A Proposed Treatment System for the Remediation of Landfill Leachate Using Algae

by
Shanna Grace Myers

A PROJECT

submitted to
Oregon State University
University Honors College

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the requirements for the
degree of

Honors Baccalaureate of Science in Environmental Engineering
(Honors Scholar)

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Abstract approved: ________________________________ ______________________

David Hackleman

Landfill leachate is a complex wastewater that contains high levels of ammonium (often over 250 ppm) and trace heavy metals (usually under 0.2 ppm). Microalgae have been shown to inexpensively and effectively treat wastewaters containing high loads of organics and nutrients, as well as adsorb metal ions. This thesis focuses on designing a treatment system for landfill leachate using a combination of algal and traditional water treatment methods for economical and reliable remediation of leachate. Scenedesmus dimorphus was grown in low dilutions of pretreated and untreated leachate to test the viability of this species for leachate treatment. Higher dilutions were not tested due to light limitation concerns. Pretreatment consisted of filtration through dried brown macroalgae Ascophyllum nodosum and distillation to reduce ammonia concentration. Nitrogen concentration and algal density were monitored using a Hach assay kit and hemocytometer cell counts, respectively. Nitrogen removal was expected in all samples, but assay results were inconclusive due to a lack of duplicate assays. S. dimorphus grew to over 5 times the original concentration within 5 days in dilutions of 5, 10, 15, and 20% untreated leachate in growth media. With adequate pretreatment and dilution, S. dimorphus shows potential for use in an algal bioreactor to treat landfill leachate.

Key Words: Bioremediation, Landfill Leachate, Algae

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APPROVED:

David Hackleman, Mentor, representing Chemical Engineering

Tyler Radniecki, Committee Member, representing Environmental Engineering

Kenneth Williamson, Committee Member, representing Clean Water Services

Toni Doolen, Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Shanna Myers, Author
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A Proposed Treatment System for the Remediation of Landfill Leachate Using Algae

Introduction

In 2012, Americans discarded approximately 135 million tons of solid waste in landfills (EPA, 2014). When liquid enters landfills and comes in contact with this solid waste, through percolation of rainfall or from existing liquid content in the landfilled wastes, leachate is formed. Landfill leachate is a toxic, foul-smelling mixture of organic and inorganic compounds; including pharmaceuticals, heavy metals, nutrients, and biocides. Landfill leachate varies in composition based on the age, annual rainfall, and accepted waste streams of its respective landfill (McBean & Rovers, 1999; Chen, 1996; Ziyang, et al., 2009). As of 2010, there were just over 1900 landfills in the U.S., and the number of landfills has been decreasing since 1988 when there were almost 8000 landfills (EPA, 2009). This trend of decreasing landfills has centralized the production of new, high-strength leachates, but landfills that have been closed still continue to produce leachate and must be monitored for a minimum of 30 years after their closure (EPA, 2012).

Treatment of landfill leachate is a multifaceted problem due to the complex makeup of leachate. While many treatments systems exist specifically to remove metals, nutrients, or xenobiotics from waste waters, there are fewer treatments systems that effectively remove all of these components from the same waste stream. Currently, common treatment methods for landfill leachate include discharge to a publicly owned treatment works (POTW), leachate recirculation, leachate evaporation, and treatment
with reverse osmosis (Johannessen, 1999; McBean & Rovers, 1999). Because of the variations in leachate composition, no single method works best for all landfills.

This paper outlines some of the existing strategies for dealing with contaminated waters, particularly strategies that utilize algal biomass, and proposes a synergistic method of treating waters containing multiple pollutants, as in the case of leachate. The economics and size requirements of such a treatment system are considered for a hypothetical landfill leachate with characteristics similar to leachate used in laboratory experiments.

**Background/Literature Review**

**Scope of the Problem**

Landfill leachate contains a mixture of toxic organics, metals, and nutrients (Table 1). This mixture, if untreated, exhibits acute and genetic toxicity (Brown, Schrab, & Donnelly, 1991). Notable chemicals in Table 1 are benzene, vinyl chloride, and chromium; all of which are well-known carcinogens (only hexavalent chromium is considered a carcinogen, but the chromium concentration in Table 1 is a combination of trivalent and hexavalent chromium concentrations).
Table 1

<table>
<thead>
<tr>
<th>Inorganic</th>
<th>Average Concentration</th>
<th>Units</th>
<th>Organic</th>
<th>Average Concentration</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>281 ppm</td>
<td></td>
<td>Acetone</td>
<td>2163 ppb</td>
<td></td>
</tr>
<tr>
<td>Antimony</td>
<td>4.52 ppm</td>
<td></td>
<td>Benzene</td>
<td>221 ppb</td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.0418 ppm</td>
<td></td>
<td>Chlorobenzene</td>
<td>128 ppb</td>
<td></td>
</tr>
<tr>
<td>Barium</td>
<td>0.8526 ppm</td>
<td></td>
<td>Chloroform</td>
<td>195 ppb</td>
<td></td>
</tr>
<tr>
<td>Beryllium</td>
<td>0.0056 ppm</td>
<td></td>
<td>p-Cresol</td>
<td>2394 ppb</td>
<td></td>
</tr>
<tr>
<td>BOD(^1)</td>
<td>3837 ppm</td>
<td></td>
<td>4,4 - DDT</td>
<td>0.1031 ppb</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.022 ppm</td>
<td></td>
<td>1,1 - Dichloroethane</td>
<td>1715 ppb</td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>0.1754 ppm</td>
<td></td>
<td>1,2 - Dichloroethane</td>
<td>1841 ppb</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>0.1679 ppm</td>
<td></td>
<td>cis 1,2 - DCE(^3)</td>
<td>330 ppb</td>
<td></td>
</tr>
<tr>
<td>Cyanide</td>
<td>0.0634 ppm</td>
<td></td>
<td>trans 1,2 - DCE(^3)</td>
<td>568 ppb</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>221 ppm</td>
<td></td>
<td>Ethyl benzene</td>
<td>274 ppb</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>0.1616 ppm</td>
<td></td>
<td>Isophorone</td>
<td>1168 ppb</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>9.59 ppm</td>
<td></td>
<td>Lindane</td>
<td>0.02 ppb</td>
<td></td>
</tr>
<tr>
<td>Mercury</td>
<td>0.002 ppm</td>
<td></td>
<td>Methylene chloride</td>
<td>5352 ppb</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>0.3255 ppm</td>
<td></td>
<td>Methyl ethyl ketone</td>
<td>4151 ppb</td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>1.88 ppm</td>
<td></td>
<td>Naphthalene</td>
<td>32.4 ppb</td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>2.67 ppm</td>
<td></td>
<td>Phenol</td>
<td>2456 ppb</td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>0.0119 ppm</td>
<td></td>
<td>Toluene</td>
<td>1016 ppb</td>
<td></td>
</tr>
<tr>
<td>Silver</td>
<td>0.0208 ppm</td>
<td></td>
<td>1,1,1 - Trichloroethane</td>
<td>887 ppb</td>
<td></td>
</tr>
<tr>
<td>Thallium</td>
<td>0.1753 ppm</td>
<td></td>
<td>1,1,2 - Trichloroethane</td>
<td>378 ppb</td>
<td></td>
</tr>
<tr>
<td>TDS(^2)</td>
<td>5691 ppm</td>
<td></td>
<td>Vinyl chloride</td>
<td>36.1 ppb</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>8.32 ppm</td>
<td></td>
<td>Xylenes</td>
<td>141 ppb</td>
<td></td>
</tr>
</tbody>
</table>

Notes: \(^1\)BOD stands for biological oxygen demand
\(^3\)DCE stands for dichloroethylene.
\(^2\)TDS stands for total dissolved solids

Leachate production in landfills is highly dependent upon rainfall, permeability of landfill covers, and the type of wastes received at each landfill (Chen, 1996; Qasim & Chiang, 1994). Because of this variability, each landfill will have different volumes and strengths of leachate. With no cover in place, landfills can generate 27,000 gallons of water per acre per inch of rainfall (Bolton, 2007). Average annual rainfall in the US is approximately 28 inches, so the average US landfill would generate just over 0.75 million gallons of leachate per acre of uncovered landfill per year (World Bank, 2014). However, subtitle D of the resource conservation and recovery act (RCRA) requires daily...
application of cover materials over landfilled wastes (Pohland & Graven, 1993; EPA, 2012). This daily cover is commonly 6” of compacted soil which keeps trash from blowing away, controls disease vectors, and minimizes odors. Daily cover also reduces landfill permeability by a small amount due to the compaction of the soil. Additionally, final covers for landfills are required to have a permeability of under $10^{-5}$ cm/s, so water infiltration into closed landfills is significantly reduced (EPA, 2012).

A major concern in leachate treatment is finances. One of the most common methods of leachate treatment is discharge to a POTW. However, POTWs must charge extra for leachate treatment. Surcharges for leachate treatment are highly variable, depending on the strength of the leachate and the capacity of the POTW to handle that leachate, and can range from $0.01 to over $1.00 per gallon (Paetsch & Macnab, 2014; McBean & Rovers, 1999; Duffy, 2005). In 2005, a typical POTW charged approximately $0.02/gallon for leachate treatment (Duffy, 2005). For a hypothetical worst-case scenario US landfill generating 0.75 million gallons of leachate per acre per year and receiving leachate surcharges of $0.02/gallon, annual leachate treatment costs (excluding leachate transportation) would reach $15,000/acre. If the same landfill were charged $0.50/gallon, annual treatment would rise to over $375,000/acre.

While leachate treatment costs can be covered by tipping fees for garbage disposal, landfills must be certain their tipping fees are also sufficient to support future treatment of leachate in closed landfill cells as landfills continue to generate leachate from decomposition and existing liquid content after closure (Duffy, 2005; McBean & Rovers, 1999; Williams, 2005). The EPA requires landfill monitoring for a minimum of 30 years after closure, and leachate treatment must continue until the volume of leachate
is considered no longer a threat to human health or the environment (EPA, 2012).

**Variations in Leachate Composition**

One of the largest challenges of leachate treatment is the variability of leachate composition. Each landfill receives different waste streams that vary during the life of the landfill, and environmental factors such as temperature and moisture content influence the decomposition rate within the landfill (Qasim & Chiang, 1994; Williams, 2005).

Leachate composition also changes based on the age of the landfill cell (Williams, 2005; McBean & Rovers, 1999). When landfilled wastes first come in contact with water, there are many readily soluble organics and nutrients. Over time, water leaches off a majority of the soluble contaminants and the concentration of most leachate constituents decreases significantly (Table 2, McBean and Rovers, 1999).

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
</table>

**Typical Leachate Concentrations for Various Constituents vs. Time**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 year</td>
</tr>
<tr>
<td>BOD</td>
<td>20,000</td>
</tr>
<tr>
<td>TKN</td>
<td>2,000</td>
</tr>
<tr>
<td>Ammonia-N</td>
<td>1,500</td>
</tr>
<tr>
<td>TDS</td>
<td>20,000</td>
</tr>
<tr>
<td>Chloride</td>
<td>2,000</td>
</tr>
<tr>
<td>Sulfate</td>
<td>1,000</td>
</tr>
<tr>
<td>Phosphate</td>
<td>150</td>
</tr>
<tr>
<td>Calcium</td>
<td>2,500</td>
</tr>
<tr>
<td>Sodium and potassium</td>
<td>2,000</td>
</tr>
<tr>
<td>Iron and magnesium</td>
<td>700</td>
</tr>
<tr>
<td>Aluminum and zinc</td>
<td>150</td>
</tr>
</tbody>
</table>

*Notes: BOD: biochemical oxygen demand, TKN: total Kjeldhal nitrogen, TDS: total dissolved solids.*

Leachate composition over time can also be represented graphically (Figure 1). Leachate composition changes with the stage of decomposition within the landfill cell creating that leachate, and it is common to split decomposition into five distinct phases (McBean & Rovers, 1999; Williams, 2005). These phases can be given different names, but the stages represented in Figure 1 are: (I) hydrolysis/aerobic degradation, (II) hydrolysis and fermentation, (III) acetogenesis, (IV) methanogenesis, and (V) and oxidation (Williams, 2005).

**Figure 1.** Landfill leachate composition in relation to biodegradation of solid wastes. From “Waste Treatment and Disposal” by Paul T. Williams, p. 203 (2005).

Hydrolysis/aerobic degradation (I) occurs when wastes are first landfilled. Aerobic microorganisms use available oxygen to break down organic wastes, and this stage usually lasts under a month (Williams, 2005; McBean & Rovers, 1999). Hydrolysis and fermentation (II) marks the beginning of anaerobic degradation. Carbohydrates and lipids are decomposed, and a large increase in ammonia concentration occurs as facultative anaerobes break down proteins (Williams, 2005). Acetogenesis (III) is typified by acetic acid and acetic acid derivative formation, and BOD levels are at their highest
during this stage (Williams, 2005). Methanogenesis (IV) generates methane for landfill gas from organic acids in the leachate. This is considered the longest phase of leachate decomposition, and may not start for 6 months to many years after a landfill is closed (Williams, 2005). Oxidation (V) begins when all biodegradable compounds in the landfill have been removed and aerobic microbes become re-established as the landfill becomes aerobic again due to infiltration of oxygenated water (Qasim & Chiang, 1994; Williams, 2005). The oxidation stage takes the longest time to reach, and potentially will not begin for over 100 years in some landfills (Williams, 2005).

