ALGAE CULTIVATION ON LANDFILL LEACHATE: EXPLORING A NOVEL NUTRIENT SUPPLY FOR SUSTAINABLE BIO-RESOURCE PRODUCTION

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2012

This work is dedicated to my wife, Haley, and my son, Kepler.

ACKNOWLEDGEMENTS

I thank my family for their whole-hearted support of my algae endeavors. It is because of their patience and love that I am blessed with the opportunity to pursue such fantastic scientific adventures.

I gratefully acknowledge the guidance and support of Dr. Wilkie, without whom I would still be studying non-microscopic subjects without a macroscopic perspective. Dr. Wilkie's tireless pursuit of sustainability is nearly unbelievable and has inspired the holistic concepts developed herein.

I also thank my thesis committee members, Dr. Phlips and Dr. Rathinasabapathi, who have been encouraging professors in my graduate experience.

Thanks are due to my Bioenergy and Sustainable Technology Laboratory colleagues, James Duncan, Jon Alldridge, Ryan Graunke, and Reginald Toussaint, for moral support, as well as philosophical and technical discussions. Thanks are also due to the undergraduate algae teams of the 2010 and 2011 BioEnergy and Sustainability Schools.

This work was supported by the Alachua County Public Works Department and the Hinkley Center for Solid and Hazardous Waste Management.

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LIST OF ABBREVIATIONS

AB	Aeration Basin
ACSWL	Alachua County South West Landfill
BBM	Bold’s Basal Medium
DEP	Department of Environmental Protection
HRAP	High Rate Algae Pond
LL	Landfill Leachate
MSW	Municipal Solid Waste
SR	Slope Reactor
TAN	Total ammoniacal nitrogen

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

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May 2012

Chair: Ann C. Wilkie
Major: Interdisciplinary Ecology

Human systems must become more sustainable if survival beyond the next several generations is intended. The most elegant template for creating and sustaining complex systems is the natural ecosystem. In natural systems nothing is wasted. Human systems should emulate this technique for sustainability. Photosynthetic algae comprise the trophic base of many ecosystems and have significant potential in contributing to sustainable biofuel production, CO_2 mitigation, and waste remediation. Municipal solid waste landfills are a common final repository for much of society's wastes. Landfills produce liquid leachates, which must be managed and remediated to prevent long-term community health impacts. In the remediation of landfill leachate, fossil energy is expended to ensure continuing environmental quality. These considerable anthropogenic deposits and the leachates which ensue, embody unutilized elemental resources. Photosynthetic algae provide a remediation alternative, which simultaneously produces bioresources with societal value from the biological assimilation of elemental nutrients within landfill leachate. In this study, a closed municipal solid waste landfill in Alachua County, FL was bioprospected for native algae

with potential in remediating landfill leachate and bioresource production. A total of 17 genera were observed from 15 sampling locations. Genera were screened for lipid production using Nile Red; notable genera include *Chlorella*, *Ankistrodesmus*, *Navicula, and Scenedesmus*. Elemental analysis shows that leachate contains all required elements for photosynthetic growth; however, in cultivation experiments leachate exhibited a strong inhibitory effect on algae growth correlated with concentration. Through investigative experimentation, toxicity within landfill leachate was attributed to the presence of unionized ammonia. Growth of algae on leachate without pH control is inhibited above a 10% landfill leachate concentration. Algae growth is supported only under the appropriate conditions of pH regulation, which can be accomplished by the addition of carbon dioxide or hydrochloric acid to the algae culture. If pH regulation is applied, growth is possible in MSW landfill leachate concentrations of 100%, a value not reported in the current literature.

CHAPTER 1
INTRODUCTION

Cultivating a Sustainable Civilization

Ecosystems are composed of communities, which continuously recycle elements through solar energy captured by photosynthesis. Autotrophs fuel the activities of all ecosystems, with photosynthetic autotrophs supplying the vast majority of the Earth's organic carbon and molecular oxygen. Human systems should aspire to model resource production and waste remediation processes by ecological concepts. Orchestrating primary producers and microbial consortia to recycle elements within human wastes while regenerating bio-resources is a progression towards human sustainability. At present, human systems are artificially supported by heavy inputs of nonrenewable energy. Fossil fuels (*i.e.* coal, petroleum and natural gas) fuel both the production of resources and the remediation of wastes for human communities. Production of resources from fossil fuels can be through direct synthesis (*e.g*. plastics), fertilizers for agricultural, or electrical generation. As a community, human civilization is artificially supported in both the creation of inputs and the treatment of waste products. This system is susceptible to collapse upon the inevitable exhaustion of fossil fuels (Shafiee and Topal 2009; Stephens *et al.* 2010). Despite this susceptibility, the human global population is expanding and consequently the amount of energy consumed and waste generated remains a growing issue, especially for future generations. Human ingenuity can be applied to support civilization after the fossil fuel era. Functional ecosystems are a model of production and remediation that human communities can apply for the production of resources and the treatment of wastes. For future

generations to thrive, ecologically inspired concepts must be developed into functional, self-supporting human systems.

Photosynthetic Foundation

Photosynthetic algae have significant potential to contribute to the global production of bioresources and the mitigation of anthropogenic wastes (Huntley and Redalje 2007; Stephens *et al.* 2010; Rawat *et al.* 2011). Depending on both the species of the algae and the cultivation conditions, algae can generate high quality proteins, unique polysaccharides, lipids, and pigments. Of particular interest is the biosynthesis of lipids, especially triglycerides for the production of renewable fuel resources, such as biodiesel and jet fuels. Lipids from algae can be converted into liquid transport fuels using conventional petroleum refining or biodiesel production techniques (Sheehan *et al.* 1998), analogous to the production of biodiesel from the lipids of other plants (Durrett *et al.* 2008). The vast majority of biodiesel currently produced in the U.S. utilizes soybean oil as the lipid feedstock. The soundness of converting food into fuels is dubious, with potential to cause shortages and price volatility in global food supplies (Tenenbaum 2008). Regardless of ethical and economic considerations, soybean oil is fundamentally incapable of meeting the U.S. diesel demand. A recent life cycle analysis shows that conversion of the entire U.S. soybean crop to biodiesel would meet only ~6% of the national diesel demand, which drops to 2.9% when the diesel consumed in the production and processing of the soybean is taken into account (Hill *et al.* 2006). Due to the use of arable land, fertilizers, potable water, and agricultural equipment, the production of biodiesel fuel via the chemical conversion of food oils is a questionable practice in terms of sustainability. Oils produced by algae, however, have the potential to substantially contribute to the production of renewable fuels (Chisti 2007; Wijffels and

Barbosa 2010; Stephens *et al.* 2010) on a scale that can potentially meet global energy demand (Huntley and Redalje 2007).

The concept of cultivating algae on a large scale for bioresources is a comparatively new idea in the agronomic history of humans. The development of techniques for the mass cultivation of algae only arose in the mid twentieth century (Cook 1949; Burlew 1953). At the time, the principal impetus for algae mass cultivation was protein production for human consumption (Burlew 1953). To this day, only several cultivation systems are used in the commercial scale production of algae. Among these are two general classes; the outdoor open pond and the closed photobioreactor (Becker 1994; Richmond 2004). Outdoor open pond reactors are generally applied in waste treatment settings and are considered more cost effective, but suffer from contamination and low productivity. The high rate algal pond (HRAP) is the classic example of outdoor open pond cultivation (Oswald 1957). Enclosed tubular photobioreactors are commonly used for the production of high value products (*e.g.* astaxanthin). Hybrid reactors blending cultivation strategies from both open and closed systems have potential in providing resources on a large scale (Huntley and Redalje 2007). Additionally, algae can be grown heterotrophically on fixed carbon substrates (*e.g.* glucose), this cultivation strategy renders the production of algae dependent on the sustainability of the carbon source, and may initiate the food vs. fuel argument against algal biofuel production.

If algae are to be cultivated for global renewable resource production a sustainable means of cultivation must be developed. A recent life cycle analysis of algae derived biodiesel show that current production methods are not yet sustainable (Lardon et al. 2009). A critical factor in the sustainability of photosynthetic resource production via

algae is the requirement of elemental nutrients, many of which are non-renewable (*e.g.* rock phosphate). Utilizing anthropogenic waste nutrients may allow for the dual purpose of remediation and resource production (Rawat *et al.* 2011), contributing to the foundation of human sustainability.

Algal BioRemediation

Inadvertent release of nutrients from human activities into natural systems can have marked impacts on the trophic state of natural water bodies (Hutchinson 1969). This anthropogenic process, known as cultural eutrophication, can have long-lasting impacts on the biological communities as well as nearby human populations. The results of cultural eutrophication are often manifested as extensive algae blooms. This outburst of photosynthetic production eventually exhausts the nutrient resources that gave rise to the bloom (typically nitrogen or phosphorus) and collapse. Fixed carbon of the algal biomass provides the biota of the benthos with a repast of organic material. Aerobic microbes quickly consume the dissolved oxygen within the water body leading to hypoxic conditions. A decrease in the available dissolved oxygen is deadly to complex aerobic organisms (fish, amphibians, arthropods, etc.). Nutrient-rich wastes significantly impact surrounding ecological systems, as seen in the numerous global examples of harmful algae blooms and aquatic dead zones. Many times in the public perception the algae blooms are the problem, however the nutrient discharge from under managed human systems are the root cause of the perturbations. Instances of cultural eutrophication are manifestations of missed opportunity in bioresource production. Incorporating bioremediation with resource production is the ultimate achievement in sustainability.

Algal assimilation of mineral nutrients from wastewaters is a well-documented phenomenon in the scientific literature (Lincoln *et al.* 1996; Hoffmann 1998; Wilkie and Mulbry 2002). Algae are recognized for their ability in thriving in polluted waters and are the primary biological force in the self-purification of streams. The use of algae to remediate pollution is often referred to as *phycoremediation* (Olguin 2003). Research has typically focused on treatment of sewage and manure effluents (Oswald 1957; Oswald 2003; Wilkie and Mulbry 2002). Treatment of anthropogenic wastewaters with algae provides both waste remediation and resource production services. Phycoremediation of wastes can give both an ecologic and economic advantage over conventionally used chemical and physical nutrient removal techniques, as these are usually energy intensive processes. The potential that algal species have in treating excessive nutrients in municipal wastewaters is demonstrated in the efficient and effective removal of nitrogen and phosphorus (Hoffman 1998; Wilkie and Mulbry 2002). Oxygen generated through algal photosynthetic activity fuels the aerobic bacterial population, which consume and oxidize organic wastes, reducing the wastewaters biological oxygen demand (BOD). Biological oxygen demand is a key criterion for the ecological impact of a wastewater on a natural ecosystem. Additionally, algae are able to uptake and sequester nitrogen (as ammonium and nitrate) and phosphorus present in wastewaters. Algae are able to scavenge these nutrients from the aqueous environment, preventing the unintentional eutrophication of natural water bodies and springs by wastewaters.

Algal production systems may be utilized to simultaneously prevent cultural eutrophication and provide resource production. Autotrophic photosynthesizers: plants,

algae, and certain bacteria, comprise the energetic base of the entire planet. If society is to re-establish and manage an integrated human ecosystem it is imperative that biological wastes are productively returned to the foundation of the global ecosystem. However, the application and implementation of algal mass cultivation is a technology that remains in the development stage.

Ecological Recycling: A Focus on Liquid Leachates from Landfill

Municipal solid waste (MSW) is abundant. On average each citizen of the U.S. generates 4.34 pounds of MSW per day (EPA 2009). Landfilling solid wastes is the most commonly practiced method of waste management. As human societies progressively move towards more sustainable waste management options, it is imperative that long-term ecological solutions are explored thoroughly for the benefit of future generations. As human populations grow in both developed and developing countries the amount of wastes accumulating within landfills also continues to grow. Landfills offer public health and environmental benefits over more primitive open dumping, reducing contact between municipal wastes and the surrounding human and wildlife populations by confining environmental impacts. Despite these sanitary and ecological advantages of landfills, accumulated wastes inevitably generate both gaseous and aqueous pollutants. Landfills can generate these pollutants for decades post closure and must be managed until the emissions of contaminants have subsided. The appropriate management of these highly mobile environmental pollutants is of critical importance in the long-term health and protection of human and wildlife communities. Landfills may provide algal cultivation with large amounts of fertilized, aqueous media and carbon dioxide- the necessary resources for the cultivation of algae bioresources on a significant scale. Renewable resource generation and carbon

mitigation occur while algae provide biological treatment of the landfill leachate. Algal biomass harvested from the cultivation system can be regionally processed into a variety of commodities, depending on local needs. Lipids extracted from the algae biomass can be processed into biodiesel or other renewable fuels, while the non-lipid residue can be used as an animal feed, applied as a soil amendment, or anaerobically digested to produce methane. Combustion of methane can provide the energy requirements for the cultivation of the algae system and may be essential in the sustainability of the production of algal bioresources and bioremediation. Algae cultivation paired with waste nutrient recycling and anaerobic digestion offers the possibility of a sustainable resource cycle (Figure 1-1).

Landfill Environmental Contaminants

A consequence of any waste collection site is the accumulation of liquids and the emission of gases from microbial and chemical degradation. These outcomes are magnified for landfill sites as both gas and liquid wastes are concentrated for ease of management. Gaseous emissions include carbon dioxide (CO_2), methane (CH_4), and a wide range of volatile organic compounds (VOCs). Carbon dioxide and CH_4 are both potent greenhouse gases. Contaminated liquids, or leachates, dissolve components within the waste as water from rainfall or biological degradation percolates through the solid refuse within a landfill cell. Landfill leachates typically have high levels of chemical oxygen demand (COD), ammonia-nitrogen, suspended solids, VOCs, xenobiotic organic compounds (XOCs), and dissolved metals (Kjeldsen *et al.* 2002). These compounds need to be reduced or removed by treatment due to their toxicity (Wiszniowski *et al.* 2006). Total ammonia nitrogen in leachate is frequently reported at

extremely high concentrations of 2,000 mg/L, total ammonia-nitrogen in leachates worldwide can range from 0.2-13,000 mg/L (Renou *et al.* 2008). Ammonium is released from decomposing organic matter high in proteins and amino acids, such as food and yard waste. High concentrations of ammonia nitrogen are toxic to biological systems. Furthermore, ammonia concentrations may persist in the leachate with time, so that ammonia has been regarded as the most problematic constituent in leachate over the long term (Kjeldsen *et al.* 2002).

Landfill leachates, if not properly managed, can cause serious negative impacts to surface and groundwater quality. Furthermore, the volumes of leachate produced are proportional to the size of the waste facility and are produced for many years even after the closure of a landfill. Unlined landfills, or an accidental breach of lined containment facilities can threaten the integrity of both surface and groundwater resources, negatively impacting the surrounding human and natural communities. Remediation of leachate-impacted areas after such an incident can be costly and take decades for full ecological restoration (Crawford and Smith 1985). Therefore, methods for effectively and efficiently collecting and treating leachates from landfills are of key interest in protecting environmental quality.

Landfill Leachates in Florida:

With numerous outstanding lakes, rivers, and a critical groundwater supply, Florida is especially vulnerable to the negative consequences of leachate contamination in both surface and groundwater supplies. As recently as 1980, open dumps were the most commonly employed method of trash disposal. Florida alone had over 500 open dumps at this time (FDEP 2001). By 1985 the state of Florida required all Class I municipal

solid waste (MSW) landfills to have liners. As of 2009, 55% of all municipal solid waste generated in Florida was landfilled (Figure 1-2).

Florida has 60 active Class I MSW Landfills (Figure 1-3). All Class I MSW landfills are required to have a liner system and to manage the leachate, which by consequence accumulates on the liner system. Class II and III are assumed not to produce leachates of environmental concern and are not required to have liners. Leachate quality can vary significantly from site to site and over time (Figure 1-4). MSW landfill leachates of Florida have a significant range of values for investigated analytes, including pH, biological oxygen demand (BOD), chemical oxygen demand (COD), ammonia-nitrogen, sulfate, chloride, manganese, and zinc (Reinhart and Grosh 1998). Many factors contribute to leachate composition such as age, moisture, and operation procedures. The compositional data (Table 1-1) collected from 39 Class I, lined landfills in Florida were divided into two categories based on estimated stage of decomposition, (*i.e*. young/acidogenic and old/methanogenic). Leachate quality is highly variable. As is apparent by the high standard deviation, leachate qualities within the state of Florida vary by both spatial and temporal influences.

Compositional parameters of landfill leachates are important factors in determining the appropriate management and treatment methods. Different methods of treatment address specific pollutants of concern with varying degrees of efficiency and cost. One of the most common methods of landfill leachate treatment in Florida is off-site discharge via sewage systems to rural publicly owned water treatment (POWT) facilities. Many treatment plants in less populated areas of Florida no longer accept landfill leachate for treatment due to biological upset, excessive corrosion, and problem

compounds in residual sludge (Englehardt *et al.* 2006). As an alternative, off-site trucking of leachate to a larger POWT can result in transportation and treatment costs of $0.20/gallon (Englehardt *et al.* 2006). Furthermore, pretreatment of leachates is usually required of landfill operators by water treatment facilities to remove specific pollutants. Alternative methods for on-site treatment of leachates may be an economical and sustainably oriented means to manage these pollutants in Florida

Landfill Leachate Remediation

The discharge of leachates into surrounding environment is a primary consequence of solid waste disposal. Open dumps gradually contaminate the surrounding areas as water moves through surface runoff and groundwater plumes. Lined landfills provide immense benefits over open dumps by confining the migration of pollutants carried by water. As a consequence of confinement, however, highly concentrated liquid leachates accumulate at the lower impermeable barrier of the landfill. The management of these concentrated landfill leachates is therefore a major concern in the assured protection of environmental quality.

Conventional methods for treating leachates to water quality standards necessary for acceptable discharge are energy and capital intensive and have been primarily adapted from municipal sewage treatment operations. These methods all have advantages and disadvantages and can be organized for convenience into the general categories of leachate transfer, physical, chemical, and biological treatment methods (Table 1-2). Treatment methods most frequently employ a combination of chemical, physical, and biological approaches designed to treat the site-specific leachate characteristics. These methods are designed to prevent the deterioration of environmental quality, but often must operate indefinitely as residuals from treatment

are re-landfilled, subsequently creating more leachate to be treated (Renou *et al.* 2008), perpetuating the problem of these pollutants for future generations. The development of on-site, sustainable leachate treatment is an area of research lacking critical evaluation. Emerging trends in leachate treatment use various combinations of biological, chemical, and physical strategies to achieve on-site leachate remediation, however, a standard, sustainable solution for dealing with landfill leachates has yet to be devised and implemented, although landfill construction continues.

Leachate Management by Transfer

Leachates can be transferred to off-site facilities or pre-treated and transferred to off-site facilities. Off-site transfer involves transporting liquid leachates, by either bulk trucking or sewage discharge, to publicly owned water treatment facilities. These water treatment facilities typically employ a biological aerobic/anaerobic activated sludge process. In Florida, the landfill operator is responsible for having a written contract with the off-site facility to discharge leachate to the plant (FDEP 2010). Off-site treatment facilities often require pre-treament by the landfill to remove specific problem components (*e.g*. ammonia). This method is increasingly scrutinized due to rising costs of fuels for trucking and resultant greenhouse gas emissions, corrosion issues of local sewer systems, and the presence of inhibitory compounds which reduce the treatment efficiency of the accepting site (Englehardt *et al.* 2006, Renou *et al*. 2008).

