an estimated total cost and operation and maintenance cost. Systems designed with a *Cladophora glomerata* specific growth rate of 0.115 day^{-1} are the high end of the costs range, and systems designed with a growth rate of 0.159 day^{-1} are on the low end of the price range. (Design flows over 10 MGD were eliminated from this table, see cost discussions section for details).
Table 7-7: Estimated Cost of proposed *Cladophora glomerata* treatment systems by flow

<table>
<thead>
<tr>
<th>Flow MGD</th>
<th>Total Cost</th>
<th>Operation and Maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>$638,000 - $826,000</td>
<td>$16,000 - $22,000</td>
</tr>
<tr>
<td>1</td>
<td>$3,310,000 - $4,601,000</td>
<td>$83,000 - $123,000</td>
</tr>
<tr>
<td>10</td>
<td>$23,399,000 - $34,590,000</td>
<td>$585,000 - $949,000</td>
</tr>
</tbody>
</table>

An example breakdown of all costs calculated for a *Cladophora glomerata* treatment system addition to a 0.1 MGD flow wastewater treatment plant can be seen in Table 7-8. Harvesting equipment costs were derived from the cost of sedimentation tank mechanical equipment; typically mechanical scrapers for rectangular tanks. The equipment was sized to fit one 20 ft. wide harvester for the small flow tank, 0.1 MGD. This cost was compared to the tank cost to obtain a percentage of the tank cost (approximately 18% of the tank construction costs). This cost percentage was applied to the 1 MGD tank and the 10 MGD tank to get their harvester equipment costs. A variable cost was determined for aerators, it was unknown how many would be needed for each size system. An upper cost estimate was determined from RSMeans data and actual product data; this value accounted for between 4.5% - 9% of the total high end costs (Reed Construction Data Inc., 2012; Siemens, 2009). Operations and maintenance costs were estimated from the operation and maintenance costs associated with sedimentations basins, and the power consumption of proposed aerators.
Table 7-8: Costs breakdown of the proposed algal treatment system for a 0.1 MGD flow wastewater plant

<table>
<thead>
<tr>
<th></th>
<th>Cost (low end)</th>
<th>Costs (high end)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concrete and Reinforcing</td>
<td>$497,388</td>
<td>$608,583</td>
</tr>
<tr>
<td>Site Grading</td>
<td>$11,276</td>
<td>$15,572</td>
</tr>
<tr>
<td>Stone Base</td>
<td>$31,949</td>
<td>$44,120</td>
</tr>
<tr>
<td>Harvesting Equipment</td>
<td>$97,310</td>
<td>$120,289</td>
</tr>
<tr>
<td>Aerators</td>
<td>$0</td>
<td>$37,400</td>
</tr>
<tr>
<td>Tank O&amp;M</td>
<td>$15,948</td>
<td>$19,714</td>
</tr>
<tr>
<td>Aerator O&amp;M</td>
<td>$0</td>
<td>$1,916</td>
</tr>
<tr>
<td>Methane Payback</td>
<td>$4,214</td>
<td>$8,429</td>
</tr>
</tbody>
</table>

7.1.3 Cost discussion

A cost comparison was made between wastewater treatment option 1: conventional nutrient removal, and option 2: algal system nutrient removal. Costs are only approximations and could vary from what is stated; this comparison is meant to evaluate the feasibility of a *Cladophora glomerata* algal treatment system not to obtain exact pricing information. Table 7-9 summarizes the costs estimate comparison between option 1 and option 2.

Table 7-9: Cost estimate comparison, wastewater treatment option 1 to wastewater treatment option 2