According to McBean & Rovers (1999) and Williams (2005), the completion of breakdown in landfills may take decades to centuries. The same authors found that leachate aging and breakdown of organic material was faster in warm climates and slower in cold, dry climates. In addition, these authors found that landfills with multiple active and closed cells might see different stages of decomposition within the landfill at the same time, resulting in complex leachate compositions.

**Existing Treatment Schemes**

Similar to how there are many different types of leachate, there are multiple options for landfill leachate treatment. Some of the more common treatment methods are POTW discharge, leachate recirculation, leachate evaporation, and reverse osmosis.

**POTW discharge.** When possible, discharge to a POTW is one of the preferred methods of leachate treatment. However, high levels of ammonia can throw microbial treatment of waste water out of balance at the POTW and magnesium can bind with ammonium and phosphate to cause scaling in pipes (Bushnell, Kolibaba, & Mills, 2011).
To reduce the potential of harm from landfill leachate, it has been recommended for POTWs to incorporate leachate in at well under 5% of the waste water stream (Qasim & Chiang, 1994). Anecdotally, leachate is often incorporated into treatment plants at less than 1% of total flow.

POTWs can charge landfills wastewater surcharges anywhere from pennies to over a dollar per gallon of leachate to make up for the extra strain on their water treatment system. This surcharge is often dependent on the strength of the leachate, and the cost does not include the price of transporting the leachate (McBean & Rovers, 1999). Leachate can be piped or trucked to the POTW, but pipelines may experience heavy wear from the leachate and extensive paperwork is necessary to truck leachate due to the EPA’s classification of leachate formed in landfills containing hazardous waste as a hazardous waste (Skinner, 1983).

In many cases, landfill leachate can be pretreated with precipitation or oxidation to reduce the strain on the POTW. This pretreatment is required by some POTWs, but in cases where pretreatment is not required it can reduce the leachate surcharges by reducing leachate strength (McBean & Rovers, 1999). Precipitation removes metals by addition of sodium hydroxide or lime to react metals into a solid particle such as a metal hydroxide and causing the particles to clump together and settle out (Armenante, 1997). Oxidation through ozonation or by addition of metal oxide catalysts can be used to remove ammonia, cyanide, organics, and bacteria from waste water (Fickes, 2011).

**Recirculation.** Another form of leachate treatment that is commonly used is leachate recirculation (McBean & Rovers, 1999; Qasim & Chiang, 1994; Williams, 2005). In recirculation, leachate is recycled back through landfill cells in order to further
break down solid waste (Figure 2). Recirculation creates more uniform leachate, promotes quicker breakdown of wastes, and increases methane production (Williams, 2005; Qasim & Chiang, 1994). Higher methane production can be beneficial, as landfill methane can be captured and burned to produce energy and generate profit for the landfill (Fickes, 2011). Recirculation can also concentrate salts from inorganic wastes to produce more toxic leachate and is not possible at all landfills as EPA regulations do not permit over a foot of leachate accumulation on landfill liners (Arthur D. Little, Inc, 1986).

**Figure 2.** Example of leachate recirculation in conjunction with landfill gas collection. (Waste Management, Inc., 2012)

**Leachate Evaporation.** Landfills which do not discharge to a POTW may choose to use leachate evaporation to reduce or eliminate the need to discharge leachate. Leachate evaporation employs waste heat from landfill gas combustion to evaporate large volumes of leachate each day (Purchwitz, 2000). According to Purchwitz, this process volatilizes organics in the process but leaves most to all metals behind in the concentrate. Evaporation can decrease air quality through vaporization of contaminants, so it may not
be an option in locations with poor air quality. Concentrate from leachate evaporation manifests as a solid or semi-solid, and can be disposed of back in the landfill unless regulations prohibit (Purchwitz, 2000).

**Reverse Osmosis.** Reverse osmosis (RO) utilizes pressure gradients to generate pure water from contaminated waste water (Gavrilescu, et al., 2012). Water is removed across an ultra-fine membrane using pressure to overcome osmotic potential, and the treated water (permeate) is separated out from residual water and contaminants (concentrate) which cannot pass through the membrane (Figure 3). Permeate from an RO system can reach high levels of purity, but pre-filtration of leachate is required and there is potential for membrane fouling by humic acids (Gavrilescu, et al., 2012). Concentrate must be treated and disposed of properly, as concentrations of toxins within concentrate are much higher than in the original leachate. The high pressures required for RO systems result in large energy inputs to the system, but recent technology for recovering energy from high pressure liquid has reduced the energy burden of RO (Lenntech).

![Figure 3. Example of an RO unit, showing membrane layers water must travel through. (Conteno, 2011)](image-url)
Limitations. Many treatment systems are restricted in their removal and breakdown of heavy metals and xenobiotic compounds. Water treated at a POTW relies primarily on sedimentation and biosorption to remove heavy metals (Chipasa, 2003). Biological degradation of xenobiotics such as pharmaceuticals is often under 50%, although photodegradation and anaerobic digestion may increase the rate of breakdown for these compounds (Bienkowski & Environmental Health News, 2013; Jones, Voulvoulis, & Lester, 2005). Leachate recirculation has the potential for partial xenobiotic breakdown (Innovative Waste Consulting Services, LLC, 2007). Recirculated leachate also has a higher economic potential to undergo metals recovery than normal leachate due to expected higher metal salt concentrations (Williams, 2005). Leachate evaporation, much like leachate recirculation, can concentrate non-volatile chemicals such as metals from the leachate (Purchwitz, 2000). Volatiles, including some pharmaceuticals, will enter the gas phase and leave the leachate unless technologies are used to return them to the concentrated leachate (Purchwitz, 2000). Heavy metals and organics that do not volatilize re-enter the landfill in solid form and can potentially leach the same contaminants again. RO also concentrates contaminants, but has the benefit of removing almost all contaminants from the clean permeate. As with other concentrated leachates, concentrate from RO systems is a high strength waste and must undergo intensive treatment or else return to the landfill (IPPC, 2007).

Bioremediation of Waste Waters

Over the past century, there has been significant interest in on-site biological treatment of landfill leachate. Bacteria have already proven effective at decomposing
carbonaceous matter and reducing biological and chemical oxygen demand (BOD and COD) in waste waters. However, traditional biological wastewater treatment does not remove significant amounts of ammonium unless nitrifying and denitrifying bacteria have been allowed sufficient time to become established (OWP, 2009).

Constructed wetlands of nitrogen tolerant species such as reeds and willows can be used to remove high levels of nitrogen and to take up metal ions (Białowiec, Davies, Albuquerque, & Randerson, 2012). It has been shown that the majority of the actual nitrification and denitrification in wetlands systems is accomplished by microorganisms such as bacteria and algae, and that up to 90% of nitrogen and 20% of COD can be removed by nitrifying bacteria (Welander, Henrysson, & Welander, 1998). Unfortunately, wetlands with dilutions of landfill leachate are at risk of salinization, and plants have to be periodically harvested and disposed of when metal concentrations become too high (AILE, 2007).

Algae have been researched for their ability to utilize nutrients in wastewater and their capacity to adsorb metals to their cell walls. Additionally, algal cells can release metals and be regenerated through NaCl washing of live cells or acid washing of dead cells (Avery, Codd, & Gadd, 1998; Tam, Wong, & Simpson, 1998). The adsorptive capability of algae has been found to vary by species, and also by pretreatment of the algal cells (Tam, Wong, & Simpson, 1998; Mathad, Angadi, & Mathad, 2001). Table 3 contains a brief compilation of select experiments where algae were used to adsorb metals; including alga used, metal species adsorbed, and maximum removal achieved.
Table 3.

**Summary of Select Known Studies on Metal Adsorption by Algal Species**

<table>
<thead>
<tr>
<th>Species of Algae</th>
<th>Metal</th>
<th>Maximum Metal Adsorption</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascophyllum nodosum</em></td>
<td>Cd</td>
<td>110 mg/g</td>
<td>Yu, Matheickal, Yin, &amp; Kaewsarn (1999)</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>72.4 mg/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pb</td>
<td>249 mg/g</td>
<td></td>
</tr>
<tr>
<td><em>Chlorella emersonii</em></td>
<td>Cs</td>
<td>66 mg/g</td>
<td>Avery, Codd, &amp; Gadd, (1998)</td>
</tr>
<tr>
<td><em>Chlorella pyrenoidosa</em></td>
<td>Mn</td>
<td>26.0 mg/g</td>
<td>Knauss &amp; Porter (1954)</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>As</td>
<td>0.31 mg/g (live cells)</td>
<td>Maeda &amp; Ohki, (1998)</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>2.12 mg/g</td>
<td>Tam, Wong, &amp; Simpson, (1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.61 mg/g (heat killed cells)</td>
<td></td>
</tr>
<tr>
<td><em>Cladophora fracta</em></td>
<td>Cd</td>
<td>0.24 mg/g</td>
<td>Ji, Xie, Li, &amp; Chen, (2011)</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>2.39 mg/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hg</td>
<td>0.23 mg/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>1.62 mg/g</td>
<td></td>
</tr>
<tr>
<td><em>Phoridium sp. (blue-green algae)</em></td>
<td>As</td>
<td>1.95 mg/g</td>
<td>Maeda &amp; Ohki, (1998)</td>
</tr>
<tr>
<td><em>Scenedesmus quadricada</em></td>
<td>Cu</td>
<td>75.6 mg/g</td>
<td>Bayramoğlu &amp; Yakup, (2009)</td>
</tr>
<tr>
<td></td>
<td>Ni</td>
<td>55.2 mg/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>30.4 mg/g</td>
<td></td>
</tr>
<tr>
<td><em>Sargassum sp.</em></td>
<td>Cr</td>
<td>3.69 mg/g</td>
<td>Esmaeili, Ghasemi, &amp; Zamani (2012)</td>
</tr>
</tbody>
</table>

*Note: metal adsorption is in units of mg metal/g biomass. Wet and dry biomass is not always specified, although dry biomass is the standard. Dry algae often has 8-9% the mass of wet algae (Sládeček & Sládečková, 1963)*

Although these studies looked at different algae and had different experimental setups, there were consistent trends between many of these studies. Adsorption of metal cations occurred primarily at the same pH range (4-6) regardless of algal species (Avery, Codd, & Gadd, 1998; Fike, 2001; Ji, Xie, Li, & Chen, 2011; Tam, Wong, & Simpson, 1998). Metal ions tend to adsorb in higher concentrations at higher pH values due to decreased competition from hydrogen ions (Bohli, Villaescusa, & Ouederni, 2013). However, it should be noted that higher pH can result in the precipitation of metals, so an ideal pH exists for each metal which results in maximum adsorption (Bohli, Villaescusa, & Ouederni, 2013). Multiple studies showed that more metal ions were adsorbed by dead...
algae that had undergone some form of heat treatment than by live algae (Parameswari, Lakshmanan, & Thilagavathi, 2009; Tam, Wong, & Simpson, 1998). It is hypothesized that heat-killing of algae removes competing protons that would be produced during metabolism by live algae (Parameswari, Lakshmanan, & Thilagavathi, 2009).

Algae are capable of utilizing dissolved carbon dioxide and nitrogen in the forms of ammonium or nitrate, making them suitable for nutrient removal from leachate (Douskova, et al., 2009; Edmundson & Wilkie, 2011, 2013; Goswami & Kalita, 2011; Larsdotter, 2006). Algae are also capable of surviving in high concentrations of ammonia. In a 1996 study of the maximum concentration of ammonia algae could handle, Tam and Wong found that *Chlorella vulgaris* could survive in ammonia concentrations of at least 1000 mg/L as nitrogen. However, this same study found that cell division for *C. vulgaris* was inhibited over 750 mg-N/L ammonia (Tam & Wong, 1996). In a study on the utilization of ammonia by *S. dimorphus*, maximum growth rate was reached at concentrations between 0.8 and 1.2 mM (11.2-16.8 mg-N/L) ammonia and maximum utilization of ammonia was 0.06 ± 0.01 g NH$_3$/g cell (Kang, 2012). Algae also utilize phosphate in waters, and a study on *Chlorella emersonii* found phosphate uptakes of approximately $2 \times 10^{-8}$ μmol P/cell under ideal conditions (Robinson, 1998).

Algae have also been grown in untreated leachate at multiple dilutions and have shown the ability to survive in most dilutions. In Florida, algae were grown in dilutions of a high strength leachate containing 1300 mg/L total ammonia-N in groundwater. Algal viability was seen in all dilutions from 0-100% leachate, and in ideal conditions the algae succeeded in removing over 90% of ammonia and over 70% of COD (Edmundson & Wilkie, 2011). However, it was noticed by the same authors in a separate study that algae
were phosphorus-limited in leachate compared to in the algal growth medium utilized in their study (Edmundson & Wilkie, 2013; Nichols & Bold, 1965).

The high bioavailability of algae and comparatively low operating cost of systems utilizing algae makes algal remediation an ideal solution for many wastewaters. However, there is a maximum ammonia concentration (above 750 mg/L) before algal growth is inhibited (Tam & Wong, 1996). There is also a concern that some leachates may be toxic to algae depending on the composition of the leachate (Cheung, Chu, & Wong, 1993). Cheung, Chu, and Wong found that organic compounds and ammonia were the determining factors for leachate toxicity, and it is possible that herbicides could be among the toxic organics which kill algal cultures (1993).