The practice of leachate recirculation has been common over the past decade as one of the cheapest methods of handling leachate volumes (Renou *et al.* 2008). In addition, recirculation of leachate increases contact between attached methanogen populations and soluble substrates in the leachate, which can lead to increased methane production as well as decreasing the time required for leachate stabilization.

Recirculation of leachates from young landfills may cause an initial increase in organic acid levels upsetting pH and inhibiting methanogenesis (Renou *et al.* 2008). The major drawback of leachate recirculation comes from the perpetual operation and management cost of transferring the liquid from the bottom of the fill to the top. In addition, the strength of some components of the leachate may increase over time. Both off-site treatment and recirculation do not actually treat pollutants within the leachate, but rather transfer the burden.

Chemical Treatment Methods in Leachate Remediation

Conventional methods employed in leachate treatment often include chemical pretreatment methods. The coagulation and/or flocculation of suspended solids within leachates can be an effective means of reducing COD by as much as 50% in older leachates, but is dramatically less effective (10-25%) in young landfill leachates (Renou *et al*. 2008). Flocculating reagents such as aluminum sulphate or ferrous sulphate are consumed in the process and can accumulate in the liquid phase or the resulting sludge. Precipitation by lime milk or sodium hydroxide is common in the removal of dissolved metal ions, especially heavy metals (Wiszniowski *et al*. 2006). Granular or powdered activated carbon can also be used as a chemical pretreatment or co-treatment step. Activated carbon participates in adsorption processes of positively charge species and can reduce both the COD and ammonium levels. The constant consumption of activated carbon tends to make this method costly and therefore, it is used primarily in the final polishing to ensure removal of heavy metals and organics. Other materials, such as zeolites and vermiculites, function similarly to activated carbon and are being investigated for adsorption applications (Wiszniowski *et al.* 2006). An emerging trend within the chemical treatment methods is the application of chemical

oxidation reactions, advanced oxidation processes (AOP). Many researchers report excellent results in COD and color reduction of leachate using oxidative chemicals such as hydrogen peroxide (H_2O_2) and hypochlorite ($HClO^-$). These reagents are consumed in large quantities in order to treat the volumes typical in landfills and are therefore not economically or energetically viable as a primary treatment method. Chemical oxidizers work especially well when paired with pH extremes (Fenton reactions) or with physical radiation processes like ultraviolet (UV), ultra-sonication (US), or ozone treatment (O3). These can reduce the volumes of oxidative chemicals needed, but require high electricity operating costs for generating the radiation forces. These AOP techniques are still experimental in their application to landfill leachate treatment.

Physical Treatment Methods in Leachate Remediation

Other than the application of different forms of physical radiation in combination with chemical oxidation, physical treatment methods usually involve transferring pollutant problems from one medium to another. In the case of filtering media, particles in solution of various sizes are trapped on the physical barrier. These barriers can be sophisticated membranes, as in reverse osmosis for the removal of dissolved ions or simply coarse fibers for removal of suspended solids. In either case, the pollutants within the leachate are concentrated on the surface of the filter medium and removed from the liquid medium. The rapid fouling of filter media is a major drawback in the application of these physical removal techniques. Additionally, as the pollutants are only transferred there remains a concentrated residual that must subsequently be treated. If disposed of in a landfill, the concentrated residuals redissolve into the liquid medium and become a leachate management issue once again. Full-scale reverse osmosis membrane treatment units have been installed for the treatment of landfill leachate in

Korea and China (Ahn *et al.* 2002, Liu *et al*. 2008, respectively), but little data is available for sustainability analysis. Nevertheless, the technology seems appropriate for guaranteeing the quality of final discharge.

Ammonia has long been recognized as a problem constituent of landfill leachates. In the unionized form ammonia (NH_3) is a volatile gas. Ammonia has a pKa of ~9.26, at which unionized and ionized species are in equilibrium in solution. Under pH conditions lower than 9.26 the ammonium ion ($NH4^+$) is the major form, which is soluble within the liquid phase. At pH conditions higher than 9.26 the unionized ammonia is increasingly the major form. Because of these molecular properties, a common practice to remove volatile ammonia gas is to raise the pH and drive air through the liquid medium causing the escape of the volatile ammonia gas into the atmosphere. The practice of diffusing coarse air bubbles through leachate to drive off aqueous ammonia to atmospheric ammonia is commonly known as ammonia desorption or ammonia stripping (Crawford and Smith 1985). Although an effective means of removing ammonia from leachate it has fallen out of favor as the transfer of ammonia to the atmosphere is not an acceptable treatment solution due to the generation of atmospheric pollution.

Biological Treatment of Landfill Leachates

Current biological methods of leachate remediation include on-site aerobic and/or anaerobic treatments, such as aerated lagoons, rotating biological contactors, activated sludge, sequencing batch reactors (SBRs), constructed wetlands, or irrigation fields. Whether treatment methods are employing microbes or plants, these options strive to provide optimal conditions for growth and reproduction of organisms involved in the treatment process. Organisms involved are used to convert dissolved organics, organic colloids, and inorganic dissolved elements into cell biomass and metabolic by-products

(e.g. CO2 from respiration). Microbial methods include both aerobic, anaerobic, and combinations of the two operating conditions. Microbial remediation methods are frequently used in on-site leachate treatment facilities. Aerobic activated sludge treatment is employed to reduce biological oxygen demand in some on-site leachate treatment facilities, and is the same method generally used to treat sewage wastewaters. These systems consist of a reactor with a community of microorganisms supplied with a constant supply of oxygen and biodegradable organic matter from the waste stream. Through aerobic metabolism, microbes assimilate the organics and dissolved minerals into cell biomass and respire CO2 (Wiszniowski *et al*. 2006). All aerobic processes generate excess sludge, which becomes a handling burden in treatment situations and is often re-landfilled. Sequential batch reactors optimize biological conditions for both carbon oxidation, nitrification and denitrification reactions. Attached biofilms can facilitate either aerobic organisms or anaerobic organisms depending on operation and leachate qualities. Commonly employed attached biofilms are the trickling filters and rotating biological contactor (RBC). Trickling filters also known as percolating filters or bacterial beds consist of a bed filled with media, such as crushed brick, corrugated PVC, or other such high surface area material. The media serves as a fixed bacterial habitat, and the bacteria biologically degrade dissolved compounds as liquid waste pass through the filter media. In leachate recirculating systems the landfill itself is an enormous anaerobic trickling filter. Anaerobic microbial consortia promote reductive chemical reactions, like methanogenesis. Aerobic trickling filters on the other hand, promote oxidation reactions like nitrification of ammonia. Rotating biological contactors are similar in biological function but employ a disc or

similar structure fixed to a rotating axis that dips in and out of the liquid medium. The microbial consortium forms on the surface of the disc and degrades the dissolved compounds (Crawford and Smith 1985).

Photosynthetic Bioremediation:

An ecologically intelligent form of remediation uses photosynthetic organisms to degrade, detoxify, or sequester pollutants, this is commonly termed phytoremediation. Applications of this remediation strategy can target diverse elemental and organic pollutants (Meagher 2000), relying primarily on the tolerances of the remediating plants. The potential of the phytoremediation strategy specifically for the treatment of landfill leachates is great, but needs optimization and development for wide-spread application (Jones *et al*. 2006). This may be due in part to the high degree of variability of landfill leachates. Terrestrial phytoremediation of landfill leachates includes primarily the intermittent field irrigation of short-rotation woody crops (*e.g*. poplar, willow, pine) and grasses. Terrestrial phytoremediation generally requires large land areas for treatment. Additionally, leachate irrigation fields can suffer from accumulation of pollutants and contamination of soils where leachates are applied (Jones *et al.* 2006). Constructed wetland treatment systems have also been investigated in the remediation of landfill leachates. Constructed wetlands passively combine both microbial-based reactions and plant driven remediation. Constructed wetlands have often had encouraging results in polishing landfill leachates, usually after initial pretreatment such as air stripping of ammonia or settling of suspended solids. Constructed wetlands have an undeniable potential in passive remediation and are therefore promising candidates for sustainable waste treatment, but are still developing in practice. Constructed wetlands suffer from the clogging of wetland bed materials by excessive microbial growth and deposition of

oxidized solutes contained within the leachate, typically requiring annual maintenance (Wojciechowska *et al*. 2010). An additional advantage of both integrated wetlands and terrestrial phytoremediation systems is the potential creation of habitat for wildlife. Integrating treatment wetlands with other remediation approaches may have long-term benefits for local wildlife.

Algae for Leachate Remediation

The use of algae for the treatment of landfill leachates is an uncultivated application for these photosynthetic microbes. Remediation of landfill leachates via algae may have a significant potential in long-term criteria pollutant abatement and simultaneous resource generation. As previously described, the generation of algal biomass may be a valuable feedstock for biofuel production. Many algae produce and store solar energy as lipids in relatively large quantities; in fact, some species are well known to store over 50% of their dry weight as lipids (Hu *et al.* 2008). Algal biofuels, however, have yet to reach large-scale levels of production (DOE 2010). One primary reason is the expense of current cultivation techniques, which rely heavily on energy inputs into the cultivation system (Lardon *et al.* 2009). Among these costs are the fertilizers needed to support photosynthetic growth and an adequate carbon dioxide supply, both of which can be supplied by a landfill. Furthermore, one of the remaining obstacles faced by large-scale algal cultivation is the demand for water, which is critical because of competing domestic and agricultural demands. Anthropogenic wastes (*e.g.* landfill leachate) may provide algal cultivation with nutrients and water. Renewable resource generation and carbon mitigation occur while algae provide biological treatment of wastes. Despite intriguing advantages, an extensive literature search reveals only one published reports investigating the utilization of algae for landfill

leachate remediation (Lin *et al*. 2007) and no reported literature on the use of landfill leachate specifically for growing algal bioresources.

Thesis Rationale

Landfill leachates are a societal burden, which may have intrinsic value as an algal culturing medium. Equally, algae may have the capacity to remediate landfill leachates and reduce the societal and environmental burden imposed. Current literature is sparse and suggests that landfill leachates must be highly diluted for algae to grow. Even at a high dilution (90%) reported growth is only moderate whereas higher concentrations are inhibitory or toxic. Landfill leachate is an extreme environment, which is not typically inhabited by algae. This is primarily due to its location in the bowels of a landfill, with no direct contact with sunlight. A lack of exploration for algae with high capacity to utilize this medium and the appropriate cultivation techniques for growing algae within this waste environment are missing from the current scientific literature. This scientific effort aims to explore novel algae for the remediation of landfill leachate as well as determine appropriate methods for cultivation within this potential algae medium.

Hypothesis

Indigenous algae of a closed municipal solid waste landfill can utilize landfill leachate for photosynthetic growth.

Objectives

The goal of this study is to explore the phycological diversity of a closed municipal solid waste landfill for algae with potential application in the production of bioresources through remediation of leachates generated by the landfill. Two primary objectives will be addressed: 1) Survey algae through bioprospecting and 2) develop techniques for the cultivation of algae on landfill leachate.

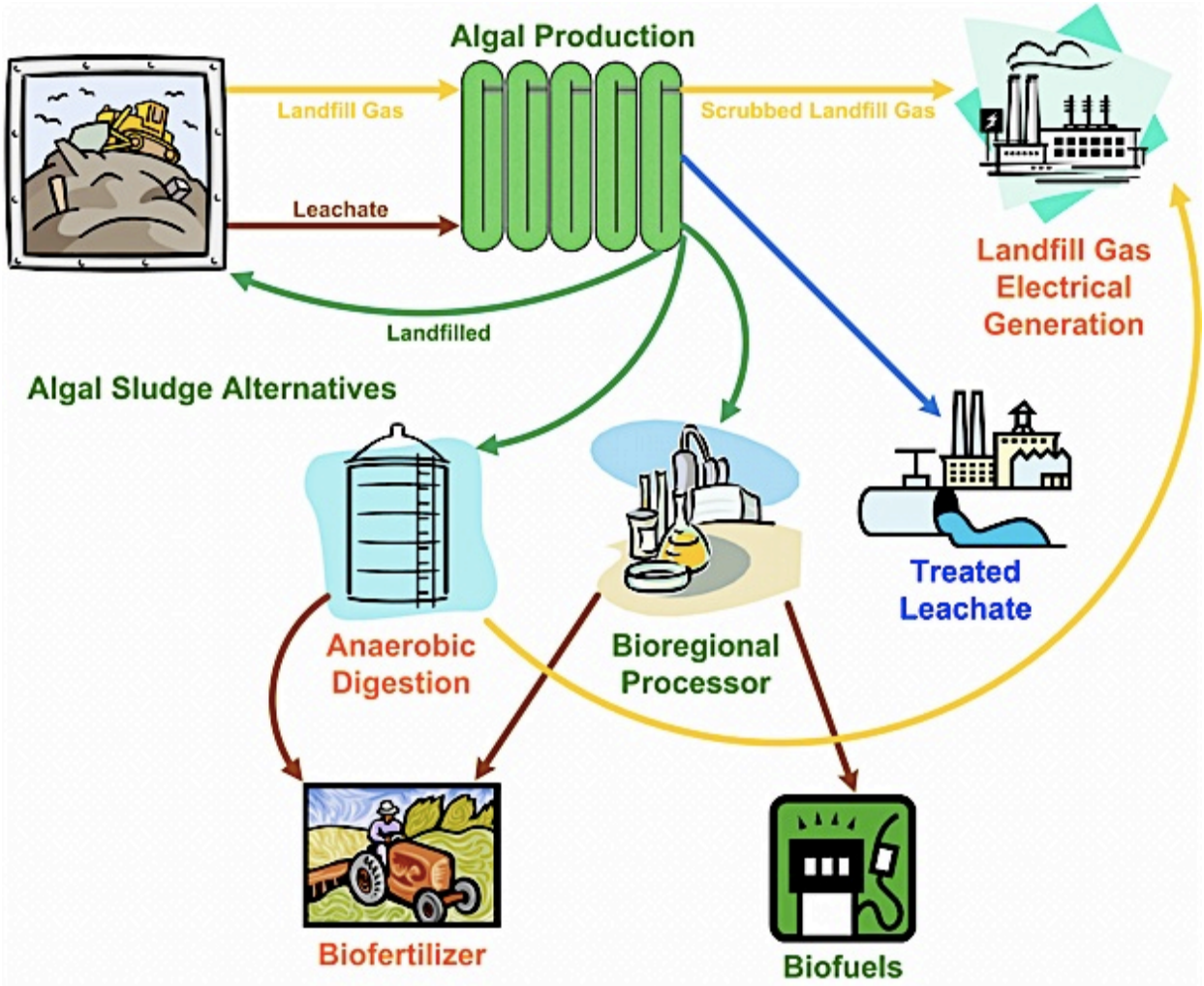


Figure 1-1. An integrated human system with continuous algae production from societal wastes; algae utilize mineral elements in landfill leachates and carbon dioxide in landfill gases while treating leachate.

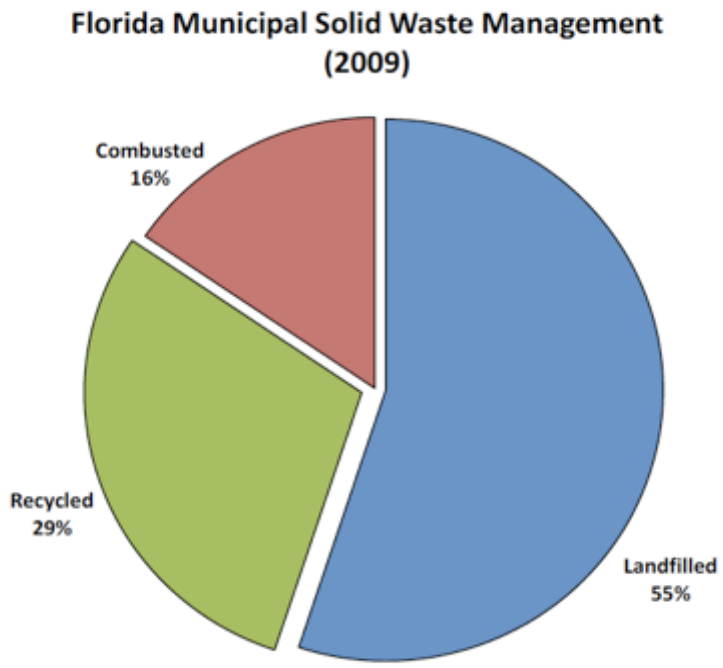


Figure 1-2. Fate of Municipal Solid Waste in Florida (Source, FDEP 2009)

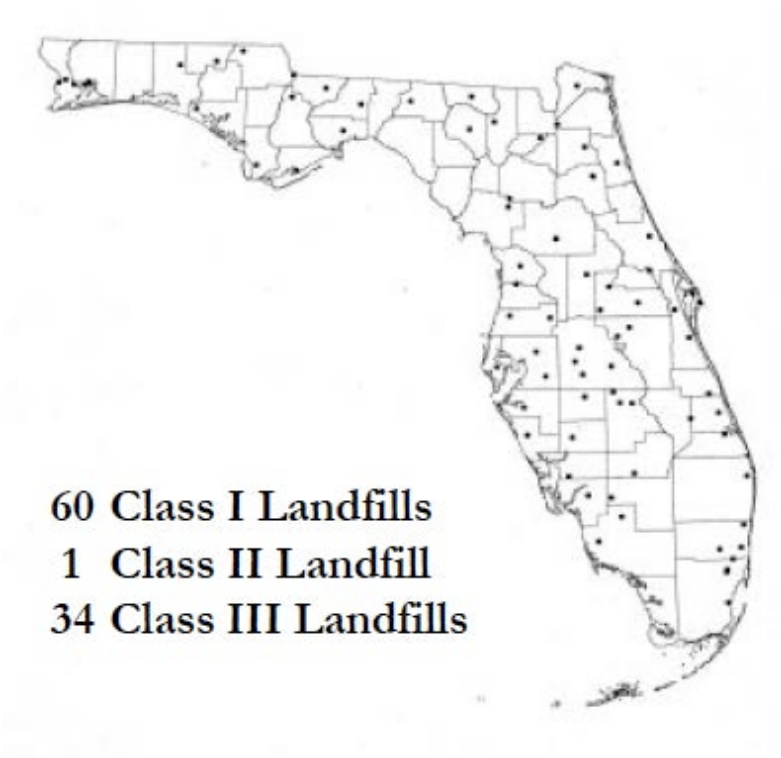


Figure 1-3. Active Landfill Locations in Florida (Source: FDEP, 2001)

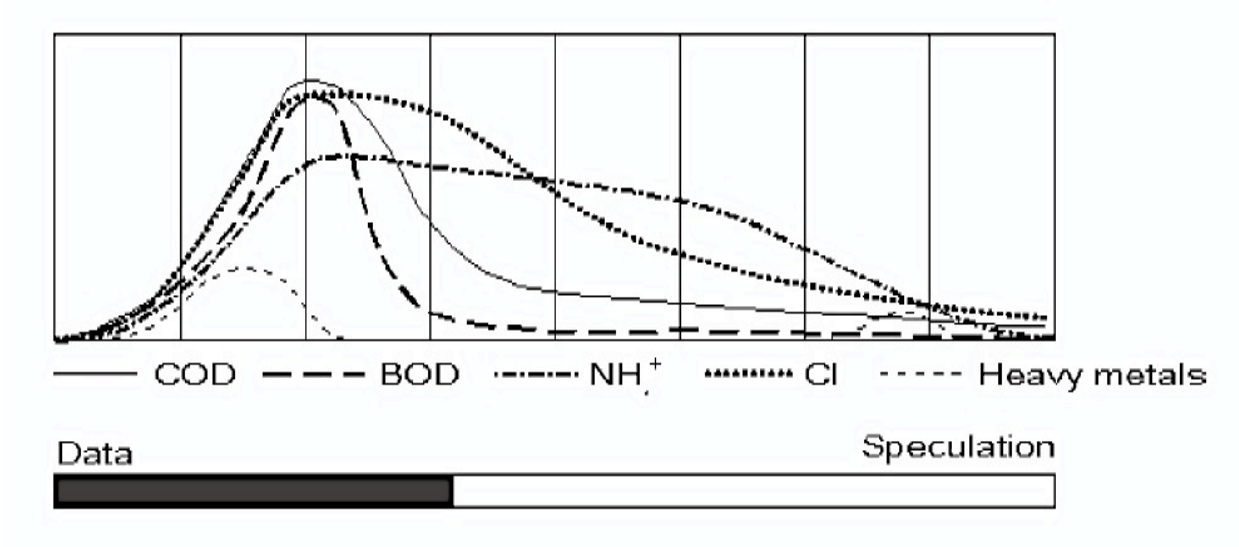


Figure 1-4. Changing Landfill Leachate Composition Over Time, Source: Kjeldsen et al. 2002.