<table>
<thead>
<tr>
<th>Flow, MGD</th>
<th>Cost 2012$</th>
<th>Option 1: Conventional</th>
<th>Option 2: <em>Cladophora glomerata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Capital Cost O&amp;M /yr</td>
<td>$1293,524 - $1,426,339</td>
<td>$638,000 - $826,000</td>
</tr>
<tr>
<td></td>
<td>O&amp;M /yr</td>
<td>$3,297 - $207,000</td>
<td>$16,000 - $22,000</td>
</tr>
<tr>
<td>1</td>
<td>Capital Cost O&amp;M /yr</td>
<td>$932,479</td>
<td>$3,310,000 - $4,601,000</td>
</tr>
<tr>
<td></td>
<td>O&amp;M /yr</td>
<td>$172,231</td>
<td>$83,000 - $123,000</td>
</tr>
<tr>
<td>10</td>
<td>Capital Cost O&amp;M /yr</td>
<td>$3,011,407</td>
<td>$23,399,000 - $34,590,000</td>
</tr>
<tr>
<td></td>
<td>O&amp;M /yr</td>
<td>$835,797</td>
<td>$585,000 - $949,000</td>
</tr>
<tr>
<td>20</td>
<td>Capital Cost O&amp;M /yr</td>
<td>$4,569,942</td>
<td>&gt; $37,000,000 - &gt; $51,000,000</td>
</tr>
<tr>
<td></td>
<td>O&amp;M /yr</td>
<td>$1,520,232</td>
<td>&gt; $89,000,000 - &gt; $120,000,000</td>
</tr>
<tr>
<td>50</td>
<td>Capital Cost O&amp;M /yr</td>
<td>$8,307,784</td>
<td>&gt; $89,000,000 - &gt; $120,000,000</td>
</tr>
<tr>
<td></td>
<td>O&amp;M /yr</td>
<td>$3,513,308</td>
<td></td>
</tr>
</tbody>
</table>
During costs estimation calculations it was decided that the algal treatment system was not going to be feasible for implementation at wastewater plants with more than 10 MGD flow. The area required for construction and cost of tanks are both too high (see Table 6-2, Table 6-3, Table 7-5, and Table 7-6). Therefore, costs above tank construction were not calculated.

Methane production from the Cladophora glomerata biomass created in option 2 was valued in Table 7-4. The value of methane gas could potentially cover 19% - 53% of O&M costs for a plant flow of 0.1 MGD, 34% - 102% for a plant flow of 1 MGD, and 44% - 144% of O&M costs for a plant flow of 10MGD. There is a wide range of predicted costs and yields, but the 1 MGD and 10MGD plant could potentially make back money each year if the conditions are right.

From Table 7-9 and the methane gas payback in Table 7-4, Table 7-10 was created; the estimated costs of each option over a 25 year design life. From a financial standpoint, a Cladophora glomerata algal treatment system could be a viable nutrient removal option for small wastewater treatment plants with flows of 0.1 MGD. The algal treatment system could also be a potential option for plants with a flow of 1.0MGD, the 25 year estimated cost is close to the 25 year estimated cost of a conventional system. For a flow of 10MGD the data is too varied for the algal system to make any conclusions. The low value of its predicted cost ($17,000,000) is similar to what conventional treatment is estimated to cost (23,900,000), but the high value is much larger ($47,000,000). Twenty-five year costs used an inflation and discount rate of 0.0%
Table 7-10: 25 year costs of wastewater nutrient removal option 1 and option 1

<table>
<thead>
<tr>
<th>Flow MGD</th>
<th>25 year costs, option 1: Conventional</th>
<th>25 year costs, option 2: Cladophora glomerata</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>$1,380,000 - $6,600,000</td>
<td>$827,000 - $1,270,000</td>
</tr>
<tr>
<td>1</td>
<td>$5,240,000</td>
<td>$3,280,000 - $6,620,000</td>
</tr>
<tr>
<td>10</td>
<td>$23,900,000</td>
<td>$17,000,000 - $47,800,000</td>
</tr>
</tbody>
</table>
Chapter 8

Conclusions and Future Work

Eutrophication of natural waters is a problem throughout the world and locally in Lake Erie. Nutrients can lead to excess growths of algae and other aquatic plants, loss of component species, and loss of ecosystem function (Bennett, et al., 2001; Aslan, 2006). In order to stop eutrophication, nitrogen and phosphorus levels entering natural waters must be decreased. For this reason, much research and effort has been focused on nitrogen and phosphorus removal systems for wastewater treatment plants. The results of this study indicate that the proposed Cladophora glomerata nutrient removal system could increase resource efficiency for wastewater treatment plants with flows up to 1.0 MGD. The system could be used as a viable nutrient removal tool. For larger facilities, the estimated initial capital costs of the algal treatment system and associated land requirements are far too significant. A Cladophora glomerata algal treatment system utilizing shallow earthen ponds instead of concrete basins could be evaluated in the future; this could potentially lower the initial capital costs of the algal treatment system.