Scope of Thesis

The purpose of this thesis was to examine the viability and inhibition of select algal strains in landfill leachate, to attempt to quantify the growth of algae in leachate, and to examine the possibilities of treating leachate with algae in a full-scale system. Testing of algal capabilities was done by growing algae in untreated and pretreated landfill leachate in lab-scale batches and observing the growth rate of those algae. The treatment system was proposed based off observed algal robustness, literature results on algal treatment, and existing treatment schemata. This treatment system is intended to be a rough framework for future researchers to work off of and modify as more knowledge becomes available in the field of algal remediation as well as in the treatment of landfill leachates.
Methods

To test the viability of algae in leachate, algae were subjected to multiple dilutions of pretreated and untreated leachate in DI water or media. Additional experiments were performed to measure the nitrogen uptake capabilities and growth rate of the algae.

Algal Strain

Two strains of algae, *Chlorella vulgaris* and *Scenedesmus dimorphus*, were cultured in a modified media based off the UTEX BG-11 recipe (2009). A side by side comparison of original and modified BG-11 media can be found in Table 4. Initial *C. vulgaris* growth was poor despite the success of colleagues at growing *C. vulgaris* in the same media. It is suspected that a lack of temperature control during initial algae cultivation may have affected *C. vulgaris* growth rates. *Scenedesmus dimorphus* was found to be a more forgiving culture under initial conditions and grew quickly, so cultivation of *C. vulgaris* was suspended and efforts were focused on growing *S. dimorphus*. 
Table 4

*Original and Modified BG-11 Media Composition*

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration: (mg/L) (mM)</th>
<th>BG-11</th>
<th>Modified BG-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>1500 17.649</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>40 0.230</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgSO₄*7H₂O</td>
<td>75 0.304</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl₂*2H₂O</td>
<td>36 0.245</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric Acid*H₂O</td>
<td>6 0.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferric Ammonium Citrate</td>
<td>6 0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferrous Ammonium Sulfate</td>
<td>- -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂EDTA*2H₂O</td>
<td>1 0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>20 0.189</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration: (mg/L) (μM)</th>
<th>BG-11</th>
<th>Modified BG-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₃BO₃</td>
<td>2.85 4.626</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MnCl₂*4H₂O</td>
<td>1.81 0.915</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZnSO₄*7H₂O</td>
<td>0.22 0.077</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂MoO₄*2H₂O</td>
<td>0.39 0.161</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CuSO₄*5H₂O</td>
<td>0.079 0.032</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co(NO₃)₂*6H₂O</td>
<td>0.0494 0.170</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoCl₂*6H₂O</td>
<td>- -</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Notes:* Original BG-11 media components as listed on UTEX website (BG-11 Media, 2009).

*S. dimorphus* was cultured in duplicate in 5 L Erlenmeyer flasks containing 1 L of modified BG-11 media. Flasks were aerated with filtered air and stirred using a magnetic stir plate. The temperature for cell cultivation ranged from 20-30 °C, and cells received on average 14 hours of light per day at a light intensity of approximately 80 W/m² of white fluorescent light. Carbon dioxide gas was bubbled in using a gas syringe to adjust pH to within 7-8 pH units. Algae for experiments were removed via centrifugation at 3200 rpm for 5 minutes, decanted, and algal pellets re-suspended to the desired concentration in DI water before addition to experimental solution. When 5 L algal flasks
began to show reduced growth, algae were allowed to settle and media was decanted and replaced.

**Leachate Pretreatment**

In order to test the effects of different leachate strengths on algae, leachate from a site referred to as “West Pond” with approximate initial properties listed in Table 5 was centrifuged at 15,000 rcf for 5 minutes and decanted to remove suspended solids. The decanted mixture was added to an Erlenmeyer flask containing dried brown algae (kelp meal) from the species *Ascophyllum nodosum* at a ratio of 0.1 L leachate/1 g algae. The kelp meal had a density of 1.26 g/cm³ and an average particle diameter of 100-149 μm as determined using an Allen-Bradley sonic sifter. Leachate and dried algae were mixed on a shaker table at a low speed for 2 hours and then allowed to settle. Once dried algae had settled, leachate was decanted into a new Erlenmeyer flask. This process was performed a total of 5 times, during which 1/3 of the leachate volume was lost to sampling and to ensuring dried algae from one flask did not carry over into the next flask (White, 2014a). The volume of leachate and mass of dried algae used during each adsorption can be found in Appendix A. After contact with dried algae, leachate was distilled in a hot oil bath that was kept at approximately 110 °C by a hot plate in order to reduce ammonia content. Leachate distilled off at approximately 5 mL/min, and distillation lasted until 35% of the leachate had been collected as distillate using a Graham condenser (White, 2014b). Total nitrogen was measured before and after distillation using Hach total nitrogen assay #2714100.
Table 5

Approximate Initial Leachate Characteristics from West Pond

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium-N</td>
<td>1090</td>
<td>mg/L</td>
</tr>
<tr>
<td>Cyanide</td>
<td>0.012</td>
<td>mg/L</td>
</tr>
<tr>
<td>TDS</td>
<td>9300</td>
<td>mg/L</td>
</tr>
<tr>
<td>Arsenic</td>
<td>167</td>
<td>μg/L</td>
</tr>
<tr>
<td>Chromium</td>
<td>225</td>
<td>μg/L</td>
</tr>
<tr>
<td>Copper</td>
<td>24.1</td>
<td>μg/L</td>
</tr>
<tr>
<td>Nickel</td>
<td>244</td>
<td>μg/L</td>
</tr>
<tr>
<td>Zinc</td>
<td>122</td>
<td>μg/L</td>
</tr>
</tbody>
</table>

*Note: TDS stands for total dissolved solids
*Source: NELAP accredited laboratory Analysis of West Pond leachate (July, 2013)*

**Nitrogen Uptake**

Three 100 mL dilutions of pretreated leachate in DI water were prepared at concentrations of 25%, 50%, and 100% pretreated leachate. A 100% media solution was also prepared. All solutions were buffered to a pH of 7-9 using dibasic sodium phosphate, and *S. dimorphus* was added to each of these solutions to an initial density of approximately 1.8 * 10^7 cells/mL. Each solution was prepared in triplicate. Cell solutions were kept in 250 mL Erlenmeyer flasks with rubber stoppers on top, and cells were suspended using a shaker table at an average speed of 95rpm. All flasks were also agitated daily by hand to ensure algae were uniformly distributed, and CO₂ gas was bubbled in using a gas syringe to keep the pH below 9. Algae were grown at 30 °C in 14 hours of light and 10 hours of dark in a light intensity of approximately 20 W/m².

Each day, flask stoppers were removed and 1 mL of solution was withdrawn from each flask and centrifuged at 10,500 rcf for 3 minutes. Total nitrogen (TN) in the distillate was measured using Hach assay #2714100. Cells centrifuged out of the 1 mL samples were re-suspended in DI water and dried on filter paper in a Precision Scientific
31604 oven at 105 °C. Cell masses were measured and recorded after 1 day of drying in the oven.

**Cell Growth**

Initial cell growth tests were performed qualitatively to confirm that algae were capable of growing in dilutions of untreated leachate. Five 50 mL dilutions of 0 (control), 5, 10, 15, and 20% untreated leachate in media were prepared. Dilutions were put in separate 250 mL Erlenmeyer flasks, and undetermined but low concentrations of algae were added to each flask. Rubber stoppers were used on each flask to reduce likelihood of spilling, but stoppers were removed daily for sampling. Flasks were grown on a shaker table at an average speed of 95 rpm and maintained at 25 °C with 14 hours of light and 10 hours dark per day. Light intensity for qualitative growth was just over 40 W/m². Upon gathering visual confirmation of cell population increases, the experiment was repeated with daily hemocytometer cell counts to quantify growth under a Motic BA300 microscope at 40x magnification. Cell counts were performed on a Neubauer chamber following the basic hemocytometer procedures outlined by Caprette (2012). The specific growth rate, $\mu$ (day\(^{-1}\)), of the algae was calculated using the following equation, where $X_i$ and $X_f$ stand for initial and final cell concentrations in cells/mL and $t_i$ and $t_f$ stand for the initial and final time in days (Shuler & Kargi, 2002):

$$\mu = \ln \frac{X_f}{X_i} / (t_f - t_i) \tag{Equation 1}$$

The initial and repeat algal dilution test employed all the same dilutions (0, 5, 10, 15, and 20% untreated leachate in modified BG-11 media), the same leachate, and a new batch of media using the same modified BG-11 media recipe. Qualitative and quantitative
experiments occurred using a different shaker table and different light intensity due to moving the experiment location. Both shaker tables were used in the 90-100 rpm range, but light intensity in the quantitative experiment was over 80 W/m$^2$, or about twice the intensity of the qualitative experiment. All lights used were fluorescent white lights.

**Results and Discussion**

**Leachate Pretreatment**

Leachate clarified in dried brown algae showed reduced turbidity and was lighter in color (Figure 4). Distillation further reduced turbidity and color, and was successful in removing over 90% of total nitrogen from the distilled leachate (Appendix A). Hach assays gave a TN concentration of 1500 mg/L for the untreated leachate and a concentration of 220 mg/L for distilled leachate. Ammonia that was distilled off was captured, and the liquid distillate contained 2800 mg/L TN (White, 2015). Ammonia captured in distillate was approximately 65% of the original ammonia concentration, with 9% of the ammonia remaining in the distilled leachate and 26% escaping to the atmosphere. Ammonia capture percentages assume that the majority of the total nitrogen measured was ammonia.
Nitrogen Uptake

Results of nitrogen uptake tests were inconclusive (Figure 5). All flasks showed a decrease in total nitrogen after one day of growth, but total nitrogen appeared to increase after 3 days. This trend is likely due to user error with the Hach assays related to the high levels of dilution that the samples required (up to a 1:20 dilution). Uncertainty was intensified by a lack of duplicate assays. To obtain conclusive results on nitrogen uptake, additional nitrogen uptake tests would need to be run and tests should be run in duplicate or triplicate to reduce uncertainty.
Figure 5. Measured total nitrogen concentrations over time. PL stands for pretreated leachate and DI for deionized water. The fourth 100% PL data point was discarded due to assay spillage. Data were collected as singular points, so no confidence interval can be associated with data points.

There appeared to be cell growth occurring in all flasks based on dry cell masses taken during the experiment (Figure 6). However, it was noticed that if cells were not well mixed when re-suspended they would form clumps and would not dry within 1 day. It is suspected that the 3rd and 6th cell masses from the 100% pretreated leachate solution were taken before the cells were fully dried. Total nitrogen and cell mass data from nitrogen uptake are available in Appendix B.
Figure 6. Measured dry cell masses from 1 mL of solution. PL stands for pretreated leachate and DI for deionized water. Cell growth was observed at 0.195, 0.129, and 0.278 mg/(mL·day) with $R^2$ values of 0.95, 0.86, and 0.85 for the control, 25% PL, and 50% PL solutions, respectively. The cell growth rate in the 100% PL solution had an $R^2$ value under 0.8, and cell growth could not be reliably quantified.

**Cell Growth**

Initial qualitative algal growth experiments showed growth in all flasks (Figure 7). Upon witnessing an increase in algal concentration for all dilutions, a quantitative test was run in the same dilutions of leachate (Figure 8). The two experiments showed different growth patterns, as can be seen by comparing Figures 7 and 8. In the qualitative experiment, the media control appeared to have the greatest amount of cell growth but in the quantitative test the 5-15% dilutions appeared to have higher concentrations of cells than the media control. Solutions with higher untreated leachate concentrations had a characteristic brown color from the leachate. Flasks in the quantitative growth experiment were believed to be seeded to the same cell density of $1.2 \times 10^6$ cells/mL, but initial cell counts were not consistent with that value. All measured growth rates and percent increases were based off the actual concentration calculated on day 1 of the experiment. Data for the quantitative cell growth test can be found in Appendix C.
Figure 7. Comparison of algae concentrations in dilutions of 0, 5, 10, 15, and 20% untreated leachate in growth media for qualitative experiment at days 1 and 5.

Figure 8. Comparison of algae concentrations in dilutions of 0, 5, 10, 15, and 20% untreated leachate in growth media for quantitative experiment at days 1 and 5.
Cell counts for each of the flasks over 5 days are shown in Figure 9. Cell populations increased to 16, 22, 10, 12, and 9.6 times the concentrations recorded on day 1 in the 0, 5, 10, 15, and 20% dilutions, respectively. Cell counts showed high variation, likely due to the tendency of *S. dimorphus* to form colonies and produce a non-uniform cell mixture.

![Figure 9](image)

*Figure 9.* Cell density in untreated dilutions over time. Error bars are for one standard deviation. The high cell density in 0% untreated leachate on day 4 may be the result of an incorrect dilution.