Table 1-1. Characteristics of Landfill Leachates from 39 Florida Landfills.

	Acidogenic		Methanogenic	
	Average	Std. Dev.	Average	Std. Dev.
pH	7.2	0.641	7.14	0.59
COD	4230	7360	912	1200
BOD	462	745	149	478
Ammonia	446	406	257	419
Chloride	1000	1380	732	742
Mn	0.767	1.39	0.659	1.44
Zn	0.238	0.269	0.158	0.382

All components in mg/L except pH, n=39 Source: Reinhart and Grosh 1998

Table 1-2. Summary of Advantages and Disadvantages of Landfill Leachate Management Options

Treatment Method	Advantages	Disadvantages
Leachate Transfer		
Off-site transfer (municipal sewage treatment facility via truck or sewer)	▪ High populations of nitrifying bacteria present at sewage treatment facilities ▪ Effective removal of BOD and ammonia nitrogen	▪ High cost of transporting liquids ▪ Corrosion issues in sewers ▪ Biological upset of treatment plant ▪ Contaminates sludge ▪ Sludge returns to landfill
Leachate Recirculation	▪ Low short-term cost ▪ Can increase methane production ▪ Improves leachate stability	▪ Perpetual operation and maintenance costs
Chemical Treatment		
Coagulation/Flocculation Precipitation Adsorption Chemical oxidation Radiation	▪ Effective removal of COD, BOD, ammonia nitrogen ▪ Rapid ▪ Improve biodegradation	▪ High cost of reagents ▪ Do not meet discharge requirements as stand alone systems ▪ Some by-products can be toxic ▪ Often need pH extremes to be effective
Physical Treatment		
Filtration Reverse Osmosis Air Stripping	▪ Effective removal of COD, BOD, ammonia nitrogen ▪ Can meet and exceed discharge requirements	▪ High cost of operation and maintenance ▪ Membrane fouling ▪ Residual concentrate ▪ Transfer of pollutant
Biological Treatment		
Microbe-based: Aerated Lagoons Activated Sludge Sequential Batch Reactors Attached Bio-films	▪ Can have low energy consumption ▪ Use natural consortia to treat wastes ▪ Effectively reduce COD, BOD, ammonia nitrogen	▪ Often do not meet discharge requirements as stand alone systems ▪ Highly variable ▪ Temperature dependent
Plant-based: Constructed wetlands Irrigation Fields	▪ Low energy consumption ▪ Use natural consortia to treat wastes ▪ Plants can sequester heavy metals	▪ Large land areas required ▪ Soil contamination
Algae-Based: Open ponds Photobioreactors	▪ Use natural consortia to treat wastes ▪ Bio-resource production	▪ Experimental ▪ Unproven

CHAPTER 2
MATERIALS AND METHODS

Leachate Physico-Chemical Characterization

Leachate samples were collected directly from mainline recirculation plumbing and analyzed in field for temperature, pH (Orion pH meter, Thermo Electron Corp.), conductivity and oxidation-reduction potential (ORP) (Hach MP-6p, Hach Co. Loveland, Ohio). A 2.5 gallon batch of leachate was collected in an airtight container after overflowing to eliminate headspace and refrigerated at 4° C. Samples were analyzed by an independent NELAC certified lab for metals and elements critical to photosynthetic growth (Table 3-3).

pH

Sample pH was measured in the laboratory using an Orion Research 520A pH meter with Orion pH electrode following standard methods 4500-H^+ (APHA 2005) for pH measurements of aqueous media. The pH probe was calibrated daily with pH 7 and 10 buffers, temperature was accounted for when taking the measurement.

Conductivity

Conductivity was measured in the laboratory on an Accumet Model 30 conductivity meter (Thermo Fisher Scientific, Waltham, MA). Temperature was accounted for in all measurements. The probe was calibrated daily with a solution of 0.01M potassium chloride following standard methods in APHA (2005).

Alkalinity

Alkalinity was measured potentiometrically in leachate samples following APHA (2005) procedures. A 0.1N solution of H_2SO_4 was standardized to a value of 0.0973N

by titrating a known concentration of Na_2CO_3 to a pH of 4.5. Alkalinity was calculated in mg $CaCO_3$ equivalents by the following equation:

$$Alkalinity\ \left(mg^{CaCO_3\ eq.}/_{L\ landfill\ leachate}\right) = \frac{mL\ H_2SO_4\ used\ \times\ N\ \times\ 50{,}000}{(50\ ml\ leachate)}$$

Total Ammoniacal Nitrogen (TAN)

Total ammoniacal nitrogen (TAN) was measured using an ion selective electrode (Orion 95-12) with an Orion IonAnalyser 701A meter. TAN levels were measured in leachate and algal cultures following standard methods 4500-NH_3 (APHA 2005). The probe was calibrated daily using decimally diluted ammonium chloride stock solution (3.819g/L anhydrous ammonium chloride). The stock solution gives 1.00ml = 1.00mg N = 1.22mg NH3. The procedure raised the pH of the sample to >11.0 with a sodium hydroxide/EDTA solution (Orion ISA pH adjusting solution). Several modifications were made to the standard methods. Leachate measurements require three-fold addition of ISA pH adjusting solution to raise the sample above 11, due to the high buffering capacity of landfill leachate. In all measurements, 300ul of ISA solution were added per 5ml sample, which was determined to be sufficient. The method was also adapted to use reduced sample volumes (5ml) and verified against standard procedures in order to take multiple samples from 125ml Erlenmeyer culture flasks. The concentration of the two species of TAN is pH dependent: $K_a = \frac{[NH_3][H^+]}{[NH_4^+]}$, as well as temperature dependent: $pK_a = \frac{0.09108 + 2729.92}{(273.2 + T)}$ (Korner *et al.* 2001).

The fraction of unionized ammonia was calculated using the following equilibrium formula:

$$\%\ Unionized\ ammonia = \frac{100\%}{\left(1 + 10^{(pKa - pH)}\right)}$$

Bioprospecting and Field Cultivation of Algae

Micro-floristic Survey of the Southwest Landfill

Bio-prospecting for native algae at the ACSWL site began in February 2010 and continued through October 2011, sampling both summer and winter algae flora. The landfill site was explored for visible signs of alga growth, which was the primary determinant in sample collection. Algae samples were taken from various locations including pooled rainwater, damp soils, leachate holding tanks, and drainage areas. Additionally, several sites with pooled water on top of the landfill were sampled. At each sampling site GPS coordinates were taken. Samples were taken in 40ml screw cap containers, refrigerated after collection and identified to genera by direct microscopic observation following the morphological key developed by Prescott (1978). Raw leachate was centrifuged at 15,000rpm (Eppendorf 5414, Westbury, NY) to determine the possible presence of algae within the landfill leachate.

Field Enrichments

Empirical investigations were conducted on-site at the ACSW landfill to evaluate cultivation techniques and the effect of dilution on the growth of algae. All cultivation systems were operated in a batch mode. All cultures of the in field enrichment trials were monitored for cell growth by analysis of chlorophyll fluorescence and cell count by hemocytometer. Total ammoniacal nitrogen, culture conductivity and pH were monitored.

Aeration basin

To evaluate the adaptability of microalgae to a high concentration of leachate, a 500L elliptical agricultural basin with an areal surface area of 0.71m^2 and a total culture volume of 400L used 100% undiluted raw leachate as the sole source of nutrient fertilizer for algal growth. An algal inoculum was added at a volumetric ratio of 10% (40L). The aeration basin (AB) technique utilized a diaphragm air pump connected to a bonded glass air diffuser. The diffuser was placed on the bottom of the cultivation basin and the rising gases forced hydraulic mixing of the culture. Mixing and air diffusion provided circulation to expose the entire culture volume to light.

Slope reactor

The other cultivation technique, termed the slope reactor (SR), was a 2000L reactor utilized a submersed water pump to circulate water to the top of an aluminum slope covered with a rough plastic resin. Water then flowed over the rough surface back into the tank. This reactor was intended to cultivate filamentous algae on its rough surface.

Mini-ponds

Concurrently to these larger experiments, a series of 20L reactors, referred to as ‘mini-ponds’ were filled with landfill leachate diluted with groundwater to give several concentrations of landfill leachate, 2.5, 25, 50, 75, and 100%. All mini-ponds were inoculated with equal volumes of a mixed consortium of algae from previous bio-prospecting trips. Mini-ponds were mixed with diffused air through bonded glass air-stones and placed in full sun.

Microscopic Observation and Microscopy of Algae

Algae were microscopically observed on a Nikon Labophot after pipetting 500µl of a well-mixed sample in five 100µl grab samples. The composite sample was then centrifuged at 15,000rpm for 10sec. The resultant cell paste was mounted on a glass microscope slide and observed under glass coverslip at 125-1250x optical magnifications. Samples were keyed to genus level following Prescott (1978). Photos were taken under bright-field or differential interference contrast illumination on a Nikon Labophot compound microscope (Nikon Corporation, Tokyo, Japan) with a Spot Insight color mosaic digital camera (Diagnostic Instruments Inc., Sterling Heights, MI). Cell measurements were obtained after pixel calibration with an optical micrometer (American Optical Co., Buffalo, NY)

Lipid Screening of Algae with Nile Red

A solution of Nile Red (9-diethylamino-5H-benzo[α]phenoxazine-5-one, Sigma) dissolved in acetone at a concentration of 250µg/ml was used to stain algae samples for fluorescent lipid observation, following Cooksey *et al.* (1987). Samples were thoroughly mixed and the same pipetting regime described for microscopic observation employed to give a 500µl composite sample. To the composite sample were added 10µl of Nile Red in acetone solution to give a final concentration of ~5µg/ml Nile Red. Fluorescent microscopy of algal cells used a 50w mercury halide illuminator and a 490nm excitation and 520nm long pass emission filter.

Laboratory Cultivation of Algae on Landfill Leachate

Light

Photosynthetically active radiation was measured using a Li-Cor LI-189 instrument equipped with a quantum senor: units are reported in $\mu E/m^2/s$. Illumination in laboratory

experiments was provided by T5 Plantmax™ fluorescent lamps to give 150µE/m^2/s lighting, provided on a 12:12 (light:dark) for initial baseline cultivation and on a 24:0 photoperiod for toxicity experimentation.

Bolds Basal Reagent Preparation

Bold's basal medium (BBM) was prepared following a modified version of Bristol's solution (Bold 1942). A modified Bold's basal medium (MBBM2) was made by adding 2% landfill leachate to a stock solution of BBM.

Isolation and Enrichment of Bio-prospected Algae

Cultures from the landfill were subjected to culture enrichment by the 1:1 addition of BBM to sample volume. Cultures were isolated to unialgal cultures by agar plate cultivation. This technique involved the 1:1 dilution of samples with 10% Bold's Basal Medium. This solution was then spread across 2% agar plates and incubated under light (150µE/m2/s) until colonies appeared, ~7-10days (Figure 2-1). Isolated colonies were lifted from agar plates and cultivated in Bold's Basal Medium under lighting until growth was visible. Isolated cultures were maintained in BBM with 2% landfill leachate. Single plating isolations yielded two isolates, *Scenedesmus* sp. (Figure 2-2, A) and *Chlorella cf. ellipsoidea* (Figure 2-2, B), which were used as experimental cultures throughout the study. *Chlorella cf. ellipsoidea* was re-isolated by a subsequent agar plating due to cyanobacteria contamination of the isolated culture.

Rapid Toxicity Test

A rapid toxicity test was developed to quickly assess the tolerances of bio-prospect algae to ACSW landfill leachate. The test used *in-vivo* chlorophyll fluorescence to assess relative growth rate over a short period of time (24hours). The test was conducted in 24-well plates with five concentrations (0, 10, 30, 50, and 100%)

of landfill leachate diluted with deionized water (0% is distilled water only). Each concentration was replicated in triplicate. Fluorescence levels of leachate without algae are taken as background fluorescence. Test plates were incubated at 25C under 150µE/m^2/s on an orbital shaker platform rotating at 140rpm.

Flask Cultivation

Both orbital shaking and aeration were used for mixing algae suspensions within cultivation flasks. Experiments mixed via shaker were cultivated in 125mL Erlenmeyer flasks, filled with 75mL total volume, and shaken continuously at 140rpm. A 20% (15ml) volumetric inoculum of either the *Scenedesmus* sp. or the *Chlorella cf. ellipsoidea* mother culture cultivated on BBM with 2% (v/v) of landfill leachate was added in flask cultivation experiments with algae. All treatments were replicated in triplicate, unless otherwise stated. Flasks were stoppered with either a polyethylene foam or black rubber stoppers depending on experiment trial; giving open and sealed flasks, respectively. Abiotic controls consisted of ACSW landfill leachate diluted with deionized water to the appropriate concentration, without algal inoculation.

Shaker-based mixing is impractical when using CO_2 to regulate the pH of the medium. CO_2-enriched cultivation therefore used aeration based mixing. 125mL Erlenmeyer flasks, filled with 75mL total volume, which were continuously sparged with 0.45µm-filtered air at a rate of 0.065L/min/flask. De-ionized water was added to cultures daily to replace volume lost by evaporation. CO_2 was sparged into cultures through diffusors to regulate pH between 6.8 and 7.2, potentiometrically using a pH controller (SMS122, Milwaukee Instruments), calibrated daily and a CO_2 regulator with a solenoid valve (Milwaukee Instruments, Rocky Mount, NC). In experiments with pH

regulation by hydrochloric acid (HCl) addition, 1M HCl was added potentiometrically to ACSW LL while mixing, until the pH reached 7.0.

Cell Counts

Cells were counted on a Brightline hemocytometer with improved Neubauer ruling (American Optical Co., Buffalo, NewYork). Two counts were taken for each data point and the average between them taken as one count, if the counts had an error greater than 10% the culture was recounted.

***In-Vivo* Fluorescent Measurement of Algal Cultures**

Algae cultures were monitored using in-vivo fluorescence with a fluorometer (Thermo, NanoDrop 3300) at 490/680nm excitation/emission. Samples were vigorously mixed before sampling to ensure a homogenous suspension of algae cells within the medium. Medium blanks were taken and subtracted from the measurement of algae cultures. A nomograph validating fluorescence with cell counts was created to verify this method of culture monitoring for both *Scenedsumus* sp. (Figure 2-3) and *Chlorella cf. ellipsoidea*. Fluorescent measurements are rapid and sensitive to the photosynthetic status of the cell and were chosen as the primary means of culture monitoring in this study.

Growth Rate

Growth rate was determined by measuring the fluorescence of the algal culture at 680nm. The rate (μ) was calculated using the equation following Kallqvist and Svenson (2003):

$$\mu = \frac{lnF680_n - lnF680_0}{n}$$

where: $F680_0$= initial fluorescence, $F680_n$= final fluorescence, n=number of days

Average growth rates were determined as the rate for the duration of the growth experiment. Maximum growth rates were determined as the greatest, single day growth rate observed between all sampling events.

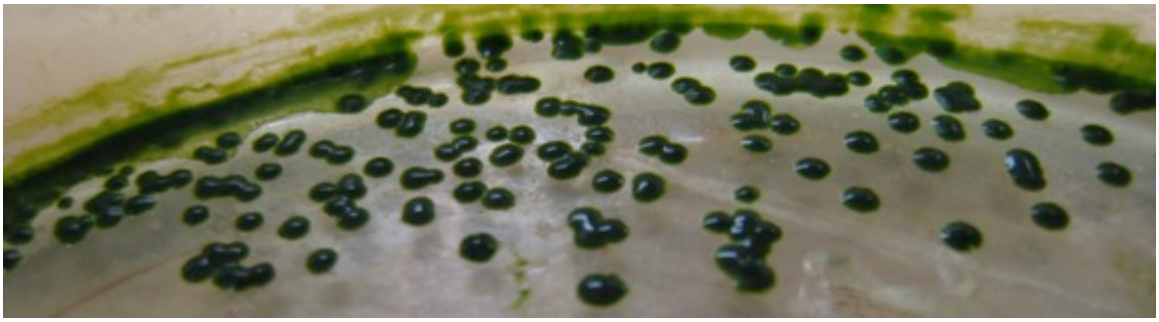


Figure 2-1. Algal colonies from the ACSW landfill growing on 2% agar in a petri dish, prior to isolation.

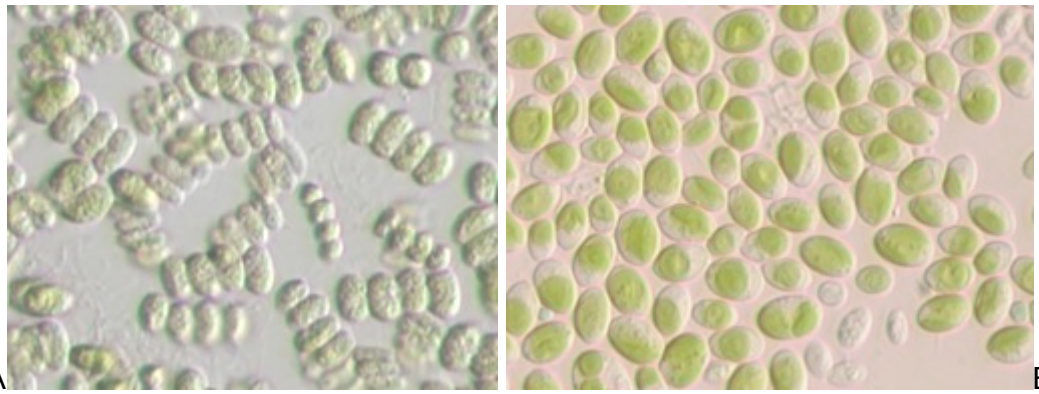
A  B

Figure 2-2. Isolated algae cultures from the ACSW landfill 500x optical magnification; A) *Scenedesmus* sp. and B) *Chlorella cf. ellipsoidea* mother cultures

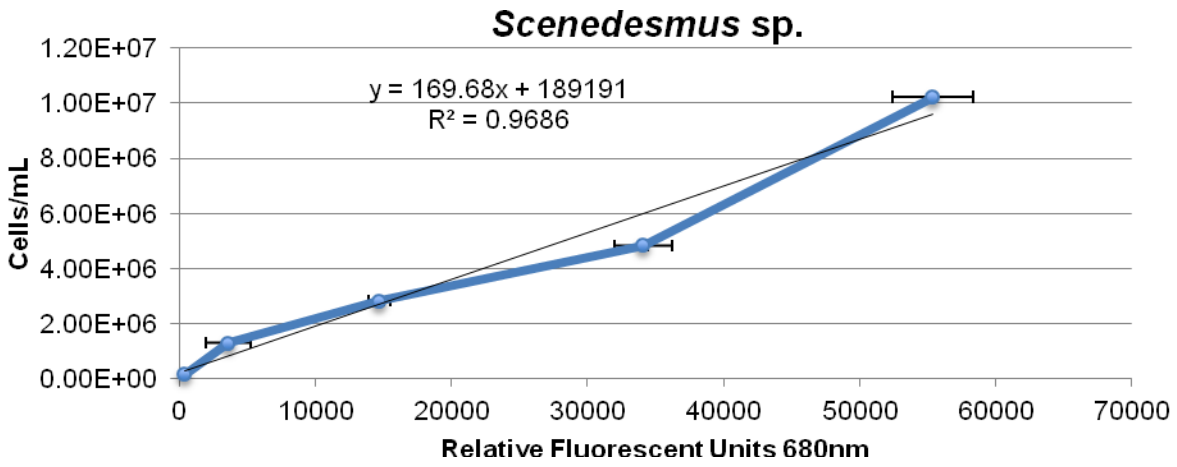


Figure 2-3. Validation of fluorescence measurement as an alternative monitoring technique to hemocytometer cell count for Scenedesmus sp.