In addition, Cladophora glomerata treatment system could potentially be used as tertiary treatment for a wastewater treatment plant that wanted to remove nutrients to a very low concentration. It is hypothesized that focusing on removing nutrients from
wastewater treatment plants could be much more effective than limiting non-point source inputs. However, removing phosphorus from wastewater using more conventional means is exponentially more expensive as effluent concentrations necessitate near 100% removal. If a plant wanted to remove orthophosphate to very low effluent levels, the algal treatment system could be a good option; even if it was placed after conventional phosphorus removal. During growth trials *Cladophora glomerata* was able to remove orthophophorus to levels below detection (< 0.1mg/L P). Adding an algal treatment system after conventional nutrient removal to remove orthophosphorus to low levels could be much more cost effective than upgrading the conventional system to reach these low levels. Future work should include growth trials with wastewater effluent from plants already using conventional nutrient removal systems to evaluate *Cladophora glomerata* as a supplementary nutrient removal tool.

Future work on using *Cladophora glomerata* in smaller scale wastewater treatment plants should include growth trials with wastewater effluent from other wastewater treatment plants to obtain a better range of growth rates and nutrient removal rates. It is also necessary to determine how the *Cladophora glomerata* might react to harvesting. It would be helpful to construct a small scale flow through system evaluating growth rates, nutrient removal rates, and harvesting effects. In addition, the ability of *Cladophora glomerata* to maintain dominance in a small scale system should be evaluated. If proven infeasible with a single macroalgeae, it might be necessary to operate a system with a mixed collection of algae, macroalgae and microalgae. Some studies suggest that these systems could be easier to culture and maintain (Hoffmann, 1998).
References


Lake Erie Millennium Network Synthesis Team. (2011). *LAKE ERIE NUTRIENT LOADING AND HARMFUL ALGAL BLOOMS: Research Findings and.* Columbus, OH: Made available by Ohio Sea Grant College Program.


Table A-1: Biomass density gDW/m$^2$ in *Cladophora glomerata*, 0.115 day$^{-1}$ specific growth rate

<table>
<thead>
<tr>
<th>Day</th>
<th>Tank Section biomass, gDW/m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.00</td>
</tr>
<tr>
<td>1</td>
<td>111.50</td>
</tr>
<tr>
<td>2</td>
<td>124.32</td>
</tr>
<tr>
<td>3</td>
<td>138.62</td>
</tr>
<tr>
<td>4</td>
<td>154.56</td>
</tr>
<tr>
<td>5</td>
<td>172.34</td>
</tr>
<tr>
<td>6</td>
<td>192.15</td>
</tr>
<tr>
<td>7</td>
<td>214.25</td>
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<tr>
<td>8</td>
<td>238.89</td>
</tr>
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<td>9</td>
<td>266.36</td>
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<td>296.99</td>
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<td>11</td>
<td>331.15</td>
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<td>12</td>
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<td>411.69</td>
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<td>14</td>
<td>459.04</td>
</tr>
<tr>
<td>15</td>
<td>511.83</td>
</tr>
<tr>
<td>16</td>
<td>570.69</td>
</tr>
</tbody>
</table>
Table A-2: Biomass density gDW/m² in *Cladophora glomerata*, 0.159 day⁻¹ specific growth rate

<table>
<thead>
<tr>
<th>Day</th>
<th>Tank Section biomass, gDW/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.00</td>
</tr>
<tr>
<td>1</td>
<td>115.90</td>
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<td>2</td>
<td>134.33</td>
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<td>3</td>
<td>155.69</td>
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<td>4</td>
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<td>209.13</td>
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<td>7</td>
<td>280.92</td>
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<td>8</td>
<td>325.59</td>
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<td>377.36</td>
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<td>437.36</td>
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<td>506.89</td>
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<td>587.49</td>
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</tbody>
</table>