Specific growth rates ($\mu$) calculated using Equation 1 were $0.7 \pm 0.3$, $0.8 \pm 0.4$, $0.6 \pm 0.3$, $0.65 \pm 0.2$, and $0.6 \pm 0.25$ day$^{-1}$, in order from 0% to 20% leachate dilution. From the growth rates observed, it does not appear the flasks were significantly substrate or toxin inhibited, but the 0% and 5% leachate dilutions did appear to have better growth rates. Observed growth rates were similar to literature values for $\mu$ in *Scenedesmus dimorphus*, which fell between 0.2 and 1.0 day$^{-1}$, suggesting mixed leachate and media provide a suitable growth environment for algae (Ribita, 2011; Goswami & Kalita, 2011).

On day 4, it was noticed that cells grown in the pure media were smaller and lighter than in the dilutions containing untreated leachate. A comparison of cells in the 0% and 5% dilutions of untreated leachate in media are shown in Figure 10.
It is possible the larger cells in 5% leachate were due to metal adsorption, but this is speculation. During cell counts, it was also noticed that new species were introduced with the untreated leachate. Organisms were seen moving across the slide in all dilutions containing untreated leachate. A time frame from a video containing mobile and immobile species and a picture of the organisms seen in the leachate are shown side by side in Figure 11.

**Figure 10.** Differences in cell appearance on day 4 in 0% untreated leachate and 5% untreated leachate from quantitative cell growth tests. Both images were taken at 40x magnification.

**Figure 11.** Microorganisms and debris found in untreated landfill leachate. On the left is a time frame from a video of a mobile leachate specimen (circled in red). On the right is an image of untreated leachate as it appeared under 40x magnification on a Motic BA300 microscope. Also of interest were what appeared to be bacillus or other rod-shaped bacteria (circled in blue) and round, immobile cells indigenous to the leachate (circled in pink).
Design

Experimental results and literature prove it is possible for algae to survive and grow in leachate dilutions. Although all leachates vary in toxicity, it is likely that most municipal leachates would be non-lethal to microalga species such as *S. dimorphus* at low to medium dilutions. Higher dilutions of leachate have the possibility to limit algal growth due to light limitation or toxins in the leachate, but the dilution of leachate at which limitation occurs will vary based on the leachate composition.

From the literature search, it is also apparent that both nutrients and metals can be removed by algae (Avery, Codd, & Gadd, 1998; Edmundson & Wilkie, 2011; Ji, Xie, Li, & Chen, 2011; Larsdotter, 2006; Tam, Wong, & Simpson, 1998). It is therefore believed that a treatment train using algae could be effectively designed to reduce leachate toxicity in a cost-effective manner and, if combined with other treatment methods, treat leachate to NPDES standards for discharge into an on-site wetlands or receiving water body.

Treatment Train Overview

The treatment train anticipated is outlined on the next page in Figure 12.
Figure 12. Layout of proposed algal treatment system. Orange lines and text represent gas streams, blue lines and text represent primarily liquid streams, and green lines and text represent streams with high concentrations of algae.
Remediation would begin with pretreatment to reduce toxicity and increase efficiency. Large suspended particles, if present, can be removed using a settling tank or fine screening. Once suspended solids have been removed, leachate would pass through metal adsorption columns using heat-killed algae. Landfills that burn landfill gases could capture waste heat from landfill gas combustion to preheat leachate to ideal conditions for algal growth (20-30 °C). Flue gas from landfill gas combustion could also be utilized as a carbon source for algal growth due to its increased CO₂ content. There is also the possibility to utilize waste heat to distill ammonia from the leachate, similar to leachate pretreatment in the experiments discussed previously in this paper. However, the distillation process would come with additional concerns such as how to cool leachate back to ideal growth temperature, and is outside the scope of this paper.

In the proposed design, algae would be treated similarly to aerobic bacteria (activated sludge) in a POTW. Algae, pretreated leachate, and any additional nutrients or diluents needed would be mixed together prior to entering a narrow, shallow channel algal bioreactor (Figure 12). Artificial light and sparged carbon dioxide provided in this channel would promote algal breakdown of ammonia. A clarifier or screen following the channel would allow algae to settle out and most of the algae would be recycled back to the blending step to maintain a high algae concentration. The remainder of the algae would be harvested as marketable biomass or for use in the metal adsorption column. An additional recycle feed after the clarification step would return treated leachate to the blending step in order ensure leachate can be treated to meet water quality standards.
Solids Removal

Before leachate can be run through any delicate equipment, suspended particles must be removed from the mixture. Most solids in leachate are dissolved and will not settle out. However, settleable solid concentrations in leachate can still be high enough to reduce the effectiveness of wastewater treatment. Average total dissolved solids (TDS) and total suspended solids (TSS) values from tests on over 70 landfills done by the EPA in 1988 were 4890 and 276 mg/L, respectively (EPA, 1988). Suspended solids are known to inhibit multiple treatment processes, and NPDES discharge standards for TSS require less than 45 mg/L average weekly concentration of TSS from secondary treatment (C.F.R., 2014; EPA, 2000). The average landfill leachate would require suspended solids removal before it could meet treatment standards. Common methods of removing suspended solids are settling basins, aerated grit chambers, and screening (Davis, 2011).

Metal Adsorption

Metal contamination of waste waters is a growing concern, and many treatment methods only temporarily remove metals from waste water. Sedimentation removes metals from water, but most of the sludges produced from sedimentation are then land applied or landfilled, allowing these metals to leach back out. Metal adsorption and recovery through regeneration allows for metals removed from waste streams to stay out of water for a longer period, and recovered metals can be sold to offset costs of leachate treatment. In this process, metal adsorption would utilize dead, heat-treated algae to remove metal ions from wastewaters.
Metal cations are more prone to adsorption at pH levels of 4-6, where the pH is high enough to reduce hydrogen ion competition but low enough to keep metals in solution, but many metals have ideal pH values for adsorption (Ramelow, Yao, & Zhuang, 1998; Bohli, Villaescusa, & Ouederni, 2013). It may be possible to obtain greater metal removal if multiple adsorption columns with different pH values are utilized. Column regeneration would occur either by washing algae with high concentrations of NaCl solution to replace metal cations with Na\(^+\) or by acid washing algae to lower pH and cause metal ions to desorb (Tam, Wong, & Simpson, 1998; Darnall & Gardea-Torresdey, 1989).

Additional benefits of metal removal include positive public perception and reduction of metal contamination and/or inhibition of produced algal biomass later in the treatment train. Algal biomass which is free of metals may be considered a higher value product, depending on the market for the biomass.

**Waste Heat Utilization**

The ideal growth temperature for algae is between 20 and 30 Celsius (Xin, Hong-ying, & Yu-ping, 2011; Ribita, 2011). Leachate pumped from landfills is likely close to average ground temperature, and in cool or moderate climates would need extra heat to reach 20 °C. At landfills that burn landfill gases, the flue gas from this combustion process reaches temperatures above 800 °C and can be utilized to preheat leachate (Edgar, 2008; SEPA, 2002). This would have the added bonus of cooling flue gas to temperatures which will not kill algae cells if landfill flue gas is utilized as a carbon source.
source for algae. To stabilize temperatures, a countercurrent heat exchanger could be used to transfer heat from the hot gas stream to the cooler leachate stream.

**Algal Remediation**

The main focus of this treatment system is the removal of excess nutrients using algae. There are thousands of known species of algae, but not all are suited to bioremediation applications. For the purpose of landfill leachate treatment, algal strains must be robust, have short doubling times (ideally a few days or less), and not produce toxins during the remediation process.

Multiple genera of algae have been studied for use in waste water applications, particularly freshwater algae from the genera *Scenedesmus* and *Chlorella* (Goswami & Kalita, 2011; Tam, Wong, & Simpson, 1998; Edmundson & Wilkie, 2011). Much like diverse bacteria in activated sludge at waste water treatment plants, algal cultures containing multiple species of algae are more resilient and have the capability to remove a greater variety of nutrients (DOE, 2014; Cardinale, 2011). These algal cultures would not remain pure, as bacteria are introduced with leachate. However, unless harmful bacteria are present in the leachate this addition of bacteria accustomed to leachate should increase the diversity and treatment capabilities of the microorganism culture.

**Mixing Tank.** At the beginning of algal remediation, incoming leachate is mixed with algae, recycled treated water, and any additional nutrients necessary to supplement algal growth in a CSTR mixing tank. Algae would need to remain in the mixing tank long enough for a uniform composition to be reached. Ideally, algae within the mixing tank could be resting in the “dark phase,” during which algae utilize stored energy to prepare
to fix addition carbon dioxide and perform cell maintenance, before entering the “light phase” in the sparged channel (Carvalho, Silva, Baptista, & Malcata, 2011; Gons, 1977).

**Sparged Channel.** Upon leaving the mixed tank, algae would travel through a long, shallow channel. This channel would need to be mixed to ensure that algae remain in suspension and to reduce mass transfer limitations. Additionally, gas containing carbon dioxide should be sparged into the reactor to guarantee algae have an adequate carbon supply. This carbon dioxide could be obtained from landfill methane combustion flue gas, as discussed previously, or else from the air. Dissolved carbon dioxide can reduce the pH of the leachate, so pH within the bioreactor would need to be monitored. From experimental evidence, it has been noticed that algal consumption of carbon dioxide results in an increase in solution pH, so carbon dioxide addition could be adjusted to maintain constant pH within the bioreactor. If the channel bioreactor is sufficiently long and narrow, it would function similarly to a plug flow reactor (PFR).

**Clarifier/Secondary Screening.** After leachate and algae have passed through the bioreactor, algae must be separated from the treated leachate. However, algae tend to have slow settling rates and algal harvest is often difficult and energy intensive. A study by Bowden and Belovich found the settling rate of *S. dimorphus* to be 0.87 cm/hr in fresh water (2013). By comparison, the average settling rate of activated sludge is often over 100 cm/hr (Dokuz Eylül Üniversitesi). If settling of algae does not occur quickly enough when tested in settling experiments, the design may need to be modified. Screening or ballast sedimentation could be implemented in place of or in addition to settling. Screening is difficult if cells are too small, as clogging of the filter often occurs, but addition of flocculants such as alum or chitin is relatively common (Divakaran & Pillai,
Ballast settling of cells can result in cells which are no longer suspended and well-mixed when they are recycled (Divakaran & Pillai, 2002). Another alternative is centrifugation of cells, but this is energy intensive and often not feasible for large-scale processes. One method of algal harvest which is particularly promising is autoflocculation, where algae flocculate out at high pH (10-11) due to interaction with magnesium hydroxide as a flocculent (Vandamme et al., 2012; 2014). Autoflocculation has the additional benefit of being reversible by slight acidification (to pH 7-8) of the solution, meaning that recycled algae would still be viable (Vandamme et al., 2012; 2014). A large percent of settled algae should be returned in a recycle feed to maintain high algal concentrations. Additional algae would be harvested and dried, then marketed as non-food biomass, used for metal adsorption, or anaerobically digested.

**Algal Biomass Utilization.** A significant benefit of algal remediation systems is the potential profit from harvested algae. Algae are cultured for multiple uses, including biofuels, human and animal food, cosmetics, bioplastics, and pharmaceuticals (Chaudhary, 2013). Ideal algae for biofuel production contain high lipid concentrations, but lipid production in algae is often optimized by limiting available nitrogen. Algae used to treat waste waters high in nitrogen are not likely to be high quality biofuel feed. Algae from a waste water application cannot be utilized as food for humans and are likely also unfit for animal food. While the bioplastics industry also utilizes lipids in algae, this industry is capable of accepting algae with lower lipid content and algae from waste water treatment applications. Algae high in nitrogen content also have the potential to be used as fertilizer. Additionally, dried algae can be utilized for metal adsorption at the start
of the treatment train or anaerobically digested to provide additional methane to combust with landfill gas (Gouleke, Oswald, & Gotaas, 1957; Ward, Lewis, & Green, 2014).

pH Regulation

At many steps in the treatment train, pH will need to be monitored and potentially adjusted. During metal adsorption, pH is important in determining which metal ions are adsorbed. Ideal pH for algae usually falls within 6 and 9. While leachate pH typically falls within a pH range of 6-8 (McBean & Rovers, 1999; Williams, 2005), treatment processes along the way may affect the pH of the leachate and adjustments may need to be made to ensure that pH remains at an ideal level for algal growth. Lower pH can be achieved by bubbling carbon dioxide through leachate and forming dissolved carbonic acid or through the addition of dilute acid. To raise the pH, dilute aqueous NaOH can be added (De Schamphelaere, et al., 2005; Vandamme et al., 2012).

Post-Treatment

Upon exiting the algal growth bioreactor, treated leachate will be too warm for discharge into most bodies of water. As such, it may be necessary to find a way to cool the treated leachate to approximately ground temperature. One solution would be treatment wetlands, which would allow for cooling of treated water to ambient temperature and provide additional water polishing. Another solution would be cooling towers, which utilize evaporation to cool water. However, cooling towers are expensive and energy intensive. An additional concern of the leachate treatment system is escape of algae or bacteria from the process into the environment. Invasive algae species should not
be used for treatment, but even with no invasive species significant discharge of bacteria or algae into waterways would likely be considered unacceptable. Effluent screening or treatment via chlorine contact would be an efficient and relatively inexpensive way to ensure minimal discharge of organisms or pathogens from the leachate treatment process.