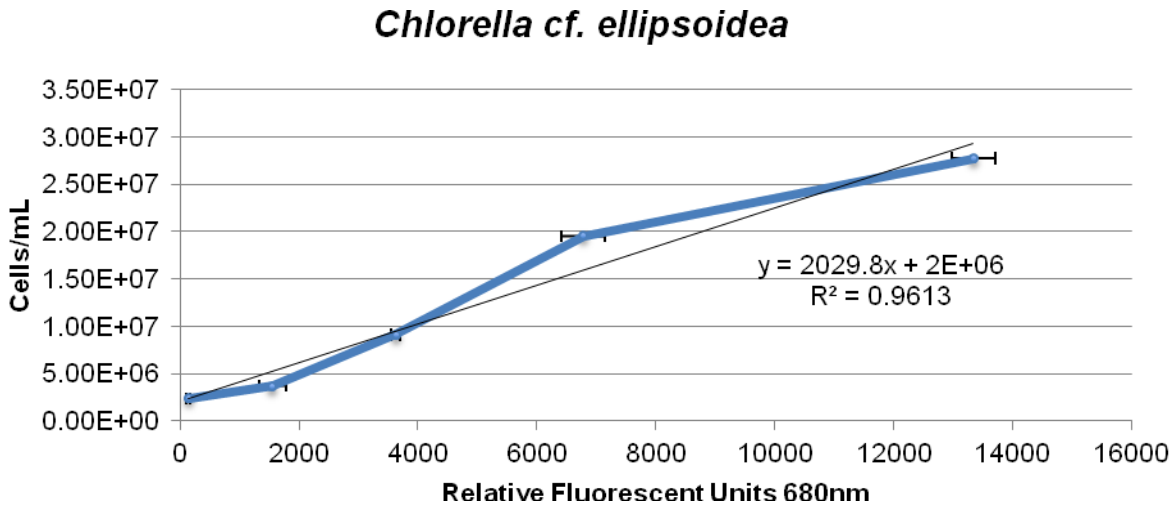


Figure 2-4. Validation of fluorescence measurement as an alternative monitoring technique to hemocytometer cell count for *Chlorella cf. ellipsoidea*

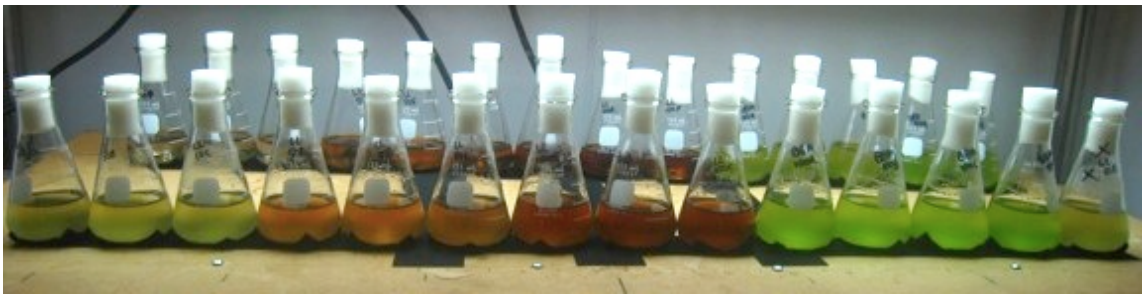


Figure 2-5. A representative set-up of flask cultivation under 150µE/m^2/s light on a orbital shaker at 140rpm.

CHAPTER 3
BIO-PROSPECTING A CLOSED MUNICIPAL SOLID WASTE LANDFILL

Alachua County Southwest Landfill: Study Site Description

The Alachua County Southwest (ACSW) landfill in Archer, Florida has been extensively used in the research of methods for sustainable landfill operations, due to its proximity to the University of Florida. The 10.9 hectare (27acre) site opened in 1988 and received ~900 metric tons of waste per month until closing in 1999. The natural terrain of the ACSW landfill is predominantly flat, typical of north central Florida. Regional climatic conditions from 1981-2011 include average annual rainfall of 121cm, average annual temperature of 20.6°C, average annual minimum and maximum temperature of 14.3 and 26.8°C, respectively. Record temperatures for the area are in excess of 38°C in summer and below -7°C in winter (NOAA, Gainesville Area).

From 1990 until 1992, leachate generated by the landfill and collected on the 60mil polyethylene liner was recirculated via infiltration ponds. Leachate was recirculated as a means of handling the volume of leachate at the facility. More than $30x10^6$ liters of leachate were recirculated to the landfill through the infiltration ponds. Recirculation of liquids can also facilitate landfill stabilization by promoting microbial degradation of wastes into methane and carbon dioxide. With the addition of groundwater (~10 $x10^6$L), the landfill was operated as a 'bioreactor' to generate landfill gas for energy production and promote waste stabilization. Methane gas generated from the microbial degradation of organics was captured and combusted to generate electricity, and at the time of this research was flared. In 1993, an alternative recirculation system consisting of seventeen horizontal injection pipes was constructed and is currently used to recirculate leachate. At this time, the ACSW landfill was producing 7.8 m^3/ha/day (837

gal/ha/day) and trucking 4.3 m^3/ha/day (460 gal/ha/day) for off-site treatment (Reinhardt and Townsend 1998). Leachate is no longer transported off-site, but alternative methods are being investigated for the eventual dewatering and long-term maintenance of the landfill facility. Algae may be able to utilize nutrients within leachate for growth while simultaneously providing biological remediation of the landfill leachate via photosynthetically driven processes.

Bio-prospecting Algae of the Alachua County Southwest Landfill

The utilization of indigenous algae was identified as an advantageous strategy for biomass production and the bioremediation of landfill leachates. Discovering novel and indigenous organisms is a method being explored in the current literature for the application of algae based resource production (Mutanda *et al.* 2011, Wilkie *et al.* 2011). Indigenous organisms are a rational alternative to the frequently employed culture collection strains, as indigenous algae have been naturally selected over time and are therefore adapted to the conditions within the particular region. Algae indigenous to the landfill site thus have inherent adaptations to enduring the local environmental conditions, including light levels, temperatures, rainfall, and seasonal changes. Additionally, indigenous algae may have evolved biological traits (*e.g*. extracellular excretions, spines) for cohabitation with site-specific bacteria, fungi, protists, viruses, and predators inhabiting the specific site of exploration. Contamination by native organisms can severely impact the growth and productivity of uni-algal cultures, and is a major limiting factor in the success of industrial algae cultivation (Sheehan *et al.* 1998). Moreover, indigenous algae cultures may have naturally formed symbiotic relationships with native bacteria, protists, and other algae, for example the vitamin B_{12} dependency of many phytoplankton (Croft *et al.* 2005). Additionally, naturally diverse assemblages

of algae provide improved stability in algae bioremediation (Cardinale 2011) and even lipid productivity (Stockenreiter *et al.* 2011). The utilization of indigenous algae with these adaptations thus allows a greater probability of success in future cultivation and remediation systems. Indigenous algae are continually adapting to local biotic and abiotic conditions; indeed, the application of bio-prospecting comes from the foundational theory of evolution through natural selection (Darwin 1859). Simply exploring the native algae biota permits access to the millennia of adaptations algae have developed to survive under local environmental conditions.

A key feature of the landfill landscape is its non-functioning leachate holding (LH) facility, composed of four cells (termed LH1 - 4). Each tank has a volume of ~90,000-gallons. When active, the LH facility was used to pretreat leachate before transfer to a publically owned water treatment facility. This was a primary target in the exploration of organisms that might be utilized for leachate treatment. Interestingly each leachate holding tank was dominated by a different group of organisms. LH tank 1 was dominated by the floating aquatic macrophyte *Lemna minor* and another floating macrophyte identified as a species of *Wolfia.* LH tank 2 was blooming with algae at the time of initial sampling and showed a range of phytoplankton genera (Figure 3-2). LH tank 2 was dominated by a *Pandorina sp.* bloom, as well as *Wolfia* sp. and showed a high degree of biodiversity with eight identified genera. LH tank 3 was inhabited sparsely by *Wolfia* sp. with little biodiversity and only two rarely occurring algal genera (*Chlorella* and *Pandorina*). Unidentified fungal clumps were commonly seen in floating in LH 3. LH tank 4 was sparsely inhabited by an unidentified flagellated colonial alga and rarely by, a ‘chlorella-like’ alga. Pooled surface water around gas well 62 (GW62)

was found to contain filamentous algae of the genera *Microspora* and *Oedogonium*. The Northwest storm water drainage (NW drainage) site was also found to have prolific filamentous algae of the genera *Rhizoclonium* and *Ulothrix*. The SW cell site was found to have a significant presence of diatoms including *Navicula* and other unidentified genera. Pooled rainwater with a striking green hue in a concrete basin was also sampled and determined to be what is tentatively identified as *Chlorella cf. ellipsoidea* (Figure 3-3B). No algae were found to inhabit raw leachate, based on centrifugation and settling techniques.

A total of 15 sites were sampled over the course of this study and are marked on a site map (Figure 3-1). Site descriptions and GPS coordinates are given in Table 3-1. A total of 17 algae genera were identified from the ACSW landfill site. Genera observed are listed in Table 3-2; among the most prevalent were *Chlorella, Navicula, Oscillatoria,* and *Scenedesmus.* Of the genera observed, several have been reported in the literature to have species with the capacity to store large amounts of oils (>30% by weight) as energy reserves including *Chlorella, Navicula, Chlamydomonas, and Scenedesmus* (Sheehan *et al.* 1998, Hu *et al*. 2008). Several genera are known to have species that produce hydrogen under the appropriate conditions including *Chlamydomonas, Scenedesmus (*Melis and Happe 2001)*, Oscillatoria* (Phlips and Mitsui 1983)*,* and *Synechococcus* (Kumazawa and Mitsui 1994). Most genera, however, have not been as extensively explored for energy production, but may produce high value compounds or afford excellent results in bioremediation.

Alachua County Southwest Landfill Leachate Characterization

In Field Characterization

Landfill leachate from the ACSW landfill is a dark brown color with a pungent odor, reminiscent of stale coffee. Leachate is effervescent upon leaving the landfill. The average temperature of the leachate directly from the landfill was ~33.5°C, the average pH was 7.4, average conductivity was 14.42mS/cm, and average reduction potential (ORP) was -200.5 mV, based on four separate samples taken on the same day.

Elemental Analysis

Raw leachate samples were taken directly from the sampling port from the mainline leachate recirculation plumbing and analyzed for critical components (Table 3-3). ACSW landfill leachate was determined to be brackish with average sodium chloride levels at 4.6g/L. The chemical oxygen demand was relatively low for landfill leachate, but was expected, as the ACSW landfill in the late methanogenic stage. Total ammonia-N levels of 986mg/L contrast with the total phosphate levels of 12.49mg/L, giving a N:P ratio of 78.9. This can be compared with the average N:P ratio of algal biomass of 5 (Healy 1973), which shows the ample supply of nitrogen present within the leachate. Although comparatively limiting, the phosphorus level is adequate to support algal growth. Other notable nutrient characteristics are the levels of potassium (777mg/L) and iron (10.5mg/L) measured within the leachate would be adequate to support algae growth. The critical photosynthetic nutrients magnesium and manganese are also present within the landfill leachate. Copper, a commonly employed algaecide, was present at levels (0.11mg/L) that may be inhibitory to some species of algae. Arsenic levels of 0.08mg/L likewise may have inhibitory effects on sensitive species of

algae, but are not expected to be universally toxic at the detected levels. Overall, observed concentrations of heavy metals were not anticipated to be toxic to algae.

Maximum Theoretical Algae Biomass Production

Algae biomass potentials were calculated by major element using average elemental values determined empirically from algae biomass (Healy 1973) (Table 3-4). Assuming algae grow unimpeded within the ACSW landfill leachate the nitrogen supply within leachate could support a maximum of 17.6g algae biomass per liter of landfill leachate. In contrast, phosphorus within the leachate could only support a maximum of 1.14g/L. Furthermore, phosphorus is not always biologically available, indicating that the level of phosphorus within ACSW landfill leachate may, therefore, be limiting in the cultivation of algae. Iron may also be a limiting factor in algae biomass production, with a maximum biomass potential of 1.77g/L. Other major elements potassium, magnesium, and calcium were not determined to be limiting elemental resources within the ACSW landfill leachate.

Algae Lipid Observations

The cultivation of oleaginous algae would provide an oil resource, which could readily be converted into a liquid petroleum replacement such as, biodiesel (Sheehan *et al.* 1998). Algae were screened for cellular lipid content utilizing fluorescent stains and epi-fluorescent microscopy. Several of the algae bioprospects examined under epi-fluorescent microscopy for lipids, showed cells storing large deposits of cellular lipids. Among those most notable are *Ankistrodesmus*, *Navicula,* and *Chlorella cf. ellipsoidea* (Figure 3-7).

Field Enrichments

Aeration Basin

The aeration basin (AB) had a working volume of 400L (Figure 3-6). The AB was filled with 360L of untreated landfill leachate and inoculated with 40L of an algal bio-prospect found on-site in previous explorations, site #9 algae growing within a concrete basin filled with rainwater. The culture was determined to be predominantly *Chlorella cf. ellipsoidea* and at the time of collection was nearly uni-algal and had significant droplets of oil within each cell (Figure 3-10C).. As there was an existing volume of this culture it was used as the sole inoculation of the AB. The high leachate concentration initially showed a poor response from the algae, with many of the cells bleaching and dissolving, with low photosynthetic activity as measured by chlorophyll fluorescence. A lag in cellular growth lasted for a period of 15 days, after which chlorophyll fluorescence began to improve and increased exponentially (Figure 3-5). At this time, many cells were observed in stages of division under the microscope; reproduction is an indication of adaptation. The culture peaked 4 days later and crashed as indicated by cell counts and chlorophyll fluorescence. After reinoculation with the same culture and volumetric percentage, cell populations reached a second fluorescence peak at 6,192 relative fluorescent units (RFU). Total ammoniacal nitrogen (TAN) dropped significantly within the first three days from 250 to 21 and finally stabilizing at 0.45 mg/L by the fourth day, this is presumed to be mostly due to volatilization from aeration and a raised pH due to algal photosynthesis. The pH of the culture was initially 8.6 and climbed to 9.1 within 24 hours of aeration and remained between 9.0 and 9.3 for the duration of monitoring. Exponential growth from days 14-20 is typical of microalgae in favorable conditions. It is curious to note that all observed algal growth occurred after the depletion of the

majority of TAN within the leachate. It is also worth noting that the observed growth is moderate even compared to natural algal systems, with no aeration inputs. A precipitous drop in chlorophyll fluorescence, as observed from day 19 to 32, is characteristic of a limiting nutrient or unfavorable conditions. The second peak in chlorophyll fluorescence and the steadily increasing cell population may indicate an adaptation of the algal culture to the leachate conditions. *Chlorella cf. ellipsoidea* remained the dominant culture organism through this experimental trial showing promise as a robust outdoor strain tolerant to landfill leachate. The aeration basin was, to the author's knowledge, the first attempt at cultivation of algae on landfill leachate in Florida.

Slope Reactor

The slope reactor was designed to explore the cultivation of filamentous algae found growing in the drainage area of the landfill (Figure 3-8). Filamentous algae may allow simplified harvesting methods, which would significantly reduce the cost of both algal-based remediation and bioproduct generation. The slope was intended as a substratum to which the algal filaments could adhere as the culture medium flowed over the surface of the slope. The SR was inoculated with blended filamentous algae including the observed genera *Oedogonium*, *Microspora*, and, *Rhizoclonium*. The cultivation of these filamentous algae was unsuccessful. Only a few small tufts of algae germinated on the surface of the slope, but quickly perished due to the intermittent functionality of the submersible pump. On day 11, during a period of pump failure, the SR bloomed a bright green. Upon microscopic observation the genus *Ankistrodesmus* spp. was dominant in the phytoplankton community. This organism remained the dominant organism throughout the field cultivation period. The culture grew linearly for

the next three sampling points and then rapidly crashed. Upon the addition of fresh leachate on day 33, culture growth resumed and reached the highest fluorescence levels recorded in field cultivation trials of 20,322 RFU (Figure 3-7). Although not significant in practice the occurrence of tufts of *Rhizoclonium* sp. after homogenizing full filaments into fragments is an interesting method worth exploring for future filamentous algae cultivation.

Mini-pond Cultivation

Mini-pond reactors with a volume of 20L (Figure 3-9) were used to test the growth of algae under different concentrations of landfill leachate. Remarkably, algae showed increases in cell populations and chlorophyll fluorescence in all dilutions of leachate. As a general trend, lower concentrations of leachate corresponded to higher maximum chlorophyll fluorescence (Table 3-4). Whereas, undiluted leachate showed poor growth and low chlorophyll fluorescence. The mini-pond with a concentration of 2.5% landfill leachate showed the highest chlorophyll fluorescence, of 14,100 RFU on the last day of the field experiment. Only the chlorophyll fluorescence of the 25% leachate of 10,052 RFU was near the fluorescent intensity of the 2.5%, all other fluorescence maximums being an order of magnitude lower (Table 3-4). Average growth rates for the study also show a distinct divide, showing a marked decrease at concentrations higher than 25% leachate concentration. The pH increased relative to the concentration of leachate, and increased over the course of the study in all concentrations. All dilutions were observed to have greater than 99% removal of TAN, at two weeks time. This removal is assumed to be mostly due to volatilization as the algal growth was not sufficient to account for TAN removal through cellular assimilation. Algae in higher concentrations of leachate showed growth only after an initial lag period. This may be due to initial levels of

inhibitory compounds that are either removed or deactivated by aeration over time. It is possible that the original inoculum perished under the high concentrations of leachate and the cultures were subsequently re-inoculated by rainfall, wind dispersal, or neighboring cultures.

Remarkably, each concentration of leachate yielded a distinct consortium of algal species; presumably better able to utilize the resources under the prevalent conditions. Large *Chlorella cf. ellipsoidea* cells surrounded by a thick cell wall dominated the lowest concentration of 2.5%. The 25% concentration was also dominated by *C. cf. ellipsoidea*, however the cell wall layer was not as pronounced and other genera such as *Selenestrum* and *Scenedesmus* became noticeable fractions of the algal population. At the 50% level, a shift in dominance of *C. cf. ellipsoidea* to a smaller, round unidentified species of *Chlorella* was apparent. At 75% leachate concentration a dramatic shift to *Ankistrodesmus* was observed. At 100% a filamentous cyanobacteria (*Lyngbya*) became widely present and were associated with pinnate diatoms forming coagulated clumps. A 100% leachate culture that was not inoculated, but ran concurrently contained filamentous and coccid cyanobacteria as well as *Scenedesmus*, *Ankistrodesmus*, *Chlorella cf. ellipsoidea,* and diatoms, however, all to a much lesser degree than the inoculated mini-ponds. It should be noted that *Chlorella cf. ellipsoidea*, *Ankistrodesmus*, and *Scenedesmus* spp. were present to some degree in all dilutions.

Discussion

Bio-prospecting the ACSW landfill site demonstrated the remarkable array of indigenous algae present in the relatively small physical area of the landfill. This characteristic is not exclusive to landfills, but can be emulated at any geographical area, as algae are found nearly everywhere. The phycological diversity of most regions has

not been explored. Just as with bacteria, insects, plants, and other life forms, novel organisms can provide entirely new industries not previously imagined. Cultivating native algae may provide a simple method for culture sourcing in algae based applications (aquaculture, remediation, CO_2 fixation, biofuel production, *etc.*). The considerable phylogenetic diversity of algae is the basis that gives bio-prospecting the capacity to find and apply novel organisms. Seventeen genera of algae were identified from only 15 sample sites taken at the ACSW landfill. It is impossible to observationally identify all organisms within an environmental sample; it is therefore presumed that many genera and species within samples taken went unobserved. These “rare” algae may have advantageous traits that could be exploited by selective cultivation through traditional enrichment and dilution techniques (Andersen 2005). Additionally, emerging techniques using genetic markers could aid in estimating the genetic diversity present, although only a handful of algal genomes have been sequenced. Finding novel algae with potential application to regional anthropogenic and ecological needs can allow a paradigm shift in both the extent and the productivity of algae cultivation. Investigating the indigenous algae of the ACSW landfill site led to the discovery of novel algae, with potential in landfill leachate remediation and resource production. Identification to the species level requires further attention to detail and more careful long-term study of the organism of interest as key morphological characteristics can change with changing environmental conditions, as noted by Bold (1950).

As demonstrated, leachate form the ACSW landfill had ample elemental resources for photosynthetic production. Elemental nutrients fertilizers such as nitrogen, phosphorus, potassium as well as other essential macronutrients for photosynthetic

growth (i.e. magnesium, sulfur, calcium, and iron) and trace metals (manganese, cobalt, copper, zinc) were present in sufficient quantities within landfill leachate. A current life cycle by Lardon *et al.* (2009) analysis of algae derived biodiesel confirmed potential of algae biodiesel production, but is not presently favorable and suggests waste nutrients as a key factor in the improvement of algal biodiesel sustainability.

Preliminary trials of algae cultivation on landfill leachate were unfavorable. Algae exhibited a marked reduction of growth, which correlated with increased landfill leachate concentration (Table 3-5). Although inhibited, algae survived within high concentrations of ACSW landfill leachate, which indicated that the inhibitory factors within the leachate may be resolved to provide the algae the capability to access the nutrients held within the leachate. Notable survivors in these field enrichments were *Chlorella* spp., *Scenedesmus* spp., and *Ankistrodesmus* spp. These genera therefore make prime candidates as algae with potential to utilize landfill leachate as a growing medium.

Figure 3-1. Bio-Prospected sites at the Alachua County South West Landfill. Red stars indicate approximate locations of sampled site. Inset shows the abandoned leachate holding (LH) facility

Table 3-1. Bio-prospected Sites at the Alachua County South West Landfill

Site Number	Site name	GPS location	Site Description
1	Sample port	N29° 30.854’ W82° 32.883’	Leachate plumbing
2	RO Unit	N29° 30.845’ W82° 32.884’	Concrete under air conditioner
3	Soil #1	N29° 30.842’ W82° 32.859’	Soil crust on top of landfill
4	Soil #2	N29° 30.791’ W82° 32.898’	Soil near leachate holding tank
5	LH#1	N29° 30.792’ W82° 32.910’	Leachate holding tank #1
6	LH#2	N29° 30.794’ W82° 32.910’	Leachate holding tank #2
7	LH#3	N29° 30.797’ W82° 32.910’	Leachate holding tank #3
8	LH#4	N29° 30.800’ W82° 32.910’	Leachate holding tank #4
9	RW basin	N29° 30.811’ W82° 32.911’	Rainwater in concrete basin
10	SW cell	N29° 30.702’ W82° 32.850’	Pooled rainwater on clay lined cell
11	GW62	N29° 30.879’ W82° 32.830’	Pooled rainwater on top of the landfill
12	NW Drainage	N29° 30.878’ W82° 32.894’	Storm water drainage, pooled
13	South Drainage	N29° 30.740’ W82° 32.900’	Storm water drainage, concrete
14	N Leak #1	N29° 30.948’ W82° 32.802’	Liner repair, leachate leak
15	N Leak #2	N29° 30.954’ W82° 32.800’	Liner repair, down slope

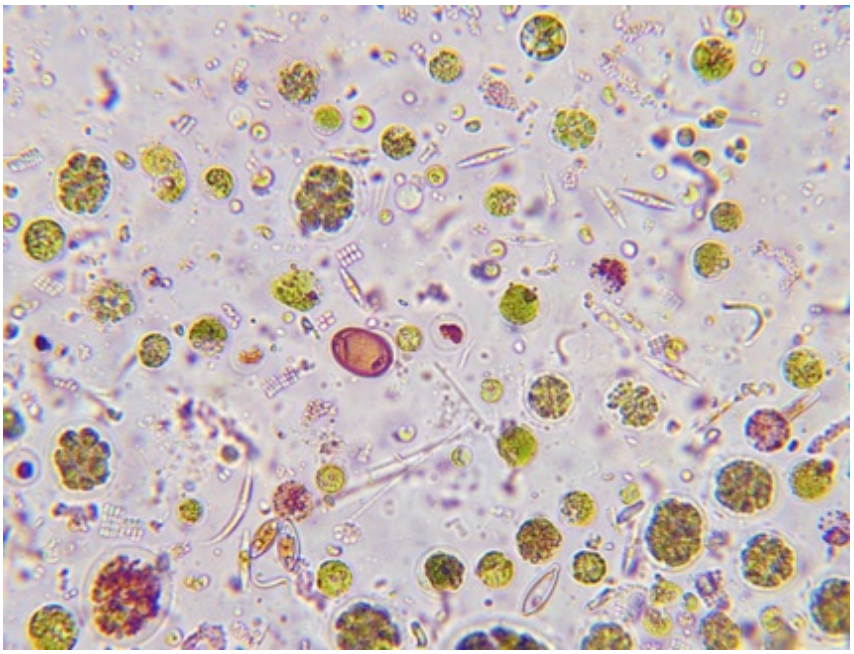


Figure 3-2. The diversity of algae within a grab sample from LH tank #2

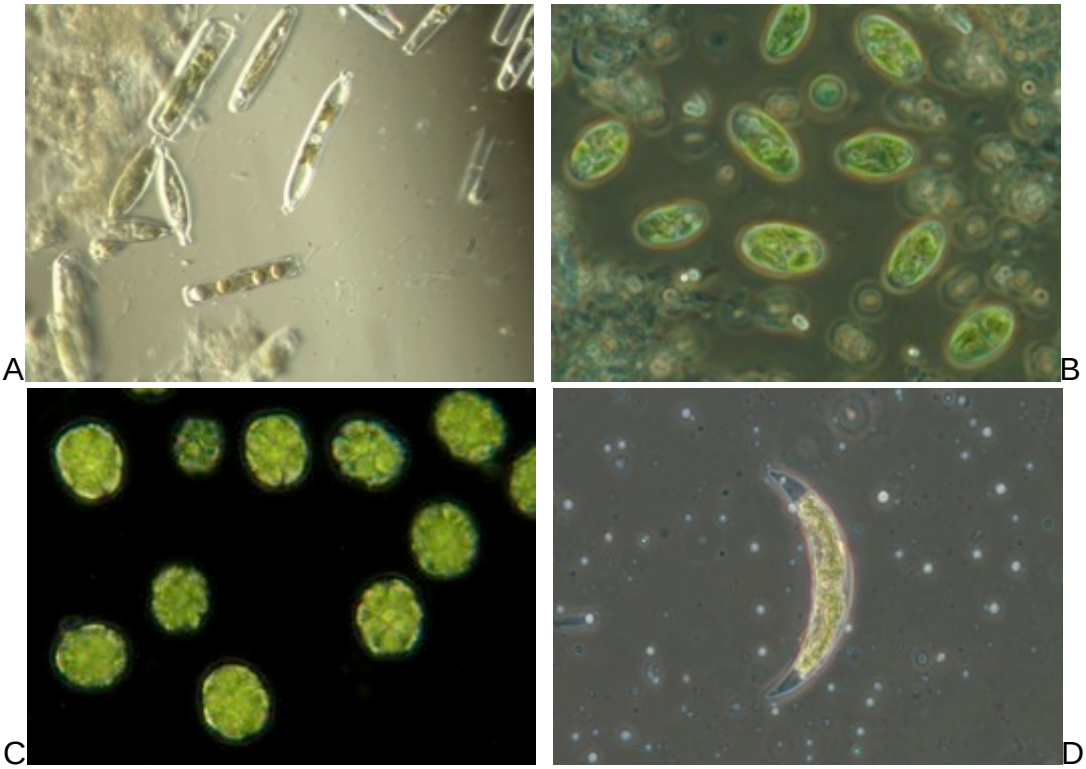


Figure 3-3. Algae Bioprospects of the ACSW Landfill A) The diatom *Navicula spp.* from pooled rainwater on soil (site #10), B) *Chlorella cf. ellipsoidea* from a concrete basin holding water (site #9), C) *Pandorina* colonies, dark-field illumination (site #6), D) *Closterium* from south drainage (site #13)

Table 3-2. Algae Genera Observed at the ACSW Landfill.

Genus	Division	Site #	Season Observed
Ankistrodesmus	Chlorophyta	6	Summer
Bumilleriopsis	Chlorophyta	2, 6	Summer
Chlamydomonas	Chlorophyta	2, 6	Winter
Chlorella	Chlorophyta	3, 5, 6, 9, 12, 13	Summer/Winter
Closterium	Chlorophyta	13	Summer
Kirchneriella	Chlorophyta	9, 6	Summer
Lyngbya	Cyanophyta (Cyanobacteria)	2, 3	Summer
Merismopedia	Cyanophyta (Cyanobacteria)	6	Winter
Microspora	Chlorophyta	11	Winter
Navicula	Bacillariophyta	10, 12	Winter
Oedogonium	Chlorophyta	11	Winter
Oscillatoria	Cyanophyta (Cyanobacteria)	2	Winter
Pandorina	Chlorophyta	6	Winter
Rhizoclonium	Chlorophyta	11, 12	Summer/Winter
Scenedesmus	Chlorophyta	9, 6, 13	Summer/Winter
Synecococcus	Cyanophyta (Cyanobacteria)	12	Summer
Ulothrix	Chlorophyta	11, 12	Winter

Table 3-3. Elemental Analysis of Leachate from the ACSW Landfill

Component (mg/L)	Average	Std. Dev.	n
TAN	967.8	198.83	5
COD	2073.33	161.66	3
Conductivity (mS/cm)	15.77	2.42	3
Alkalinity	5,448.8	25.7	3
Sodium	2713.33	857.34	3
Chloride	1933.33	152.75	3
Iron	10.50	4.95	3
Arsenic	0.08	0.05	3
Chromium	0.08	0.03	3
Copper	0.11	0.08	2
Potassium	777.00	287.09	2
Phosphorus	12.49	3.55	2
Magnesium	88.00	N/A	1
Calcium	110.00	N/A	1
Boron	32.00	N/A	1
Manganese	0.11	N/A	1
Zinc	0.06	N/A	1

Table 3-4. Algal biomass potential by element found in ACSW landfill leachate

Element	Concentration (mg/L)	Average % of algal biomass (Healy 1973)	Biomass Potential (mg/L)
Nitrogen	967.8	5.50	17,596
Phosphorus (PO_4)	12.5	1.10	1,135
Potassium	777.0	1.73	44,913
Magnesium	88.0	0.56	15,714
Calcium	110.0	0.87	12,644
Iron	10.5	0.59	1,780

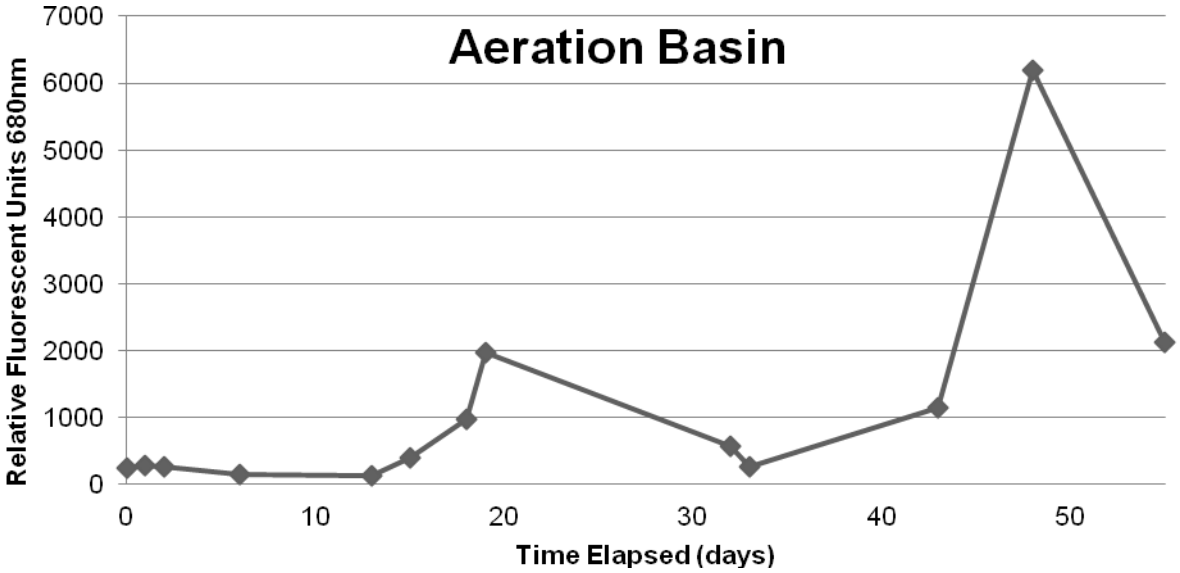


Figure 3-4. Chlorophyll fluorescence of algae within the aeration basin (AB)

Figure 3-5. The aeration basin (AB) field cultivation unit

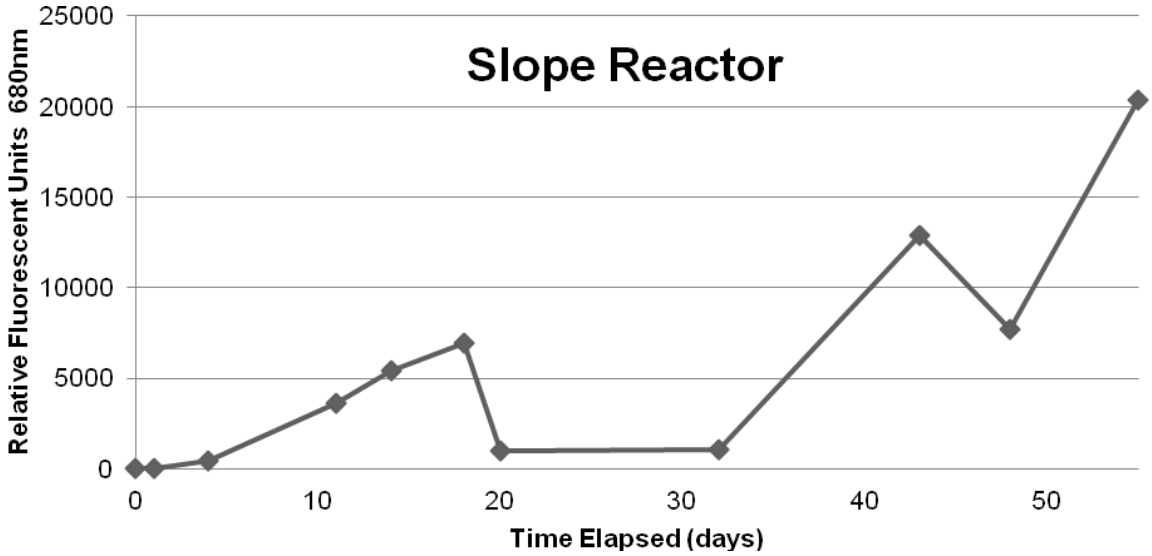


Figure 3-6. Chlorophyll fluorescence of algae within the sloped reactor (SR)

Figure 3-7. The slope reactor (SR) field cultivation unit

Table 3-5. Growth Rates, Maximum Fluorescence, and Genera of Mini-pond Cultures

Landfill Leachate %	Average Growth Rate	Maximum Fluorescence	Genera observed
2.5	0.14	14,112.5	*Chlorella*
25	0.13	10,052.3	*Chlorella, Selenestrum*
50	0.05	1,680.8	*Chlorella, Scenedesmus*
75	0.07	2,382.9	*Ankistrodesmus, Chlorella, Scenedesmus*
100	0.04	1,449.7	*Ankistrodesmus, Chlorella, Scenedesmus, Lyngbya, Navicula*

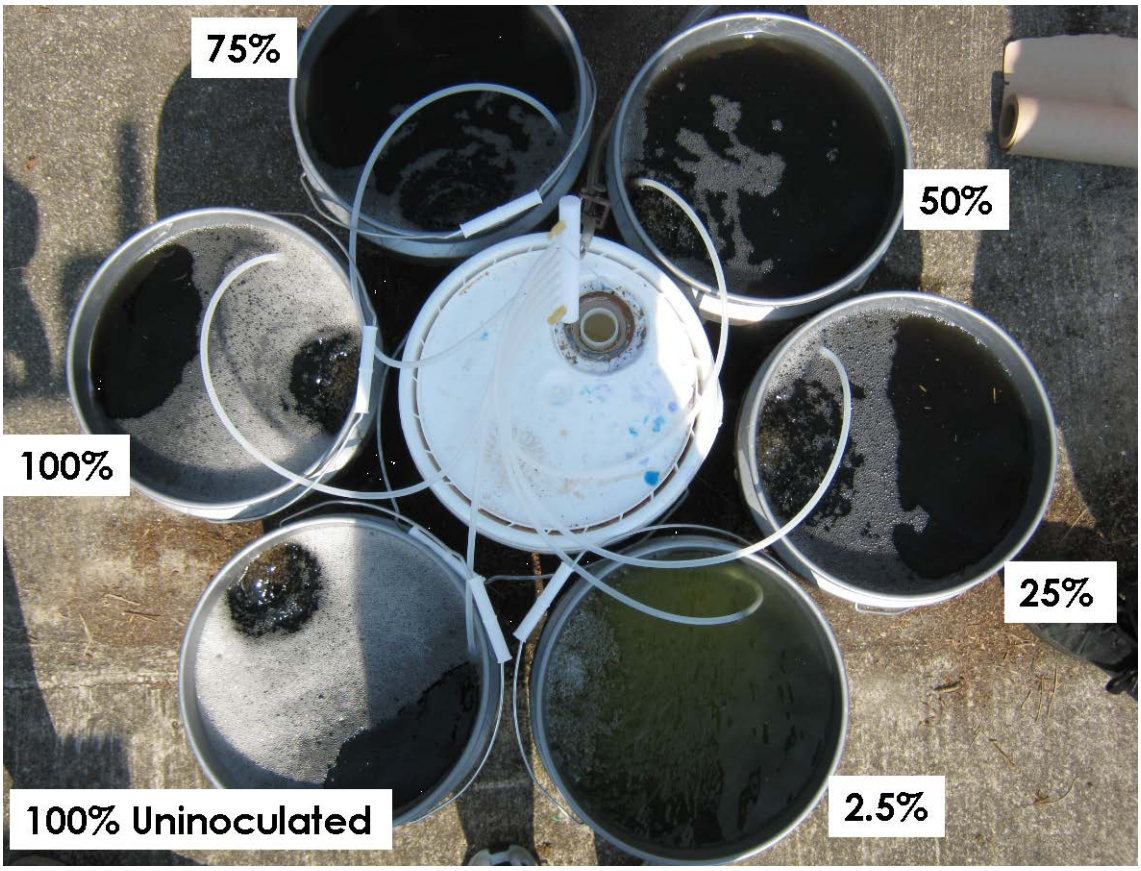


Figure 3-8. Mini-pond cultivation of algae at various dilutions of landfill leachate