**Energy and Pumping**

Energy use within treatment plants result in significant operation costs, sometimes accounting for 30% of total operation costs (EPA, 2006). For landfills that produce electricity on site, this may not be as significant of an issue, but energy cost is still important for all plants as electricity used for treatment cannot be sold or used for other purposes. The main energy uses within the treatment system would be pumping leachate from the landfill to the head of the treatment process, providing light to grow algae, pumping the recycled algae feed, drying algae, and sparging the algae channel.

**Example Sizing and Cost Estimate**

**Example Leachate Composition and Flow Rates**

For the example treatment system, assumed leachate characteristics were:

Table 6

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
<th>Units</th>
<th>Characteristic</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate</td>
<td>28*10^6</td>
<td>Gal/year</td>
<td>Temperature</td>
<td>13</td>
<td>°C</td>
</tr>
<tr>
<td>TDS</td>
<td>8,000</td>
<td>mg/L</td>
<td>TSS</td>
<td>100</td>
<td>mg/L</td>
</tr>
<tr>
<td>Oil and Grease</td>
<td>13</td>
<td>mg/L</td>
<td>pH</td>
<td>7.7</td>
<td>-</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>1,000</td>
<td>mg-N/L</td>
<td>Total Phosphorus</td>
<td>10.0</td>
<td>mg-P/L</td>
</tr>
<tr>
<td>BOD₅/COD</td>
<td>1,400/5,700</td>
<td>mg/L</td>
<td>Hardness</td>
<td>2,100</td>
<td>mg/L</td>
</tr>
<tr>
<td>Arsenic</td>
<td>170</td>
<td>μg/L</td>
<td>Iron</td>
<td>8,000</td>
<td>μg/L</td>
</tr>
<tr>
<td>Chromium</td>
<td>200</td>
<td>μg/L</td>
<td>Manganese</td>
<td>5,600</td>
<td>μg/L</td>
</tr>
<tr>
<td>Copper</td>
<td>20</td>
<td>μg/L</td>
<td>Nickel</td>
<td>200</td>
<td>μg/L</td>
</tr>
</tbody>
</table>

*Notes: BOD₅ stands for biological oxygen demand over 5 days, COD stands for chemical oxygen demand.*
Climate and regulations for the example system were based loosely off climate data and regulations in Corvallis, Oregon, but rainfall was assumed to be evenly split between all months. The system design therefore does not take into account the differences in operation between wet and dry seasons, and additional calculations would be required to size for wet and dry flows.

All costs are in US dollars based off of the value of currency in 2014, unless otherwise stated. Operating and maintenance costs are not included but are expected to be 6% of the total capital costs each year not including the costs of labor, chemicals, electricity, and residuals disposal (EPA, 1998).

**Solids Removal**

The TSS concentration for the example waste stream is too low for significant suspended solids removal through settling. It is likely any settleable solids have already settled during leachate storage. Should leachate storage time be significantly decreased by on-site treatment of leachate, it may be necessary to remove suspended solids prior to treatment. For this design, it is assumed that TSS will not increase due to on site treatment. However, were suspended solids a concern, a spiral screen (Figure 13) with openings of 6 mm could be installed to ensure no large particles enter the treatment system.
**Size.** If no screen is installed, no additional space is necessary. If a screen is installed, the exact dimensions of the screen would depend on the company the screen is purchased from. Compared to most POTW units, the plan area required for a screen would be minimal. Per specifications for a treatment plant in Indiana, a single spiral screen was estimated to take up a space of about 3' by 17', or 51 ft² (DLZ, 2014).

**Cost.** For the proposed system with no solids removal necessary, no costs would be incurred. However, in case a screen becomes necessary the expected cost should be considered. In 2006, the city of Hamilton, Montana had an engineering firm perform a cost analysis on multiple types of wastewater treatment equipment. The Hamilton facility operated at an average flow of 0.74 MGD in 2006, and according to the analysis the cost of a single 6 mm spiral screen to meet their plant’s needs was around $80,000 for the spiral screen, totaling to just over $170,000 in capital costs including installation and electrical controls (HDR Engineering, Inc., 2006).
For leachate flowing at 28 million gallons per year, or an average flow of 0.08 MGD, it is expected a smaller screen would suffice. However, this smaller screen may not be any cheaper. Assuming the initial screen costs are the same as the city of Hamilton facility and assuming a second screen is needed so the first screen can be taken offline for maintenance, the screens would cost close to $340,000 in 2006 dollars. A 2006 dollar is worth approximately $1.18 2014 dollars, so the price would be closer to $400,000 in 2014 dollars if all components of the screen (parts, contractor overhead, and installation) inflated at the same rate. Alternatively, one spiral screen could be implemented with a bypass channel containing a manually cleaned screen (Davis, 2011). This configuration would cost closer to $200,000 in 2014 dollars (HDR Engineering, Inc., 2006).

**Metal Adsorption**

Some of the notable metals in the leachate used for experiments were arsenic, chromium, copper, nickel, and zinc. Additionally, iron and manganese are known to be in leachate in high concentrations (EPA, 1988). Nickel is the only one of these metals with no EPA maximum contaminant level (MCL) or recommended concentration for drinking water. The MCLs and recommended concentrations for drinking water for arsenic, chromium, copper, iron, manganese, and zinc are 10 μg/L, 100 μg/L, 1.3 mg/L, 300 μg/L, 50 μg/L, and 5 mg/L, respectively (EPA, 2009). Effluent permit levels may be higher than the MCLs because discharge to large, well-mixed waterways is effective at reducing concentrations. However, effluent permits may also be lower than MCLs in waterways which are already severely limited by these metals. Therefore, the MCLs will be considered a goal concentration for metals removal. For arsenic, chromium, iron, and
manganese, drinking water levels are below metal concentrations seen in the landfill leachate used in experiments and metal removal would be recommended.

For this example, metal removal will consist of a metal adsorption column containing heat killed algal cells. Exact values of metal adsorption capacities for each algal species will vary, so metal adsorption capacities are based on estimates and assumptions.

**Size.** In order to correctly size the metal adsorption column, the following assumptions must be made about the leachate stream and the algal cells:

- Dried algae capable of adsorbing 1 mg/g As, 3 mg/g Cr, 30 mg/g Fe, and 20 mg/g Mn in mixed metal matrices (loosely based off Table 3)

- All sites are species-specific (in reality, metal ions can compete for many of the same binding sites. However, for the purpose of this estimation maximum metal adsorption capacities were decreased to account for the competition)

- Maximum removal of any of the metals is 90% of the influent concentration, past this point it is assumed that there will not be enough of a concentration gradient between the leachate and the algae to result in further metal adsorption

- Influent leachate has arsenic, chromium, iron, and manganese concentrations of 170, 200, 8000, and 5600 µg/L, respectively (Table 6)

- Breakthrough of metal concentration will occur when the algae has reached 90% capacity for any of the metals

- Dried algae can be regenerated to 90% efficiency approximately 10 times before the adsorption capacity decreases significantly (Volesky, Weber, & Park, 2003; Brinza, Dring, & Gavrilescu, 2005; Liu, et al., 2011)
• Dried algae density is the same as the *Ascophyllum nodosum*, density of 1.26 g/cm$^3$ seen in the leachate pretreatment experiment

To treat metals to desired levels, the concentration of arsenic would need to decrease by 140 µg/L, chromium by 100 µg/L, iron by 7700 µg/L, and manganese by 5550 µg/L. The flowrate of leachate is assumed to be 28 million gallons per year, or about 12,000 L/hr.

For these decreases in concentration at 90% algal adsorption efficiency, based on the adsorption capacities assumed above, the limiting concentration would be manganese which would require 3.7 kg of biomass per hour of leachate flow. If there were 6 columns regenerated every 6 hours (4 columns in operation, 1 column regenerating, and 1 backup column at any given time), each column would require 5.6 kg of biomass. This biomass would have to be replenished approximately every 10 days and, assuming the backup column also receives new biomass every 10 days, 1215.5 kg of dried algae would be needed each year. This would be approximately 3.33 kg of algal biomass each day, which is possible but potentially high.

If each column contained 5.6 kg of biomass at 1.26 g/cm$^3$ density and with a freeboard of 40% to allow biomass expansion during upflow operation or backwashing to regenerate the column, columns would need to be at least 6.2 L in size. These columns would take a minimal plan area.

**Cost.** The cost of constructing and installing the columns will depend on whether columns are constructed in house or ordered. Because the application and size of the column are uncommon, the columns would likely be constructed on site and the main cost would be labor. The algal biomass could be produced on site or purchased for $1.43-$4.07/kg (Everwood Farm, 2015; North American Kelp, 2015). The regeneration of algal
biomass would require either concentrated brine or acid. It would be ideal if metals could be recovered and sold and the salt or acid reused. Pumps and energy for regenerating and maintaining the columns would also be a key expense for the metal adsorption process.

### Heat Transfer

Leachate and carbon dioxide entering the algal treatment step must be at appropriate temperatures for algal growth, or approximately 20-30 °C (Xin, Hong-ying, & Yu-ping, 2011; Ribita, 2011). For this example, a temperature of 30 °C is desired. One potential method of raising leachate temperature would be to install heat exchangers in the treatment plant. An example of a shell and tube heat exchanger is shown in Figure 14. The heat exchangers could utilize flue gas from landfill gas combustion, which exits at high temperature, so long as flue gas is not highly corrosive. Landfill gas is burned at over 800 °C but it is expected that captured flue gas will have lost a significant amount of heat before capture for heat transfer utilization (SEPA, 2002; Edgar, 2008).

![Figure 14. Shell and tube heat exchanger. (Tetsa Industry and Trade)](image)

**Size.** It is outside the scope of this thesis to attempt to size heat exchangers for
this application with precision, and such a size estimate would be expected to come from a consulting firm bidding to design the heat exchanger required. Heat exchangers can be ordered from many different companies and designed to meet almost any specifications or size constraints. One of the most important variables determining heat exchanger size is heat transfer duty. The heat transfer duty of a heat exchanger is the amount of energy that exchanger needs to add or remove from a fluid. The heat transfer duty can be calculated using the equation below (Welty, Wicks, Wilson, & Rorrer, 2007).

\[ Q = \dot{m} \cdot C_p \cdot \Delta T \]  

(Equation 2)

Variables for Equation 2 are defined as follows:

- \( Q \) = heat transfer duty (kW)
- \( \dot{m} \) = mass flow rate of fluid (kg/s)
- \( C_p \) = specific heat capacity of fluid (kJ/(kg*K))
- \( \Delta T \) = change in temperature for fluid (K)

In this process, the fluid of interest is landfill leachate, which must be raised to 30 °C. To use Equation 2 to calculate heat transfer duty necessary to heat leachate, the following assumptions must be made:

- Influent landfill leachate temperature is constant at 13 °C, the approximate annual average ground temperature in Corvallis (Zheng, Hunt Jr., & Running, 1993)
- Landfill leachate is flowing at 28 million gallons per year, or 0.0034 m³/s
- Influent flue gas temperature is constant at 600 °C after losing a significant amount of heat to surrounding air
- Leachate properties are approximately the same as water and remain constant.

Density (\( \rho \)) = 997 kg/m³ and specific heat capacity (\( C_p \)) = 4.19 kJ·(kg·K)⁻¹
• Flue gas properties are approximately the same as air and can be averaged at a median temperature of 300 °C. Density ($\rho$) = 0.607 kg/m$^3$ and specific heat capacity ($C_p$) = 1.046 kJ·(kg·K)$^{-1}$ (Welty, Wicks, Wilson, & Rorrer, 2007)

Equation 2 is worked out below to give a required heat transfer duty of 240 kW.

$$Q = 0.0034 \frac{m^3}{s} \cdot \frac{997 \text{ kg}}{m^3} \cdot 4.19 \frac{kJ}{kg \cdot K} \cdot (303K - 286K) = 240 \text{ kW}$$

This heat duty of 240 kW can then be applied to flue gas using Equation 2 and an ideal outlet temperature of 30 °C to solve for an approximate gas flow rate of 0.66 m$^3$/s (1400 CFM). A landfill gas emission of 1600 CFM is considered average for landfill gas combustion programs, so this seems a reasonable value (Waste Management, 2010).

$$240 \text{ kW} = \dot{m} \cdot \frac{0.607 \text{ kg}}{m^3} \cdot 1.046 \frac{kJ}{kg \cdot K} \cdot (873K - 303K), \quad \dot{m} = 0.663 \frac{m^3}{s}$$

In an analysis project detailing heat exchanger size and cost for an ocean thermal energy conversion plant, a heat transfer duty requirement of 12,700 kW corresponded to a floor area of 762 m$^2$, or 8200 ft$^2$, using 50 heat exchange units (Taniguchi, 2006). Although the correlation between heat duty and size is not necessarily linear, a heat exchanger requiring 53 times less power could reasonably require 50 times less space, or a space under 160 ft$^2$, and potentially only one heat exchange unit. While this value is in no way a guarantee of the size that would be needed, it is highly probable that a size of less than 600 ft$^2$ could provide the heat transfer duty needed.