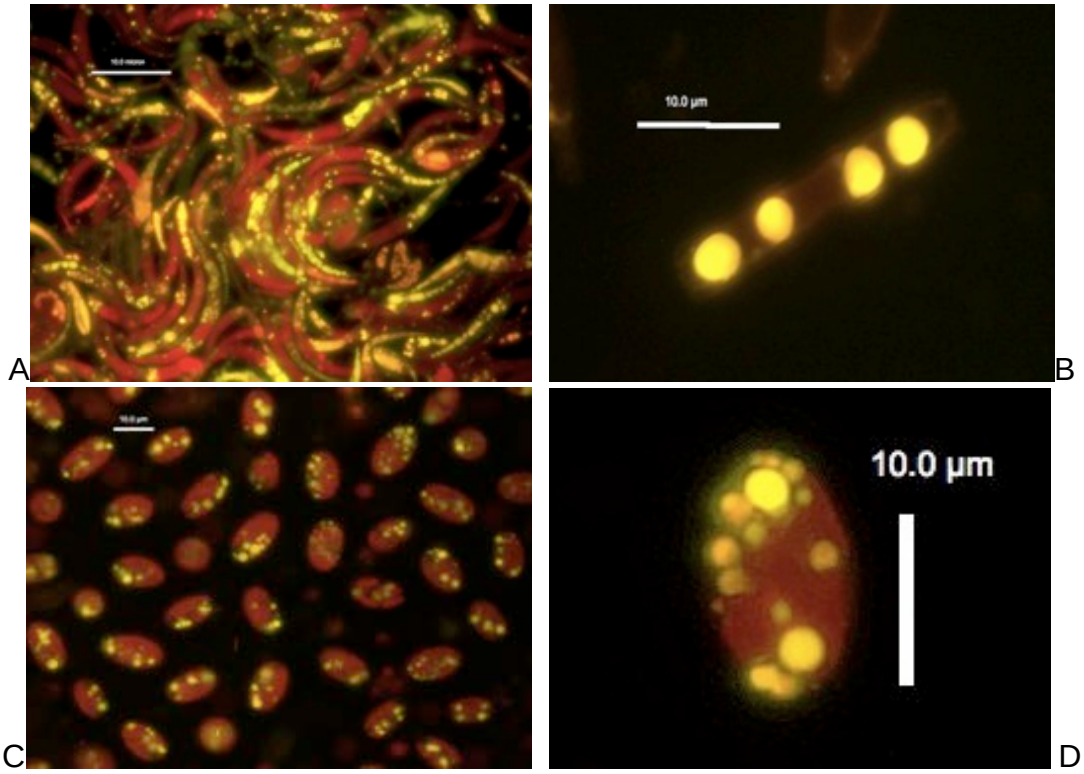


Figure 3-9. Lipids in Algae Bioprospects of the ACSW Landfill, Nile Red fluoresces yellow in lipids, red is chlorophyll auto-fluorescence. A) *Ankistrodesmus* sp., B) *Navicula sp.* C) *Chlorella cf. ellipsoidea,* D) *Chlorella cf. ellipsoidea,* single cell Scale bars = 10microns

CHAPTER 4
ALGAE CULTIVATION ON LANDFILL LEACHATE

Evaluation of Landfill Leachate as a Cultivation Medium

The growth in this set of cultivation experiments establishes a baseline growth rate for the algal culture *Chlorella cf. ellipsoidea* (AB3), an isolate of *Chlorella cf ellipsoidea* derived from the aeration basin (AB) of the field cultivation experiments. In order to evaluate the growth of an algal culture on landfill leachate it is necessary to first have a baseline comparison with a standard nutrient medium. Bold's basal medium (BBM) was chosen as a standard algal medium with all essential nutrients provided for algae growth (Andersen 2005).

Bold's Basal Medium Baseline

The cultivation of the *Chlorella cf ellipsoidea* (AB3) on dilutions of BBM with deionized water showed that both 50 and 100% concentrations showed little difference in the average and maximum growth rates (Figure 4-1). Average growth rates for 50 and 100% BBM are 0.29 and 0.33, respectively. Maximum growth rates for 50 and 100% BBM are 1.40 and 1.55, respectively and occur within the first 48 hours. A reduction to 10% BMM causes a reduction of ~48% in the average growth rate and a 33% decrease in the maximum growth rate of the culture (Table 4-1). This represents a baseline growth rate for the cultivation on BBM under the defined conditions within the laboratory.

Landfill Leachate as a Cultivation Medium

As a direct comparison, landfill leachate (LL) was diluted with deionized water to concentrations of 10, 50, and 100% and used and a medium for the cultivation of *Chlorella cf ellipsoidea* (AB3) under identical laboratory conditions (Figure 4-2).

Leachate concentrations at 50 and 100% were toxic under laboratory conditions. Cultures in these high concentrations of landfill leachate exhibited a rapid decline in chlorophyll fluorescence within the first 12 hours and a complete loss of chlorophyll fluorescence after 48hours, which persisted for the duration of the experiment. In contrast, landfill leachate at a concentration of 10% showed linear growth for the duration of the experiment (Figure 4-2). Average growth rates of *Chlorella cf. ellipsoidea* (AB3) in both 10%BBM and 10%LL are remarkably similar, μ=0.17 and 0.20, respectively (Table 4-1, Figure 4-3). The growth in 10%BBM occurs immediately and the maximum rate (μ_{max}=1.05) is within the first 24 hours of cultivation. After the initial rapid growth the culture declines slightly and then plateaus at 72hours and remains in a stationary phase for the remainder of the experiment, presumably due to Liebig's law of the minimum. Cultivation in 10%LL shows slow growth in the first 24hours, which may be associated with a lag phase of cellular adjustment to the new medium. The maximum growth rate of algae within 10%LL, which is only half that of the maximum of 10%BBM, is reached between 48 and 72hours (μ_{max} =0.45).

Total Ammoniacal Nitrogen

Levels of total ammoniacal nitrogen (TAN) within all LL cultures decreased over time (Figure 4-4). The cultures with algae did not differ from the abiotic control treatments, indicating that in this experiment algae did not serve as a mechanism for TAN remediation. Instead the most probable explanation is that TAN is volatilizing from the culture as gaseous ammonia (NH_3).

pH

Algal blooms in surface waters are often associated with dramatic pH increases (Cole 1994). Diurnal fluctuations from pH 7 to 10 are not uncommon in natural waters with high populations of photosynthetic algae. As algae photosynthesize, carbonic acid is scavenged from the water faster than it can diffuse into solution from the atmosphere. As expected, the pH of all cultures rose dramatically within the fist 12 hours of cultivation. The pH of cultures in 50 and 100%BBM, rose from 6.5 to approximately 10, while cultures in 10% BBM rose from 6.7 to 9.7 within 24hours (Figure 4-5). This pH increase corresponded to an increase in chlorophyll fluorescence, which is in agreement with the well-described mechanism of pH increase due to carbonic acid scavenging via algal photosynthesis (Cole 1994). Interestingly, the pH in 10%LL rose from 7.62 to 8.6 with no observed increase in chlorophyll fluorescence. This contradicts the photosynthetic mechanism for pH change previously described. Furthermore, the pH in 50 and 100% LL also rose from ~7.8 to 9.0, which corresponded to a decrease in chlorophyll fluorescence (Figure 4-6). Evidently, an alternate mechanism is needed to understand the change in pH of algae cultures within the landfill leachate medium. It was observed that the pH change within the abiotic landfill leachate treatments closely mirrored the landfill leachate treatments with algae. Only 10%LL showed a deviation from the abiotic control pH at 48hours, which corresponded to the period of maximum growth within this culture. The mechanism for the observed rapid initial pH change within cultures growing on landfill leachate is thus predominantly within the leachate medium and not caused by the photosynthesis of the algae. Physico-chemical mechanisms will be explored in more detail in the experiments of a subsequent section.

Based on these baseline cultivation experiments it is concluded that landfill leachate under the defined laboratory conditions was a poor growth medium. Due to the rapid decline in chlorophyll fluorescence in cultures growing on 50 and 100%LL it can be surmised that there is a toxicity factor within the LL, which dilution to 10% concentration made tolerable to the algae. At these low concentrations LL showed promise as a medium for cultivation as consistent growth was observed over the course of the study. Further investigations will aim to elucidate the cause of toxicity and cultivation strategies to avoid toxic effects.

Elucidation of Toxicity Factors

Compositional Comparison of Landfill Leachate and Bold's Basal Medium

Landfill leachate (LL) is generally considered toxic, high concentrations of salts, heavy metals, xenobiotic organics, and ammonia nitrogen can all contribute to the toxicity level; depending on the organism tested, concentration and duration of exposure (Kjeldsen *et al.* 2002, Ward *et al.* 2002, Plotkin and Ram 1984). Cultivating algae on landfill leachate requires the resolution of the cause of toxicity. Once the primary toxicant is determined, methods for fostering cultivation to avoid the biological impacts can be devised. Elemental constituents within the LL were compared to those found within Bold's basal medium (BBM), to assess potential toxicants (Table 4-3). Potential inhibitors based on elemental analysis of LL to BBM found that the most notable potential inhibitors, due to comparative concentration are total ammoniacal nitrogen, sodium, and chloride. High concentrations of total ammoniacal nitrogen, sodium, and chloride characteristic of landfill leachate may be inhibitory depending on specific tolerances of the organism under cultivation.

Interspecies Toxicity Comparison

Two algae isolates from the ACSW landfill were tested to compare the interspecies tolerances of the two organisms to ACSW landfill leachate. The two tested organisms, *Chlorella cf. ellipsoidea* (AB3) and *Scenedesmus sp*. (ISO2), were originally derived from landfill field cultivation experiments. In both species, toxic effects of the landfill leachate on algae chlorophyll fluorescence occur within 24 hours of experimentation, allowing a rapid test for tolerance to the leachate. Similar to previous results, *Chlorella cf. ellipsoidea* shows a tolerance only at a concentration of 10%, above which all concentrations are toxic (Figure 4-7). *Scenedesmus sp*. shows a tolerance of leachate up to 30% (Figure 4-8), above which all concentrations are toxic. From this rapid screening incubation, it can be established that *Scenedesmus sp.* can tolerate higher concentrations of ACSW landfill leachate than *Chlorella cf. ellipsoidea*.

Sodium chloride toxicity test

The sodium and chloride content of the two media were notably different. Bold's basal medium, a freshwater solution, had sodium levels 81.6mg/L and chloride levels of 126mg/L. In contrast, the sodium content of ACSW LL measured approximately 2,713mg/L and the chloride content measured 1,933mg/L, a 33-fold and 15-fold difference, respectively (Table 4-2). The sizable difference in absolute sodium and chloride content indicated that salinity is a possible mechanism for the observed toxicity exhibited by the higher concentrations of LL on algae. Organisms isolated from the landfill, a non-marine site, may or may not have a biological tolerance for such high sodium and chloride levels. High sodium and chloride were therefore identified as possible factors in the toxicity exhibited by cultures grown in high concentrations (>10%) of landfill leachate. Testing was undertaken with pure sodium chloride salts dissolved in

deionized water at concentrations comparable to those found in the ACSW LL. These tests elucidated the effect of sodium chloride on the two species of algae under laboratory cultivation conditions. Toxicity tests on the two species isolated from ACSW landfill showed that neither *Scenedesmus* sp. nor *C. cf. ellipsoidea* exhibited toxicity symptoms due to NaCl levels of 5.84g/L, 125% those found in landfill leachate (~4.65g/L), although *C. cf. ellipsoidea* appears to be more salt sensitive than *Scenedesmus* (Figures 4-9 and 4-10).

Ammonium chloride toxicity tests

A significant point of contrast between ACSW LL and BBM was the nitrogen level of the two media. On an elemental basis, ACSW LL had 967.8mg/L N and BBM had 41.2mg/L N, a 23.5-fold difference. Besides concentration, the chemical form of nitrogen within the two media is different. Nitrogen within leachate is predominantly in the reduced ammoniacal form (NH_3/NH_4^+) whereas in BBM nitrogen is found only as the oxidized nitrate (NO_3^-) form. These significant differences were examined in relation the observed toxicity of landfill leachate. Experimental tests were undertaken to examine the toxicity of nitrogen in the ammoniacal form at concentrations comparable with ACSW LL. As chloride had already been disproved as a toxicity factor for the two experimental cultures (Figures 4-9 and 4-10), the pure salt ammonium chloride was used as a testing reagent for determining the impact of ammoniacal nitrogen. Tests under laboratory conditions showed no toxic effects in *Chlorella cf. ellipsoidea* nor *Scenedesmus* sp. at 1000mg/L-N as NH_4Cl (3.189g/L) (Figures 4-11 and 4-12). However, the pH levels of the cultures in the NH_4Cl medium remained nearly neutral for the duration of the test. Under neutral pH conditions, the majority of TAN is in the ionized form which is considered less toxic (Kallqvist and Svenson 2003). Thus, the

experiment only tested the toxicity of the charged ammonium ion (NH_4^+), which was not toxic to either experimental organism even at high concentrations equivalent to those found in ACSW LL.

In order to test the toxicity of the unionized fraction of ammoniacal nitrogen (NH_3), also called unionized ammonia or free ammonia, the experiment was repeated with an increased concentration of unionized ammonia. The two experimental algae were subjected to the same experimental conditions, except the medium pH was experimentally elevated to the pK_a of ammonium/ammonia (9.26 @ 25 °C) with 1M NaOH. At the pK_a value, the ionized and unionized forms of ammoniacal nitrogen are present in equal fractions. Both experimental algae cultures showed an immediate toxic effect from the increase in the unionized ammonia concentration (Figures 4-13 and 4-14). The toxicity effect closely resembled the toxic effect observed in previous landfill leachate experiments, in which relative fluorescence decreased significantly within 24hours (Figure 4-2). Furthermore, the pH of 9.26 is also closely resembled the experimentally measured pH of the both the 50 and 100%LL in previous experiments. The results obtained during this experiment were remarkably different than those observed within the culture that remained at a neutral pH, but had an equivalent concentration of ammoniacal nitrogen. The results obtained elucidate the primary toxicity factor within landfill leachate as unionized ammonia. Unionized ammonia is a widely recognized toxicant. It plays a larger role in cellular toxicity than its charged counterpart due to the neutral molecular charge, which allows the permeation of cell membranes.

Landfill Leachate: Dynamics under Laboratory Conditions

The following experiments were undertaken to translate the previous findings of ammonia toxicity in a pure salt medium to the complex salt solution of the ACSW LL. The variable within the experiment is the manipulation of the landfill leachate to atmospheric exposure, *i.e.* sealed and open flasks. The experiments first addressed the nature of the LL medium without algae under sealed and open flask conditions, referred to as '*abiotic'*. Experiments then addressed the impact of the LL media on the experimental algae cultures under the same two conditions (sealed and open flasks), in order to confirm unionized ammonia as the primary toxicant. The experiment is designed to show the difference between landfill leachate exposed to the atmosphere and under limited exposure to the atmosphere, which was found to modulate the pH of the medium.

Dynamics of 'Abiotic' Landfill Leachate

The *abiotic* trials without the addition of algae were under identical conditions as those used in the algae cultivation trials (i.e. light, shaking, *etc.*). Each variable had five replicates. Parallel to initial cultivation trials, the pH of the landfill leachate exposed to the atmosphere quickly rose in pH from initial values of 7.8(±0.02) to a value of 8.9(±0.02) within 24 hours. Maximum pH recorded was 9.12(±0.01) after 72hours (Figure 4-15). Leachate samples in sealed flasks with limited atmospheric exposure rose to a maximum value of 8.0(±0.04) within 72hours (Figure 4-15). Relative concentrations of unionized ammonia within the leachate were calculated by the dissociation constant, pH, and temperature of the sample following Kallqvist and Svenson (2003) and Korner *et al.* (2001). The concentration of unionized ammonia within the closed flasks remained relatively stable throughout the experiment; at

72hours approximately 25% of TAN is present as unionized ammonia (Figure 4-16). Under open flask conditions, however, the concentration of unionized ammonia rises to over 50% of the total ammoniacal nitrogen concentration at 72hours (Table 4-17). There is a substantial rise in unionized ammonia in open flasks within the first 24hours, which agrees well with the rapid onset of observed toxicity effects in both algae cultures (Figures 4-7 and 4-8).

Dynamics of Landfill Leachate with Algae

Experiments examining the dynamics of ACSW LL under laboratory conditions were repeated with the addition of algal inoculum. *Chlorella cf. ellipsoidea* (AB3) and *Scenedesmus* sp. were cultivated on 50% landfill leachate under conditions of atmospheric exposure (open) and limited atmospheric exposure (sealed). Open flasks inoculated with *C. cf. ellipsoidea* decreased in fluorescence from 19,970 ±186 relative fluorescent units at 680nm (RFU) to 159 ±22 RFU in 72 hours, indicating toxic conditions (Figure 4-18). Open flasks inoculated with *Scenedesmus sp.* decreased in fluorescence from 13,410 ±2,867 RFU to 1,463 ±383 in 72 hours, likewise indicating toxic conditions (Figure 4-21). This observed toxicity was contrasted with the survival of both algal cultures within the sealed replicates, which maintained high relative chlorophyll fluorescence until the end of the experiment, indicating a nontoxic environment. Open flask cultures showed the same physico-chemical pH trend as those found in abiotic treatments (Figures 4-19 and 4-22), while sealed cultures had muted pH changes. Changes still occurred within sealed cultures presumably due to the opening of flasks at periodic sampling events. The dramatic impact of sealing flask the on the biology of two different species of algae gave evidence of mechanism that reduced toxicity of 50%LL, a concentration determined to be toxic. Substantial

difference in culture fluorescence in the open and sealed flasks supports the hypothesis that unionized ammonia is the primary toxicant within the landfill leachate. The results demonstrate the biological impact of unionized ammonia caused by a rise in medium pH.

Effect of pH Control on Algae Cultivation in Landfill Leachate

In previous experiments, medium pH determined the relative concentration of the unionized ammonia and therefore toxicity to two tested species of algae. Thus, pH control should allow the ammoniacal nitrogen within the leachate to remain ionized (NH_4^+) and nontoxic (Figure 4-9 and 4-10), allowing the cultivation of algae on ACSW landfill leachate. Experiments in flasks using pH control were mixed by filtered air as opposed to previously implemented shaker mixing, which may have implications on the growth of algae cultures. The media of experimental treatments were potentiometrically controlled by the addition of gaseous CO_2, which dissociated in water to form carbonic acid (H_2CO_3) and lowers the pH. *Chlorella cf. ellipsoidea* (AB3) and Scenedesmus sp. (ISO2) were grown under CO_2 regulated pH conditions in 50% and 100% landfill leachate. The pH levels of control cultures were not regulated. Regulation of pH by CO_2 addition in 50% landfill leachate showed adaptability and growth in both *C. cf. ellipsoidea* (AB3) (Figures 4-23 and 4-24) and *S. sp* (ISO2) (Figures 4-25 and 4-26). Both control cultures did poorly, exhibiting the previously observed toxicity effects of 50%LL, however, *S. sp* (ISO2) culture had moderate growth, even without pH control (Figure 4-25). This is most likely due to the aeration of the medium, which drove gaseous unionized ammonia out of the liquid phase until tolerable levels were reached. Regulation of pH via CO_2 within 100%LL showed growth in S. sp. (ISO2), but a very weak growth in *C. cf ellipsoidea* (AB3) (Figures 4-27 and 4-28).

In addition to CO_2, inorganic acids can be added to regulate the pH of the landfill medium. The pH of ACSW LL was potentiometrically adjusted to 7.0 with 1M hydrochloric acid (HCl) to reduce the relative concentration of unionized ammonia. Under pH regulation with HCl, both experimental algae cultures grew well on 100%LL (Figures 4-29 and 4-30), showing improved growth compared to the 100%LL control without pH control and even over the cultures with pH regulation via CO_2. *Chlorella cf. ellipsoidea* was able to tolerate 100%LL with HCl pH regulation, whereas this culture was not able to tolerate 100%LL with CO_2 addition. Results of HCl addition in 100%LL showed an increase in culture growth in both species more stable than the addition of gaseous CO_2, resulting in higher growth rates and maximum chlorophyll fluorescence (Figures 4-29 and 4-30).

Discussion and Summary

Baseline cultivation trials with the indigenous alga isolate *Chlorella cf. ellipsoidea* (AB3) demonstrated ACSW landfill leachate to be inferior to Bold's basal medium as a solution for the cultivation of algae. This result was expected based on current literature examining the growth of algae on landfill leachate (Lin *et al.* 2007; Cheung *et al.* 1992). Diluting landfill leachate with deionized water gave growth results similar to diluted BBM, an intriguing result giving credence to the possibility of utilizing landfill leachate as a novel medium for algae cultivation. Average growth rates in 10%LL slightly exceed those within the 10%BBM, however maximum growth rates of algae within 10%LL were roughly half of those in 10%BBM. Furthermore, rapid toxicity screening showed similar inhibitory effects of leachate above a 10% concentration on two experimental algal isolates, *Chlorella cf. ellipsoidea* (AB3) and *Scenedesmus* sp. (ISO2). Diluting leachate

by 90% with groundwater, however, would be an imprudent use of water resources. It was therefore necessary to elucidate the cause of toxicity within landfill leachate.

Due to the non-marine source of algae cultures tested within the laboratory, there was a significant possibility that the sodium chloride levels within ACSW LL could have been inhibitory to culture growth. This was quickly dismissed as a driver for toxicity as both *Chlorella cf. ellipsoidea* (AB3) and *Scenedesmus* sp. (ISO2) cultures were tolerant of NaCl levels higher than those within landfill leachate. Another suspected toxicant was ammonium, due to the high levels of total ammoniacal nitrogen within the landfill leachate when compared with BBM. Additionally, previous literature has identified ammonia as an environmental toxin (Szumski *et al.* 1982). Tests with pure salts of ammonium chloride at levels of TAN equivalent to those of landfill leachate demonstrated that algae were not only tolerant of these high levels of TAN, but actually grew under them, albeit moderately. An interesting observation on the cultivation of algae on ammonium chloride was that the pH did not rise during photosynthesis. This is theoretically explained, as the influx of ammonium ions into algal cells is counter balance with the efflux of H+ ions out of the cell to maintain ionic balance (Britto and Kronzucker 2002). It is due to the neutral pH of the medium TAN levels remained in the charged ammonium form, which is less toxic (Kallqvist and Svenson 2003). Further experiments proved that base addition (NaOH), shifts the TAN equilibrium to unionized ammonia, revealed a rapid toxic effect in both experimental algae cultures. The toxic effect in both cultures was remarkably similar to the toxicity observed within landfill leachate. Un-ionized ammonia was therefore proposed as the primary causative agent of toxicity within landfill leachate.

Experimentation then focused on the regulation of ammonia toxicity, in order to promote the cultivation of algae within high concentrations of landfill leachate. The unionized form of ammonia, which is the primary toxicant, exists in high concentrations only at elevated pH levels. It is well known that algal photosynthesis elevates the pH in natural water bodies (Cole 1994). Previous experiments, however, exhibited medium pH elevations and resultant toxic effects on algae cultures without photosynthetic-induced pH changes (Figure 4-15). Thus another mechanisms for pH rise will be proposed, which can explain the pH changes within the abiotic treatments and thus the toxic effect on the algae treatments. Landfills are primarily under anaerobic conditions, facilitating the fermentation of organic compounds to carbon dioxide and methane. Following Henry's law a high concentration of carbon dioxide causes the leachate solution to equilibrate with the carbon dioxide rich environment of the landfill. Thus landfill leachate has a high concentration of dissolved inorganic carbon. When the leachate leaves the landfill, dissolved carbonic acid concentration is greater than the atmospheric carbon dioxide concentration and equilibration by carbonic acid leaving solution occurs over time. Thus carbon dioxide escapes from the solution in order to equilibrate with the atmospheric concentration of carbon dioxide, as seen in the effervescence of leachate when sampling. As carbon dioxide escapes it shifts the carbonate equilibrium removing carbonic acid and raising the pH of the solution. As the pH of the solution rises, the total ammoniacal nitrogen equilibrium, which is strongly pH dependent, shifts towards the unionized form of ammonia. The pKa of the solution is temperature dependent but at 25°C is 9.26. Thus, as the pH of the landfill leachate

rises due to carbon dioxide escape a greater percentage of TAN is present as un-ionized ammonia, which is toxic.

Based on the empirical investigations undertaken it is concluded that the alkalinity of landfill leachate combined with high TAN concentrations are the primary drivers of toxicity in the cultivation of algae on landfill leachate. Therefore, as experimentally proven, if the pH is artificially maintained at neutral, by addition of CO_2 gas or HCl, the TAN remains largely in the ionized form and is thus non-toxic to the algae, allowing cultivation at high concentrations of landfill leachate (50 and 100%). No existing literature has been found that demonstrates the addition of CO_2 gas or HCl in the cultivation of algae on landfill leachate, which indicates this is a novel application for these widely known mechanisms of pH control.

The literature that does exist on the cultivation of algae on landfill leachate is sparse and focused primarily on toxicity testing. Two of the most relevant references (Lin et al. 2007 and Cheung et al. 1992) maximum growth rate as well as the concentration of leachate used and the concentration of TAN within the leachate. A comparison was made between this study and the current literature. It is demonstrated in this work that providing algae cultures with pH control allows growth in high concentrations of landfill leachate at rates not yet reported (Table 4-4). *Scenedesmus* sp. (ISO2) was more tolerant of landfill leachate than *Chlorella cf. ellipsoidea.* C. cf. ellipsoidea grew well in 50%LL, but poorly on 100%LL. Scenedesmus sp. (ISO2) exhibited robust growth on both 50 and 100%LL. Cultivation conditions and pH control mechanisms are not optimized in this study, indicating that improvements in growth rates and tolerances are likely. Growth in ACSW LL with only pH modification, provides

firm evidence for the ammonia mechanism of toxicity within landfill leachate and also the inherent value of nutrients present within the landfill leachate.

Figures and Tables

Table 4-1. Comparison of Growth Rates in Bold's Basal Medium and Landfill Leachate

Medium	Average Growth Rate, μ	Maximum Growth Rate, μ_{max}
100% BBM	0.33	1.40
50% BBM	0.29	1.55
10% BBM	0.17	1.05
10% LL	0.20	0.45

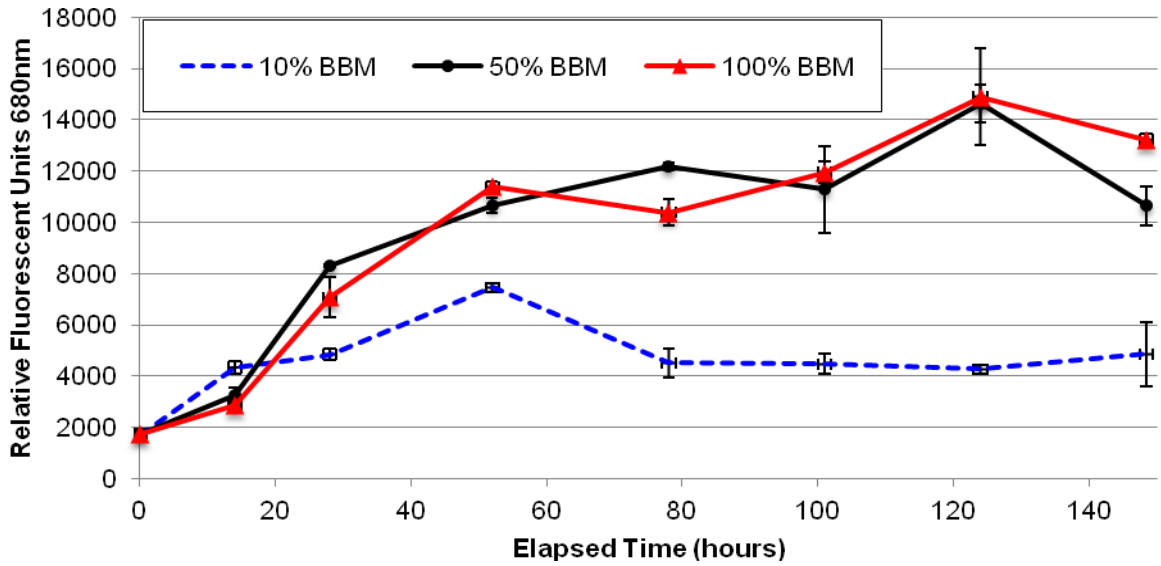


Figure 4-1. Baseline growth of *Chlorella cf. ellipsoidea* (AB3), on Bold's basal medium, error bars represent standard deviations of triplicate treatments.

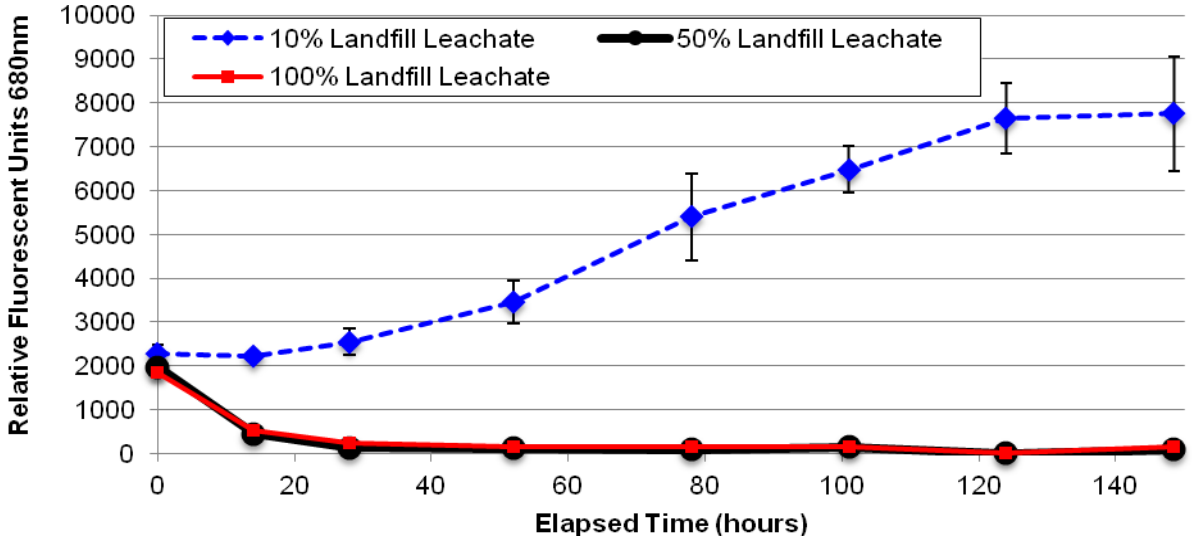


Figure 4-2. Growth of *Chlorella cf. ellipsoidea* (AB3) on leachate from ACSW landfill. Error bars represent standard deviations of triplicate treatments.

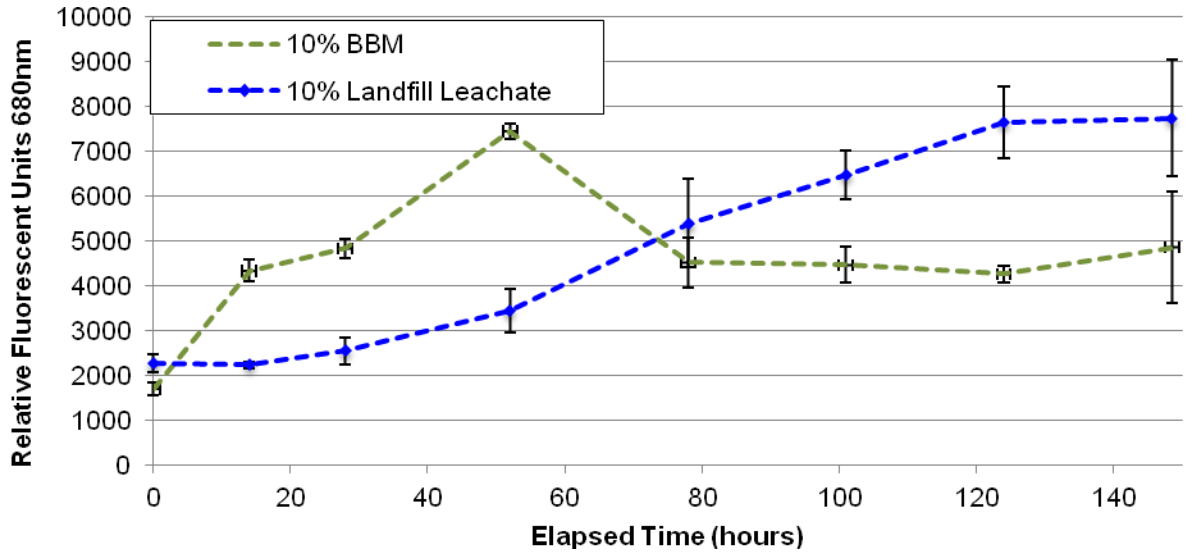


Figure 4-3. Comparison of *Chlorella cf. ellipsoidea* (AB3) growth in 10% Bold's basal medium and 10% landfill leachate, error bars represent standard deviations of triplicate treatments.

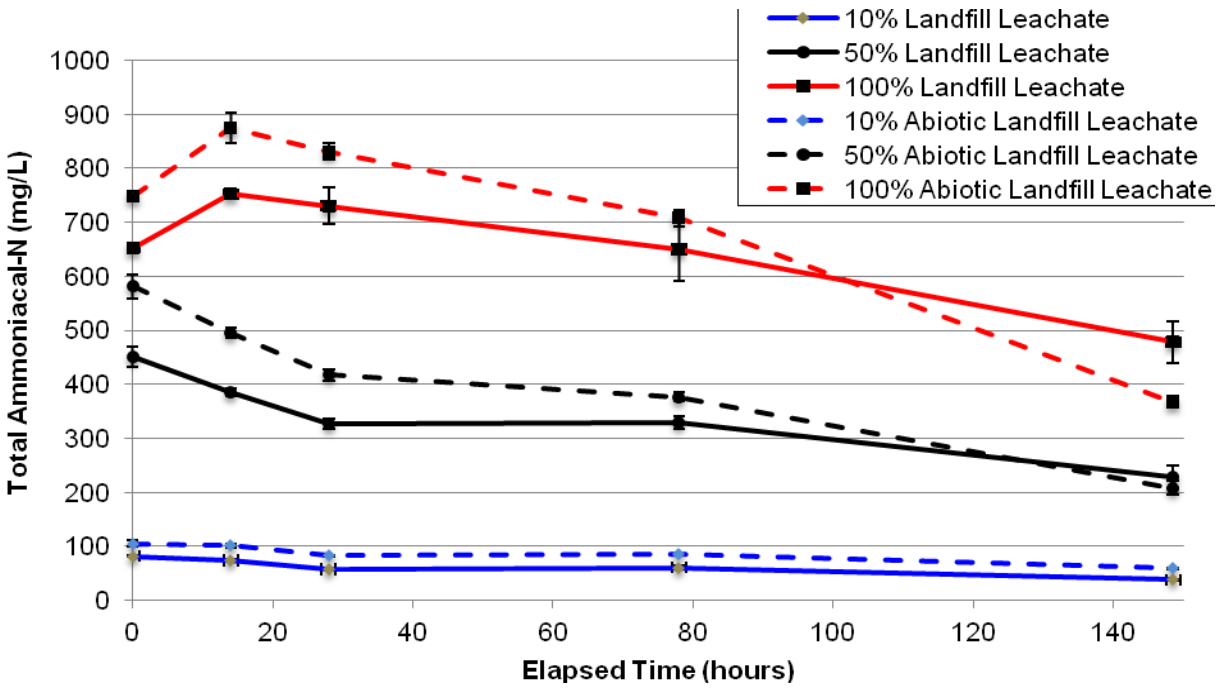


Figure 4-4. Ammonia volatilization in under cultivation conditions, error bars represent standard deviations of triplicate treatments.

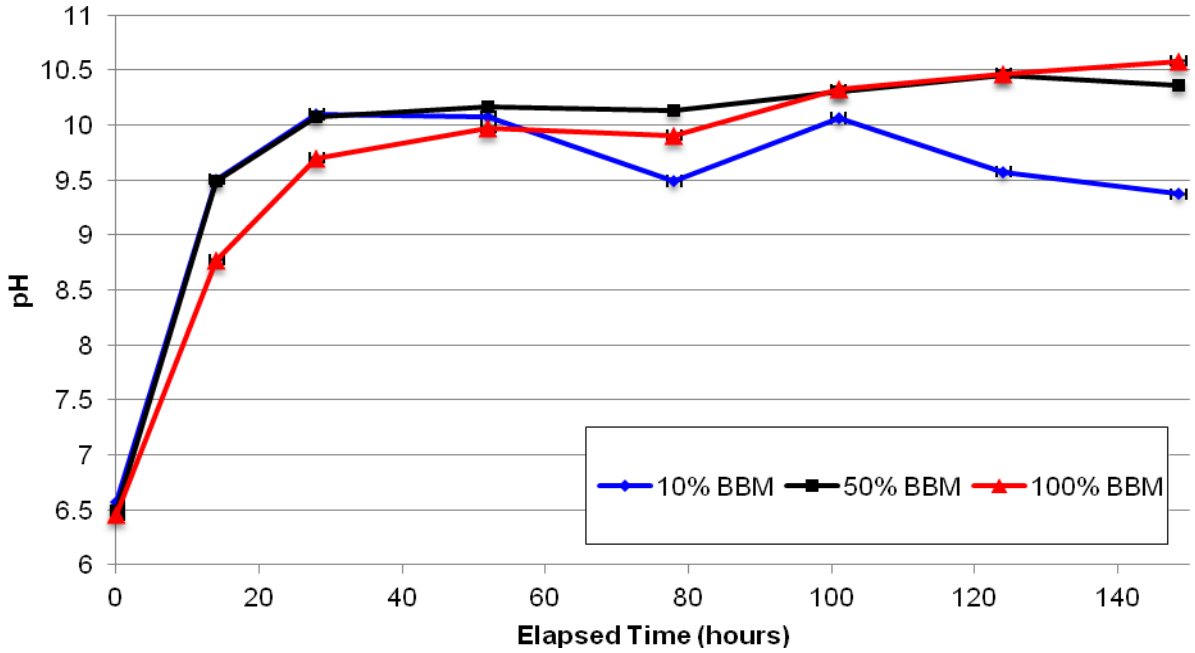


Figure 4-5. Dynamics of pH During Cultivation on Bold's Basal Medium (BBM), error bars from triplicate replications are present but not visible.