**Cost.** Price ranges for heat exchangers will depend on the specifications given to the company designing the heat exchanger. In the above mentioned analysis from Taniguchi, the 12,700 kW heat exchanger was expected to cost between $30 and $45 million in 2006 dollars (2006). These prices were again broken up by unit, with single
heat exchange units costing anywhere between $9,000 and $900,000 in 2006 dollars (depending on the unit and its size). It can again be expected that a significantly lower price would be required for a significantly lower energy requirement, so a very rough estimate of $500,000 in 2006 dollars, or about $600,000 in 2014 dollars, seems reasonable for a 240 kW heat exchanger. However, this cost estimate includes a lot of assumptions and should in no way be taken as a guaranteed price.

**Mixing Tank and Sparged Channel**

The focal point of the algal treatment system is the algal growth reactor, which in this case is a sparged channel. The treatment capacity of algae determines the size of reactor and recycle flows necessary to treat the leachate. A target effluent ammonia level of 3 mg/L was selected as a rough estimation based off acceptable ammonia discharge levels of 1-4 mg/L from a NPDES permit in Idaho (Davis, 2011; EPA, 2005).

**Size.** In order to size the sparged channel and mixing tank, the following assumptions were made:

- Algae will follow Monod kinetics using the following parameters:
  - Specific growth rate ($\mu$) = 0.67 day$^{-1}$ based off average specific growth rate found in cell growth experiment results. This is similar to an average 0.7 day$^{-1}$ specific growth rate found in a study by Ribita (2011)
  - Nitrogen is the limiting substrate
  - Half saturation constant for nitrogen ($K_S$) = 0.005 g/L (Ribita, 2011)
  - Yield coefficients from nitrogen ($Y_{X/N}$) = 11 g/g, based off of a combination of observed results in a study by Ribita and the average
molecular composition of algae (Ribita, 2011; Ebeling, Timmons, & Bisogni, 2006).

- Negligible cell death \( (k_d = 0) \), assuming that virtually no death of algae occurs as a result of toxins or of nutrient shortage and that algae is harvested before the end of its life span

- Channel functions as an ideal PFR assuming that the length:width ratio is over 50 (Reynolds & Richards, 1995)

- Maximum cell concentration of approximately 5 g/L, beyond which it is believed light would be unable to penetrate deep enough into the solution for sufficient algal growth (Ribita, 2011). Algal concentration must remain well below the maximum to assume no cell death.

- Algae concentration in PFR is 2-3 g/L, similar to the average concentration of bacteria in activated sludge basins in POTWs (Tchobanoglous & Schroeder, 1985; NMSU, 2007)

Mass balance equations for cell and substrate concentrations in a plug flow reactor with no mass accumulation are shown in equations 3 and 4 (Shuler & Kargi, 2002).

\[
\frac{dX}{dt} = \frac{\mu_{mS}}{(K_S+S)} X \\
\frac{dS}{dt} = -Y_S \frac{\mu_{mS}}{(K_S+S)} X
\]

(Equation 3)  
(Equation 4)

For PFR sizing equations, variables are defined below:

- \( X, S \) = cell, substrate concentration (g/L)
- \( \mu_{m} \) = maximum specific growth rate (day\(^{-1}\))
- \( K_S \) = half saturation constant (g/L)
- \( Y_S \) = yield (mass cell/mass substrate)
- \( t \) = time (days)
As can be seen in Equations 3 and 4, cell and substrate concentration are interdependent. Additionally, multiple variables could be manipulated to ensure nitrogen removal in the channel. Given sufficient time, algae could consume all influent nitrogen. However, this could result in an algal population well above what is sustainable. It is ideal to have a treatment plant which can treat influent streams as quickly as possible, and this could be achieved by increasing the initial cell concentration. Once again, cell concentration needs to stay within values that could be reasonably expected.

A Matlab model was generated from equations 3 and 4, and the output from this model can be seen below in Figure 15. In this model, a PFR with a starting cell concentration of 2.5 g/L algae reduced ammonia nitrogen concentration by 75.4 mg-N/L within 12 hours. This would theoretically result in an increase of algal population of 0.8 g/L, maintaining a population well under 5 g/L and keeping the average concentration within 2-3 g/L (per assumptions).

![Figure 15. Matlab model of changes in substrate and cell concentration for a PFR starting with 2.5 g/L algae cells and 75 mg/L ammonia (substrate).](image-url)
For the majority of the reaction within the PFR, cell growth and substrate consumption would be observed to occur at their maximum rate (due to no substrate inhibition and no cell death). In order to treat ammonia to under 3 mg-N/L, the influent stream would need to be diluted by recycle and/or flow augmentation to approximately 78 mg/L ammonia as nitrogen. Substrate consumption should not be significantly limited by a lack of substrate until ammonia levels fall below the half saturation value of 5 mg/L, so lowering initial concentrations of ammonia should not reduce algal efficiency and may prevent algae from seeing toxic effects of too much ammonia.

Using the algal cell mass and substrate concentrations from the Matlab model, a mass balance was performed on the PFR, mixing tank, and clarifier (pictured below in Figure 16). The total flowrate of liquid entering and leaving the system must be consistent, and it is assumed that no accumulation of substrate or cells is occurring.

The amounts of cells to be wasted and recycled were determined based on the total mass of cells produced. The concentration of algae leaving the PFR was predicted by the Matlab model to be 3.3 g/L, and an initial concentration of 2.5 g/L algae was a set variable. This change in concentration of algae would result in accumulation of biomass, so the same mass of algae produced must be wasted. This is shown in Equations 5 and 6 below (mass balances on the clarifier and mixing tank, respectively), which can be

![Figure 16. Flow arrangement for mixing tank, PFR, and clarifier, with all values listed.](image)
combined to form Equation 7. The variables $Q$ and $X$ stand for flowrate in L/min and cell concentration in g/L, respectively. The subscript $f$ denotes the value is from the end of the PFR, $i$ denotes the start of the PFR, $w$ denotes waste stream, and $r$ denotes recycle stream.

$$QX_f = Q_wX_w + Q_rX_r \quad \text{(Equation 5)}$$

$$QX_i = Q_rX_r \quad \text{(Equation 6)}$$

$$Q(X_f - X_i) = Q_wX_w \quad \text{(Equation 7)}$$

The average concentration of settled sludge from POTWs is between 4 and 10 g/L (Metcalf & Eddy, 2003). If it can be assumed that the density of settled algae is 8 g/L, then the values of $X_w$ and $X_r$ can be set to 8 g/L. $Q_w$ can then be solved for as approximately 10% of the total flowrate, $Q$. Additionally, $Q_r$ would equal 31.25% of $Q$.

The total flow can then be determined using a substrate mass balance and flow balance around the mixing tank, shown below in Equations 8 and 9. In these equations, the subscript $0$ denotes flow first entering the system (pretreated leachate). The subscript $r_2$ represents a second recycle stream coming from after the clarifier (with no algae concentration) in order to reduce the influent ammonia concentration from 1000 mg/L ($S_0$) to 78 mg/L ($S_i$) within the PFR. In addition, $M$ denotes a media stream to add any missing nutrients for the algae as well as to help dilute the leachate to an ammonia level which can be more rapidly treated by the algae. Ammonia content in this media stream would be 0 mg/L.

$$Q_0S_0 + Q_rS_r + Q_{r_2}S_{r_2} = QS_i \quad \text{(Equation 8)}$$

$$Q_0 + Q_r + Q_{r_2} + Q_M = Q \quad \text{(Equation 9)}$$

The substrate in recycle feeds is equal to the effluent substrate of 2.6 mg/L. Since $Q_r$ was already determined to be 31.25% of $Q$ and $Q_0$ is set to 202 L/min, the value of $Q_{r_2}$
can be established as a function of $Q$ and $Q_M$. The total mass balance for flow (Equation 10) constrains possible values for $Q$ based off of $Q_M$. $Q_e$ is the effluent flowrate.

$$Q_0 + Q_M = Q_W + Q_e$$  \hspace{2cm} (Equation 10)

If media is supplied at the same flowrate as the influent (202 L/min), the total flowrate for the PFR would be approximately 2665 L/min. Equation 10 would be satisfied, as the sum of the influent and media (404 L/min) would be greater than the wasted stream (266.5 L/min), resulting in an effluent flow of 137.5 L/min. Since the hydraulic retention time in the PFR must be about 12 hours for the treatment to occur to levels modeled, the total volume of the reactor would need to be just under 2000 m$^3$.

Since the reactor will be light limited if it is too deep, the depth should likely not be any more than 0.5 m. Additionally, the length:width ratio of the PFR needs to be over 50 to ensure that the reactor can be modeled as plug flow. This would give the reactor a plan area of 4000 m$^2$ or 43,000 ft$^2$ (approximately 1 acre). If the PFR is 5 meters wide, the length of the PFR would be 800 m (well over 50:1 ratio for length to width).

The “sludge age” ($\theta_c$) of the algae within the reactor, or how long it spends in the reactor on average before being wasted, was estimated using Equation 11 (Davis, 2011).

$$\theta_c = \frac{\text{mass algae}}{\text{algae wasting rate (mass/day)}} = \frac{vX_{\text{avg}}}{Q_WX_W}$$  \hspace{2cm} (Equation 11)

The approximate “sludge age” of the algae would be about 1.9 days, which is low compared to activated sludge basins where the average sludge age is 3-15 days (Davis, 2011). However, the main reason for longer retention times in bacteria systems is that slower growing organisms such as nitrifiers will wash out if the retention time is shorter than the time it takes them to reproduce (Davis, 2011). The doubling time of *Scenedesmus dimorphus* is approximately 1 day, which was calculated by deriving
Equation 3 to form Equation 12. In the derived form, the term \( \frac{\mu_m S}{K_S + S} \) is simplified to \( \mu \), the effective growth rate (in the case of \( S \gg K_s \), \( \mu = \mu_m \)), and \( t_d \) stands for doubling time, or the time when cell concentration \( X \) is twice the original concentration \( X_i \). With a doubling time of 1 day, the algae population would double at least once during 1.9 days of cell retention. Equations 5-12 from this section can be seen worked out in Appendix D.

\[
\ln\left(\frac{X}{X_i}\right) = \ln(2) = \mu \cdot t_d \quad \text{(Equation 12)}
\]

Because the total flow rate into the PFR is known at 2665 L/min, the size of the mixing tank can be determined to attempt to give algae a beneficial light:dark cycle. In experiments, 14 hours of light to 10 hours of dark was conducive to algae growth, so a corresponding light:dark cycle of 12 hours light to 8.5 hours of dark would hopefully produce similar results. To achieve a retention time of 8.5 hours in the mixing tank at 2665 L/min, the tank would need to be about 1400 m\(^3\) in volume. This mixing tank can be deeper than the PFR, so the plan area required can be much less than for the PFR. For a depth of 4m, the plan area of the mixing tank would be 350 m\(^2\) or under 3800 ft\(^2\).

**Additional Parameters.** Algae also require a carbon source in order to grow. In this case, the carbon source is bubbled in carbon dioxide from flue gas. According to literature cell stoichiometry, 5.75 moles of carbon dioxide are needed per mole of ammonia utilized (Ebeling, Timmons, & Bisogni, 2006). Since 75.4 mg-N/L ammonia is consumed during the 12 hours in the reactor, 1.36 g/L carbon dioxide would be needed during this same time frame. The solubility of carbon dioxide in water at 25 °C and 1 atm is 1.5 g/L, and at 30 °C the solubility is about 1.25 g/L, so there is a chance that the initial carbon dioxide requirement would not be met (National Center for Biotechnology Information, 2015). If flue gas is utilized to transfer carbon dioxide, the carbon dioxide
content in this flue gas is typically 13% so 500 CFM of landfill gas at STP would be needed at 100% gas transfer efficiency (Appendix D). However, transfer efficiency of carbon dioxide to water will not be 100% and if the maximum flowrate available is 1400 CFM the required carbon dioxide transfer efficiency for flue gas would be just under 40%. The mass flow rate of carbon dioxide necessary would be 220 kg/hr.

It may be of additional interest to look at the phosphorus balance in the reactor. Algal cells can accumulate around 13 mg phosphorus per cell, but can also potentially accumulate extra phosphorus for times of phosphorus deficiency (Larsdotter, 2006). Since cells grow by a concentration of 0.8 g/L, over 10.4 mg/L of phosphorus could be consumed during the reaction. The influent phosphorus concentration to the PFR can determined using Equation 13 below, but is dependent upon setting a phosphorus concentration for the media. For a hypothetical situation of complete phosphorus uptake, the media phosphorus concentration would need to be 127.2 mg/L. However, unless limitations are noticed in algae it would be preferential to not add nutrients to the PFR.

\[
Q_0P_0 + Q_TP_T + Q_{r2}P_{r2} + Q_MP_M = QIP_i \quad \text{(Equation 13)}
\]

**Cost.** From the Wastewater Handbook, the cost per installed m$^3$ of aeration basin was about $200-300 in 2006 dollars or $230-350 in 2014 dollars (van Haandel & van der Lubbe, 2007). For a 2000 m$^3$ aerated PFR at $350/m, the tank would cost $700,000. However, this price does not include the aeration grid for carbon dioxide diffusion or the cost of running the diffusers. The mixing tank would also require capital outlay, but could likely be obtained at under half the price of the aeration basin due to the smaller size and the simplicity of the mixing tank design. Mixing would also require energy input.
Clarifier and Autoflocculation

At the end of the PFR, algae need to settle out to enter waste or recycle streams. In a study on autoflocculation with \textit{S. obliquus} and \textit{P. tricornutum}, settling rates of 22-360 cm/hr were observed depending on the size of the algal cells (Spilling, Seppälä, & Tamminen, 2014).

**Size.** To size a clarifier utilizing autoflocculation the following assumptions were made:

- Algae settle out at 50 cm/hr, assuming the algae are smaller than \textit{P. tricornutum} in the study by Spilling, Seppälä, & Tamminen (2014)
- Flowrate entering the clarifier is 0.042 m$^3$/s (2750 L/min)
- A circular clarifier will be used following recommended side water depth:tank diameter ratios from Davis in Water and Wastewater Engineering (2011)

In order for algae to settle out at a settling velocity of 50 cm/hr the overflow rate must be less than 0.5 m/hr. For an overflow rate of less than 0.5 m/hr, a plan area of 302.5 m$^2$ or more is needed. For a circular clarifier, that would result in a diameter of about 20m. For a 20m diameter tank, the recommended sidewall depth would be 4m, resulting in a tank volume of 1,250 m$^3$ or approximately 44,000 ft$^3$ (Davis, 2011).

**Cost.** From the EPA cost estimating manual, the cost for a secondary clarifier that is 50,000 ft$^3$ would be approximately $164,000 in 1975 dollars (EPA, 1976). By adjusting using the consumer price index from 1974 and 2014 (53.8 and 236.7, respectively), an adjustment factor of 4.4 is obtained and can be used to calculate a cost of $722,000 in 2014 dollars (U.S. Department of Labor Bureau of Labor Statistics, 2015; Bielefeldt, 2002). In this case, the consumer price index was used instead of applying inflation.
directly due to the significant difference in time between when the cost estimate is provided for and when the cost estimate is desired for.

**Algal Biomass Utilization**

Based on the conditions within the PFR, approximately 3000 kg/day of algae is wasted from the treatment process. These algae have the potential to be utilized as a resource instead of a waste stream, and could save a significant amount of money.

**Economic Benefits.** Dried unidentified algal strains for varied non-food use sell for approximately $15/lb (Buyalgae, 2014). Assuming that the dried mass of the algae is 8% the wet mass and 240 kg/day (528 lb/day) is sold, up to $2,900,000 could be made from algae each year. However, this estimate ignores the price of shipping the algae and assumes that algae are not sold through a retailer. The actual value of algae may end up closer to $1-5/lb, or $200,000-$1,000,000/yr.

Algae could also be anaerobically digested to provide methane for energy generation. This digestion can produce 6 to 8 cubic feet of gas per pound of algae digested over the course of 30 days (Gouleke, Oswald, & Gotaas, 1957). The same 1957 study on digesting algae found that the percent methane produced was approximately 60%, which is higher than the percent of methane in landfill gas. The energy value of landfill gas is 350-600 Btu per cubic foot of gas (EPA, 2011). Using these values and assuming they are for wet algae, 3000 kg of algae produced in one year could be digested for 13-32 million Btu (3800-9500 kWh). At an industrial value of approximately 6-7 cents per kWh, this would amount to $200-660/year. Although the monetary value of the
electricity is lower than the value of harvested biomass, a facility that has an existing digester may find this option desirable.

One of the greater values of dried algae may be the use of algae produced on site in place of ordering an adsorption material. If algae are used to remediate metals in leachate pretreatment, the amount of money saved will be dependent on what material it is replacing. Activated carbon cost varies depending on the purity and purpose of the activated carbon, but prices for bulk granular activated carbon ranged from $1000-$4000/ton, or $0.50-2.00/lb (Slaughter, 2011; Cook & Tweet, 2015). Prices for bulk brown algae or kelp meal tended to range from $1350-$3700/ton, or $0.68-1.85/lb (Everwood Farm, 2015; North American Kelp, 2015). Although these adsorption materials may cost less per pound than the value of the dried algae, should the dried algae perform better than other materials or should it be difficult to get into the algal biomass market then there is a potential use of biomass produced on site.

**pH Regulation**

Leachate entering pretreatment in the sample treatment system has a pH of 7.7. This pH is on the high end as far as metal adsorption is concerned, so it may be necessary to lower the pH to 6 in order for more adsorption to occur. The exact amount of pH regulation and alkalinity adjustment during the process would depend upon the exact makeup of the leachate, including the hardness and alkalinity, and it is likely that pH and alkalinity would be monitored by continuous pH monitors and titrations in order to determine the exact amount of chemical addition necessary. Assuming that most of the pH adjustment can be managed by adjusting the carbon dioxide flow to the algae, the
chemical costs would be negligible. However, it may be necessary to pilot the leachate treatment system in order to support that claim.

Post-Treatment

Due to the microorganisms present in landfill leachate and in order to reduce the chance of releasing algae to the environment, it may be necessary to disinfect the treated leachate before discharging it. Chlorination is a relatively simple method of removing viable bacteria and algae from waters, but the concentration of chlorine needed would depend on the number of bacteria and algae cells that are in the effluent. Additionally, residual chlorine would need to be removed before effluent could be discharged. Post-treatment would likely be decided on a case-by-case basis, depending on the requirements for the receiving water body and the number of organisms present in the effluent.

Energy and Pumping

As mentioned before, the energy costs for a POTW are often up to 30% of the total operational costs (EPA, 2006). It is assumed that the algal remediation system would see similar energy costs, but there will be an additional energy burden of providing sufficient light to algal cells. If the light requirement for algae is 80 W/m², the higher end of light intensity provided to cells in the experimental section, then for a 4000 m² plan area 320 kW of light would need to be provided. If these lights need to be on all of the time, than the approximate energy use per year would be almost 3 million kWh. Using the same value of electricity from above (6-7 cents/kWh), this could result in energy
costs of $160,000-200,000 per year. Therefore, it may be wise for algal treatment plants to look into utilizing landfill gas combustion energy produced nearby or to look into solar, wind, or other alternative energy sources.

An additional concern for the algal treatment system is to ensure that the algal recycle process does not lyse and kill algae cells (as well as that algae do not clog whatever pumping or piping system is used for recycle). Any pump designed for mixed liquor return in a POTW will be designed to handle significant suspended solids and it is suspected this pump would not destroy live algae, but it may be a good idea to test the viability of algal cells in the pumping system prior to purchasing pumping equipment.

**Cost and Sizing Summary**

A summary of the approximate costs, economic returns, and size requirements for the algal treatment system can be found in Table 7.

<table>
<thead>
<tr>
<th>Unit Operation</th>
<th>Approximate Cost</th>
<th>Economic Return</th>
<th>Approximate Size Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids Removal</td>
<td>$200-400k</td>
<td>n/a</td>
<td>52 ft²</td>
</tr>
<tr>
<td>Metal Adsorption</td>
<td>Highly variable</td>
<td>Potential to sell harvested metals (value unknown)</td>
<td>negligible</td>
</tr>
<tr>
<td>Heat Exchanger</td>
<td>~$600k</td>
<td>n/a</td>
<td>&lt; 600 ft²</td>
</tr>
<tr>
<td>Mixing Tank</td>
<td>~$300-400k</td>
<td>n/a</td>
<td>3,800 ft²</td>
</tr>
<tr>
<td>PFR</td>
<td>$700k</td>
<td>n/a</td>
<td>43,000 ft²</td>
</tr>
<tr>
<td>Clarifier</td>
<td>$722k</td>
<td>n/a</td>
<td>3,300 ft²</td>
</tr>
<tr>
<td>Biomass Utilization</td>
<td>Costs associated with harvesting of algae, not calculated</td>
<td>$200-20,000/yr</td>
<td>n/a</td>
</tr>
<tr>
<td>pH adjustment and Post-Treatment</td>
<td>Dependent on treatment method</td>
<td>n/a</td>
<td>Dependent on treatment method</td>
</tr>
<tr>
<td>Energy and Pumping</td>
<td>0.3 * Total Operational Costs + $160-200k/yr for lights</td>
<td>n/a</td>
<td>Expected similar to POTW plan area for pumping</td>
</tr>
</tbody>
</table>
By comparison, a pre-packaged WWTP capable of meeting 1979 discharge standards for approximate 1 MGD would cost approximately $1.7 million in 2014 dollars to construct (EPA, 1979). To improve an existing 2.3 MGD (non-packaged) facility for greater nutrient removal cost one treatment plant over $20 million in 2014 dollars (Carollo, 2014).

Considerations and Potential Limitations

As a full-scale implementation of this design does not yet exist, it is expected that there will be unforeseen challenges and drawbacks to the system. However, many of the issues that may present themselves can be anticipated in order to mitigate their effects.

One of the largest uncertainties of this system will be the leachate itself. Leachate strength will vary with time and precipitation. Modeling and prediction of the expected leachate concentration is difficult due to the interactions of old cells (which change based on age and rainwater infiltration) and new cells (which change more dramatically with infiltration and also change based on incoming waste composition). To help make rough predictions about leachate composition, historical data of the changes in leachate composition based on rainfall and age should be analyzed. Knowledge of the approximate percentage of leachate coming from new cells can also help in calculating expected variance.

An additional problem in leachate composition within unmixed tanks such as most holding ponds is the “last in first out” phenomenon, where water from the inlet (in this case, pumped in from the landfill liner) reaches the outlet (pumped out for treatment) sooner than water which has been in the tank for a longer period of time (Duer, 2011). In
unmixed leachate holding containers, there is no guarantee that changes in leachate composition will be reflected by the leachate withdrawn for treatment. If leachate holding tanks and ponds are mixed, a more uniform leachate composition can be attained and changes in leachate can be moderated by the full volume of the leachate. The leachate composition would also be easier to anticipate as a function of precipitation, but mixing would require energy input and maintenance.

The most important safeguards against variations will be monitoring and designing the system with a substantial factor of safety. Algae should be fed less than the predicted maximum concentration of leachate at all times, and test pilots with algae and leachate should be done with high strength leachate. A backup culture of algae should also be available to re-seed algal bioreactors at any point if the algal population dies out.

Another concern for the proposed design is the effect of seasonal variation in temperature and leachate production. If leachate is not adequately insulated, there is a potential for leachate to freeze during cold winters and halt the treatment process. A lack of insulation can also result in different seasonal algal growth. In locations with distinct wet and dry seasons, it may be difficult to run year-round treatment. Because algae will need to be provided artificial light to ensure light penetration, there should not be significant seasonal light limitations based on sunlight. However, assuming that light can penetrate through the whole volume of a 0.5 m deep reactor is likely overly optimistic, and it may be necessary to look into alternate algae growing schemes such as horizontal algae tubes.

As with POTW activated sludge basins, if algal bioreactors are shut down their live cultures are lost. As existing activated sludge colonies are easier to obtain than algal
colonies which are accustomed to landfill leachate, it is important to find a way to keep the treatment process running at or above a minimum flow rate year-round. Adequate heating and insulation must be available to keep leachate above freezing during winter treatment system operation. Leachate storage during the wet months can allow for some flow during dry months, but storing large volumes of leachate is likely neither feasible nor desirable for most landfills. Leachate production during dry months occurs mostly from decomposition within the landfill, and tends to be higher strength than leachate formed during the wet months. It is possible to dilute dry month leachate by a larger factor in order to make up part of the difference, and the remainder of this difference could be made up by increasing the percentage of the treated leachate that is recycled through the bioreactor or by supplementing with growth media.

Potential high temperature loads and algal release have been previously discussed. However, both must be considered in plant design. Many waterways have strict temperature load regulations, and water that is at ideal growth temperatures for algae (20-30 °C) will be too warm for discharge. During cold months, this can be remediated by allowing water to cool in wetlands prior to entering the waterways. However, during summer months the temperature will be harder to reduce and may require energy-intensive treatment. Algal release can be prevented by removing or killing all viable algal cells before they exit the treatment plant. Chlorination of treated water prior to discharge would be one method of ensuring that viable cells do not escape, and additional options include microfiltration, UV light irradiation, and thermal shock.

Finally, structural concerns may exist if leachate has corrosive properties. If this is the case, pipes and tanks which come in direct contact with leachate will need to be built
from materials that do not corrode. Leachate also contains inorganics that can cause scaling in pipes, so maintenance may need to occur more often in a treatment plant containing leachate. It may also be necessary to confirm that leachate will behave similarly to water before that assumption is used in treatment plant design.

The above list of considerations is not extensive, and additional concerns will exist for each treatment plant. Many site specific concerns can be addressed through pilot scale testing using the algae and leachate that will be used in a specific system.

**Conclusions and Further Work**

From the experiments performed, the possibility of algal treatment of leachate is evident. The alga *Scenedesmus dimorphus* has shown the ability to survive in diluted untreated leachate, and algae are well known to utilize ammonia in waters for growth. Although additional treatment steps are necessary to ensure water meets quality requirements and construction of a treatment plant would require significant capital outlay, algal treatment of landfill leachate has the possibility to reduce the annual cost of leachate treatment for landfills and in time pay for itself.

To ensure the success of algal treatment of leachate, additional tests would be necessary for the leachate utilized in any remediation application. Nitrogen uptake per gram of cells per hour and maximum cell growth rate at different dilutions of leachate would allow for approximate sizing of reactors in design applications, and values for metal removal would be useful for economic evaluation of the metal adsorption process. Any site considering algal remediation would have to initiate pilot testing with their site’s leachate and selected algal strains due to the variations between leachates and species of
alga. Specifically, sites would need to know constituents present in their leachate in order to choose pretreatment as necessary. Sites would also need to know growth rates of the selected algal strain in different dilutions of leachate in order to approximate growth in the bioreactor, and nitrogen utilization within the desired leachate composition would be necessary to size reactors and recycle feeds.

Future work on this subject includes the maximum metal uptake capacity and metals recovery rate for kelp meal and heat killed *S. dimorphus* in order to assess feasibility of using dried algae for metal removal. A bench scale study of the entire proposed treatment system would help to find treatment capacity of the proposed system for leachate used in this study, and analysis of additional pollutants treated by algal remediation would be useful in assessing the final quality of leachate.

As regulations on water quality continue to require cleaner water, the search for inexpensive but effective water treatment methods has turned its focus to bioremediation. Although additional research is necessary to implement this treatment system, algal remediation of landfill leachate has the potential to turn a toxic waste stream into a resource. With adequate pretreatment and dilution, microalgae could thrive in landfill leachate and serve a double purpose of treating high strength waste streams while producing marketable biomass for non-food uses.
References


EPA. (2000). Wastewater Technology Factsheet. 3.


**PROPOSED ALGAL LANDFILL LEACHATE TREATMENT SYSTEM**

*Constructed Wetlands for the Treatment of Landfill Leachates* (pp. 9-10). Boca Raton, FL: CRC Press LLC.


PROPOSED ALGAL LANDFILL LEACHATE TREATMENT SYSTEM


Appendix A

Leachate Pretreatment Data

<table>
<thead>
<tr>
<th></th>
<th>Flask 1</th>
<th>Flask 2</th>
<th>Flask 3</th>
<th>Flask 4</th>
<th>Flask 5</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Leachate (g)</td>
<td>1207.5</td>
<td>1106.4</td>
<td>1039.9</td>
<td>911.1</td>
<td>863.9</td>
<td>804.9</td>
</tr>
<tr>
<td>Mass Kelp Meal (g)</td>
<td>12.1</td>
<td>11.1</td>
<td>10.4</td>
<td>9.1</td>
<td>8.6</td>
<td></td>
</tr>
</tbody>
</table>

Leachate and kelp meal masses from pretreatment (White, Acid Washed Brown Algae Meal Adsorption of Metals by Serial Batch Adsorption, 2014a)

*Note: The density of untreated leachate was 0.99 g/mL; leachate clarified by algae had a similar density.*

<table>
<thead>
<tr>
<th></th>
<th>Mass of leachate (g)</th>
<th>Total nitrogen concentration (mg-N/L)</th>
<th>Nitrogen Mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarified leachate</td>
<td>1000</td>
<td>1525</td>
<td>1540</td>
</tr>
<tr>
<td>Distilled leachate</td>
<td>621.9</td>
<td>219</td>
<td>137.6</td>
</tr>
<tr>
<td>Distillate</td>
<td>350.4</td>
<td>2844</td>
<td>1006.6</td>
</tr>
<tr>
<td>Sum of distillate and</td>
<td>972.3</td>
<td>-</td>
<td>1144.2</td>
</tr>
<tr>
<td>distilled leachate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not in treated leachate</td>
<td>27.7</td>
<td>-</td>
<td>395.8</td>
</tr>
</tbody>
</table>

Nitrogen and mass balance from distilled leachate (White, Removal of Ammonia by Distillation of Clarified Leachate, 2014b)

*Note: The density of clarified leachate was approximately 0.99 g/mL; distillate and distilled leachate had similar densities.*
Appendix B

Nitrogen Removal Data

<table>
<thead>
<tr>
<th>Time from start of experiment (days)</th>
<th>0.1</th>
<th>1.1</th>
<th>2.3</th>
<th>3.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen (mg-N/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>345</td>
<td>301</td>
<td>250</td>
<td>350</td>
</tr>
<tr>
<td>25%</td>
<td>300</td>
<td>213</td>
<td>212</td>
<td>321</td>
</tr>
<tr>
<td>50%</td>
<td>828</td>
<td>459</td>
<td>436</td>
<td>933</td>
</tr>
<tr>
<td>100%</td>
<td>1800</td>
<td>406</td>
<td>406</td>
<td>1988*</td>
</tr>
</tbody>
</table>

Measured total nitrogen in diluted, centrifuged samples from algae grown in 25-100% pretreated leachate in DI water and 0% leachate in BG-11 media (control).

*Assay was spilled during assay preparation so chemical:solution ratios were not the same as in other experiments. This datum was discarded.

<table>
<thead>
<tr>
<th>Time from start of experiment (days)</th>
<th>0.1</th>
<th>1.1</th>
<th>2.3</th>
<th>3.1</th>
<th>4.3</th>
<th>5.3</th>
<th>6.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell mass (mg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>1.41</td>
<td>1.78</td>
<td>2.11</td>
<td>2.14</td>
<td>2.28</td>
<td>2.48</td>
<td>2.72</td>
</tr>
<tr>
<td>25%</td>
<td>1.35</td>
<td>1.28</td>
<td>1.73</td>
<td>1.72</td>
<td>2</td>
<td>1.96</td>
<td>2.01</td>
</tr>
<tr>
<td>50%</td>
<td>1.57</td>
<td>1.12*</td>
<td>1.83</td>
<td>1.85</td>
<td>2.17</td>
<td>2.6</td>
<td>3.09</td>
</tr>
<tr>
<td>100%</td>
<td>1.31</td>
<td>1.56</td>
<td>4.38**</td>
<td>1.3</td>
<td>2.03</td>
<td>4.56**</td>
<td>2.27</td>
</tr>
</tbody>
</table>

Measured dry cell masses from 1 mL of 25-100% pretreated leachate in DI water and 0% leachate in BG-11 media (control).

*This datum is potentially artificially low due to a noticeable amount of filter paper left behind on the ceramic drying plate which held the filter paper.

**Cells were clumped together when placed on drying filter and it is believed that cells had not fully dried when these measurements were taken.
## Appendix C

### Quantitative Growth Data

<table>
<thead>
<tr>
<th>Time from start of experiment (days)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>0%</td>
<td>5%</td>
<td>10%</td>
<td>15%</td>
<td>20%</td>
</tr>
<tr>
<td>Average cell density (cells*10^6/mL)</td>
<td>0.56</td>
<td>0.45</td>
<td>1.11</td>
<td>0.79</td>
<td>1.15</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.49</td>
<td>1.53</td>
<td>3.71</td>
<td>2.58</td>
<td>3.15</td>
</tr>
<tr>
<td>Average cell density (cells*10^6/mL)</td>
<td>2.96</td>
<td>5.96</td>
<td>7.66</td>
<td>7.08</td>
<td>2.84</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.89</td>
<td>4.15</td>
<td>4.09</td>
<td>3.80</td>
<td>2.38</td>
</tr>
<tr>
<td>Average cell density (cells*10^6/mL)</td>
<td>5.52</td>
<td>10.4</td>
<td>14.24</td>
<td>12.5</td>
<td>9.28</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>3.01</td>
<td>3.01</td>
<td>2.39</td>
<td>3.85</td>
<td>3.94</td>
</tr>
</tbody>
</table>

Average cell densities and standard deviations for days 1-5

<table>
<thead>
<tr>
<th>0% Leachate Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1 2 2 average 2.625 Cells b) 7 1 4 4 average 3.31 cells</td>
</tr>
<tr>
<td>2 1 5 6 dilution 20</td>
</tr>
<tr>
<td>0 2 4 4 density 8.4*10^6 cells/mL</td>
</tr>
<tr>
<td>5 0 2 5 std dev 1.93</td>
</tr>
</tbody>
</table>

Sample cell count for 0% leachate dilution on day 5. Cell densities were determined using cell count numbers, dilution factor, and known hemocytometer volume of 6.25 * 10^-6 mL.
Appendix D

Worked Out Equations from Sizing Section

Given values and solved values from Equations 3 and 4 (Matlab):

\[ \mu = 0.67 \text{ d}^{-1} \]
\[ X_i = 2.5 \text{ g/L} \]
\[ X_f = 3.3 \text{ g/L} \]
\[ X_w, X_r = 8.0 \text{ g/L} \]
\[ S_0 = 1000 \text{ mg/L} \]
\[ S_i = 78 \text{ mg/L} \]
\[ S_r, S_r2 = 2.6 \text{ mg/L} \]
\[ Q_o = 202 \text{ L/min} \]
\[ Q_M = 202 \text{ L/min} \]
\[ \Theta_h = 12 \text{ hours} \]

Equation 5: \[ QX_f = Q_WX_W + Q_rX_r \]

Equation 6: \[ QX_i = Q_rX_r \]

Equation 7: \[ Q(X_f - X_i) = Q_WX_W \]

\[ \frac{Q_r}{Q} = \frac{2.5 \frac{g}{L}}{8.0 \frac{g}{L}} \Rightarrow Q_r = 0.3125Q \]

\[ \frac{Q_W}{Q} = \frac{(3.3 \frac{g}{L} - 2.5 \frac{g}{L})}{8.0 \frac{g}{L}} \Rightarrow Q_W = 0.10Q \]

Equation 8: \[ Q_oS_0 + Q_rS_r + Q_r2S_r2 = QS_i \]

Equation 9: \[ Q_o + Q_r + Q_r2 + Q_M = Q \]

Equation 10: \[ Q_o + Q_M = Q_W + Q_e \]

\[ 202 \frac{L}{\text{min}} + 0.3125Q + Q_r2 + 202 \frac{L}{\text{min}} = Q, \quad Q_r2 = 0.6875Q - 404 \frac{L}{\text{min}} \]

\[ 202 \frac{L}{\text{min}} \left(1000 \frac{mg}{L}\right) + 0.3125Q \left(2.6 \frac{mg}{L}\right) + \left(0.6875Q - 404 \frac{L}{\text{min}}\right) \left(2.6 \frac{mg}{L}\right) = Q \left(78 \frac{mg}{L}\right) \]

\[ Q \approx 2665 \frac{L}{\text{min}}, \quad Q_r = 832.8 \frac{L}{\text{min}}, \quad Q_r2 = 1428.2 \frac{L}{\text{min}}, \quad Q_W = 266.5 \frac{L}{\text{min}}, \quad Q_e = 137.5 \frac{L}{\text{min}} \]
Equation 10: \( \theta_C = \frac{\text{mass algae}}{\text{algae wasting rate (mass/day)}} = \frac{\nu x_{\text{avg}}}{Q_w x_W} \)

\[
V = Q \cdot \theta_h = 1980 \text{ m}^3 \approx 2000 \text{ m}^3
\]

\[
\theta_C = \frac{2000 \text{ m}^3 \left( \frac{2.5 g}{L} + \frac{3.3 g}{L} \right)}{266.5 \left( \frac{L}{\text{min}} \right) \left( \frac{1440 \text{ min}}{1 \text{ d}} \right)} = 1.83 \text{ d}
\]

Equation 11: \( \ln \left( \frac{x}{x_i} \right) = \ln(2) = \mu \cdot t_d \)

\[
t_d = \frac{\ln(2)}{0.67 \text{ d}^{-1}} = 1.03 \text{ d}
\]

**CO₂ requirement calculations:**

From a cell stoichiometry of \( \text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P} \), the molar equation for cell growth using ammonia and carbon dioxide would be:

\[
16 \text{NH}_4^+ + 92 \text{CO}_2 + 92 \text{H}_2\text{O} + 14 \text{HCO}_3^- + \text{HPO}_4^{2-} \rightarrow \text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P} + 106 \text{O}_2
\]

(Ebeling, Timmons, & Bisogni, 2006)

The molar mass of nitrogen is approximately 14 g/mol, and the molar mass of carbon dioxide is approximately 44 g/mol, so a molar ratio of 16 moles ammonia to 92 moles carbon dioxide when the ammonia uptake is 75.4 mg/L as nitrogen would translate to:

\[
75.4 \frac{\text{mg(N)}}{L} \cdot \frac{1 \text{ mmol(N)}}{14 \text{ mg(N)}} \cdot \frac{92 \text{ mmol(CO}_2)}{16 \text{ mmol(N)}} \cdot \frac{44 \text{ mg(CO}_2)}{1 \text{ mmol(CO}_2)} \approx 1360 \frac{\text{mg}}{L} \text{CO}_2
\]

At STP, 1 mole of carbon dioxide (44 g) would have a volume 22.4 liters. For a leachate flowrate of 2665 L/min and 13% carbon dioxide in flue gas, the flue gas flowrate needed would be:

\[
1360 \frac{\text{mg}}{L} \text{CO}_2 \cdot \frac{22.4 L}{44,000 \text{ mg}} \cdot 2665 \frac{L}{\text{min}} = 1845 \frac{L}{\text{min}} \text{ CO}_2
\]

\[
1845 \frac{L}{\text{min}} \text{ CO}_2 \cdot \frac{1 \text{ ft}^3}{28.3 L} = 64 \frac{\text{ft}^3}{\text{min}} = 64 \text{ CFM} \text{ CO}_2 \cdot \frac{\text{flue gas}}{0.13 \text{ CO}_2} = 501 \text{ CFM flue gas}
\]

\( 501 \text{ CFM} \approx 500 \text{ CFM flue gas} \)