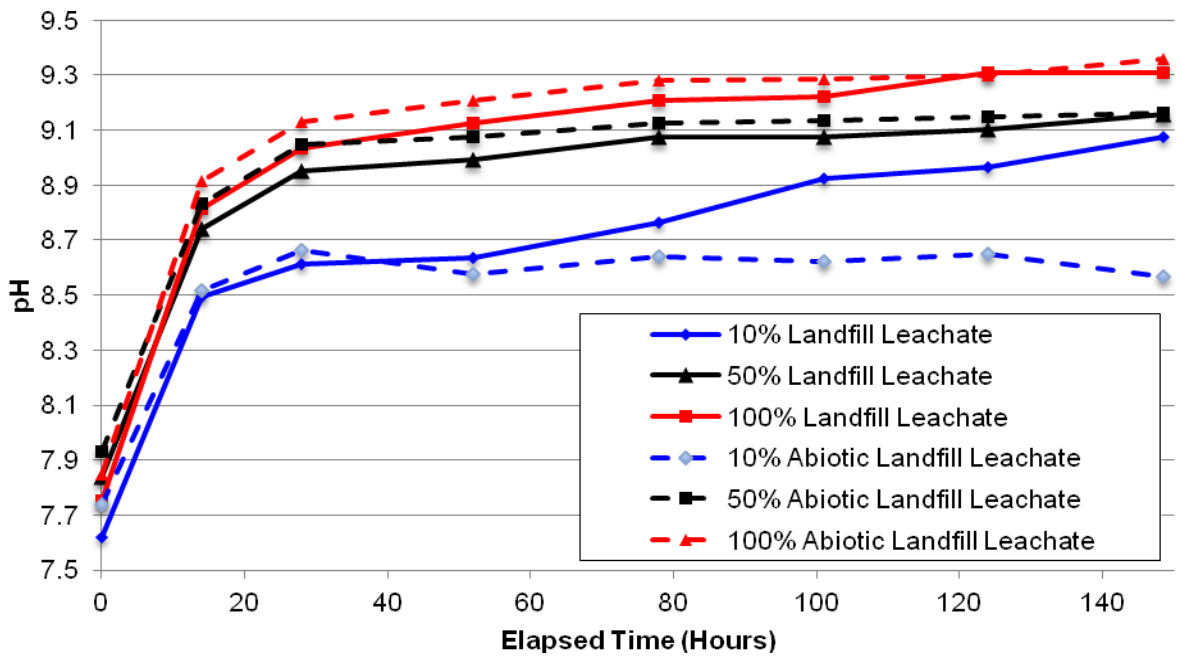


Figure 4-6. Dynamics of pH During Cultivation on Landfill Leachate, error bars from triplicate replications are present but not visible.

Table 4-2. Comparing Chemical Composition of ACSW landfill leachate with BBM

Component (mg/L)	Landfill Leachate	Bold's Basal Medium
Macronutrients		
Nitrogen		
__Ammonia-N*	967.8	_
__Nitrate-N	_	41.2
Phosphorus (PO_4)	12.5	163.1
Potassium	777	170.3
Magnesium	88	7.4
Calcium	110	6.8
Iron	10.5	1
Sodium	2,713.3	81.6
Chloride	1,933.3	126
Micronutrients		
Manganese	0.11	0.50
Copper	0.11	0.02
Zinc	0.06	0.50
Cobalt	0.07	0.01

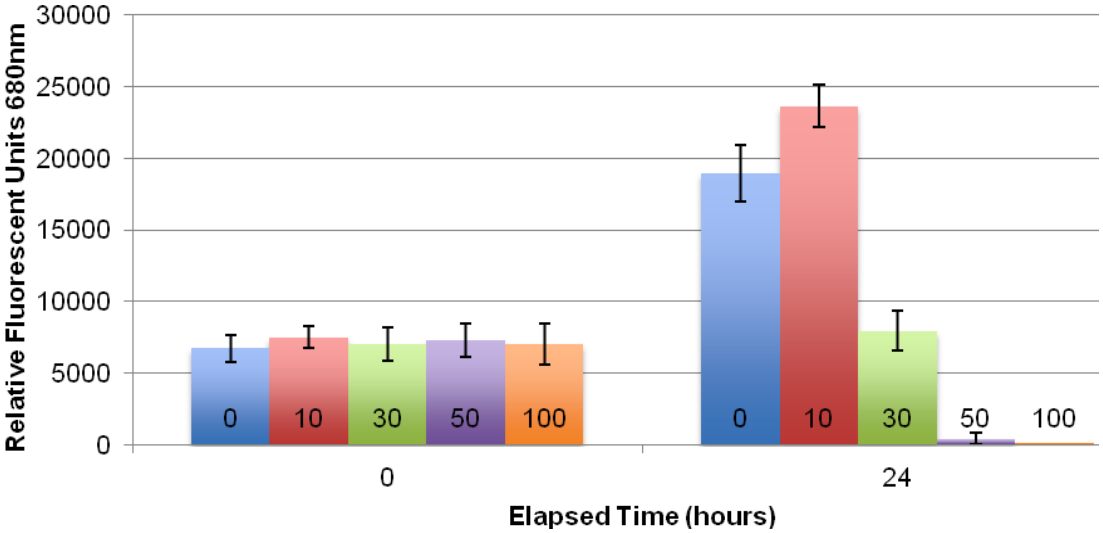


Figure 4-7. Toxic effect of 24hour landfill leachate incubation on Scenedesmus sp.

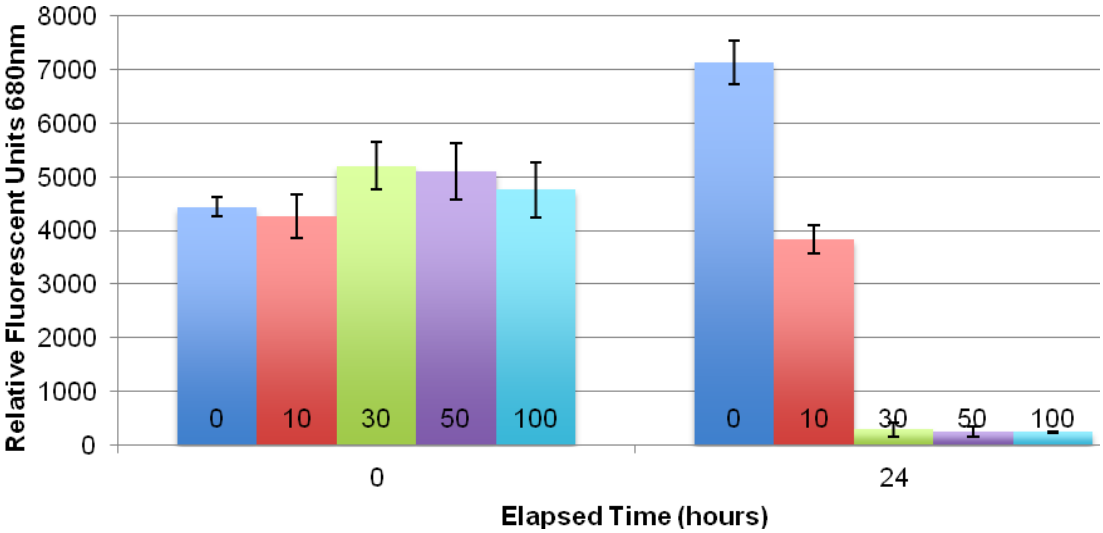


Figure 4-8. Toxic effect of 24hour landfill leachate incubation on *Chlorella cf. ellipsoidea*

Table 4-3. Sodium and Chloride concentrations in Bolds Basal Medium (BBM) and Landfill Leachate (LL)

Element (mg/L)	BBM	ACSW LL
Sodium	81.6	2,713
Chloride	126	1,933

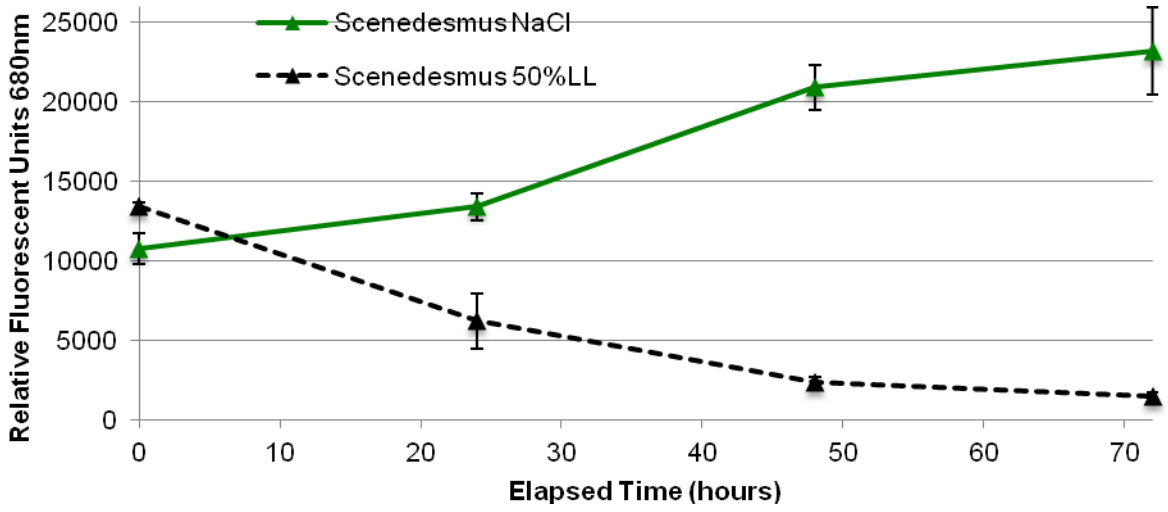


Figure 4-9. Effects of Sodium Chloride (5.84g/L) on *Scenedesmus* sp., error bars represent standard deviation of triplicate cultures.

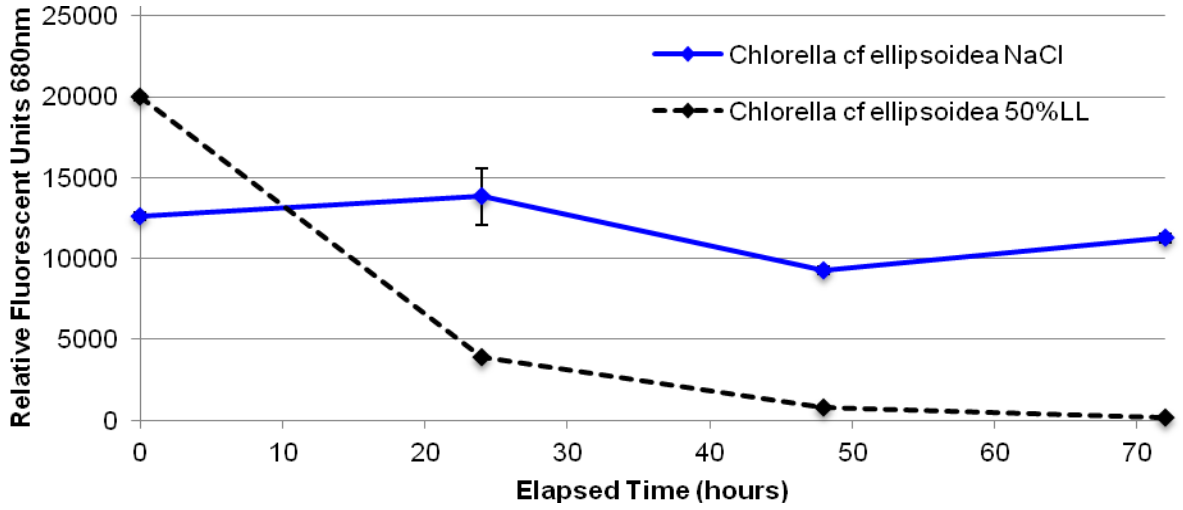


Figure 4-10. Effects of Sodium Chloride (5.84g/L) on *Chlorella cf. ellipsoidea*., error bars represent standard deviation of triplicate cultures.

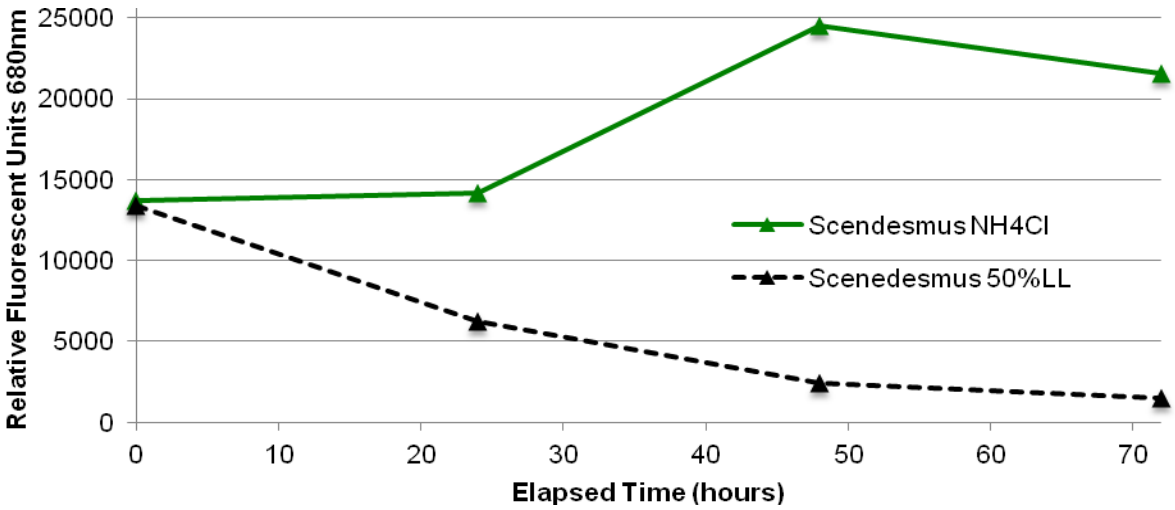


Figure 4-11. Effects of Ammonium Chloride (3.82g/L, 1000ppm-N) at pH 7.0 on *Scenedesmus* sp., error bars represent standard deviation of triplicate cultures.

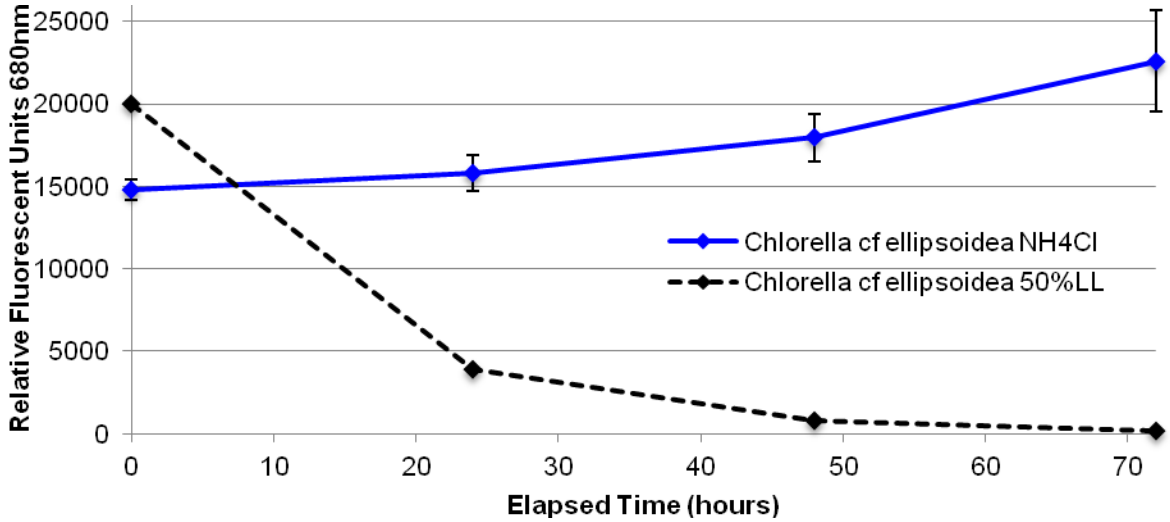


Figure 4-12. Effects of Ammonium Chloride (3.82g/L, 1000ppm-N) at pH 7.0 on *Chlorella cf. ellipsoidea*, error bars represent standard deviation of triplicate cultures.

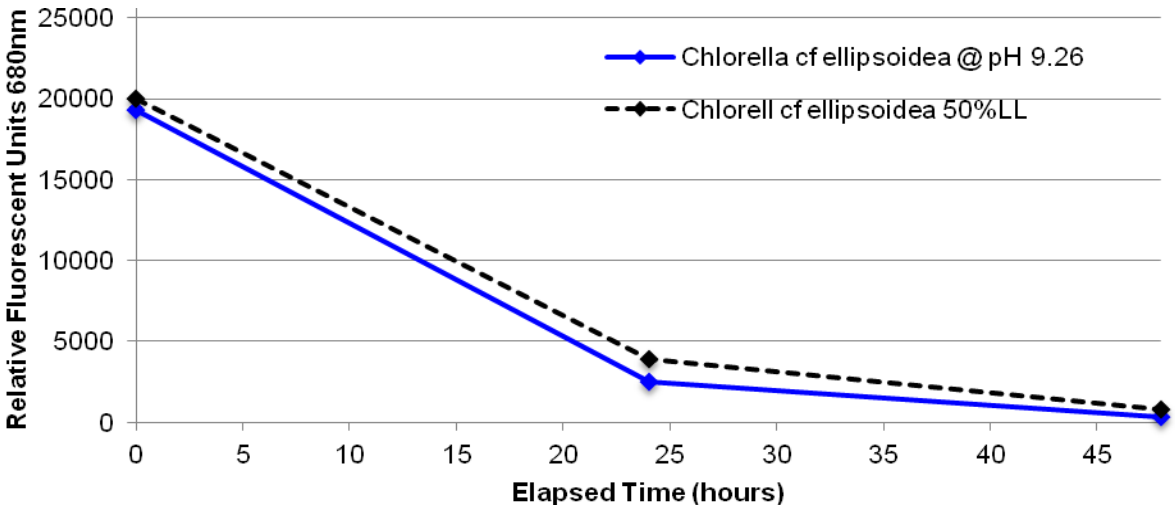


Figure 4-13. Effects of Ammonium Chloride (3.82g/L, 1000ppm-N) at pH 9.26 on *Chlorella cf. ellipsoidea*, error bars represent standard deviation of triplicate cultures.

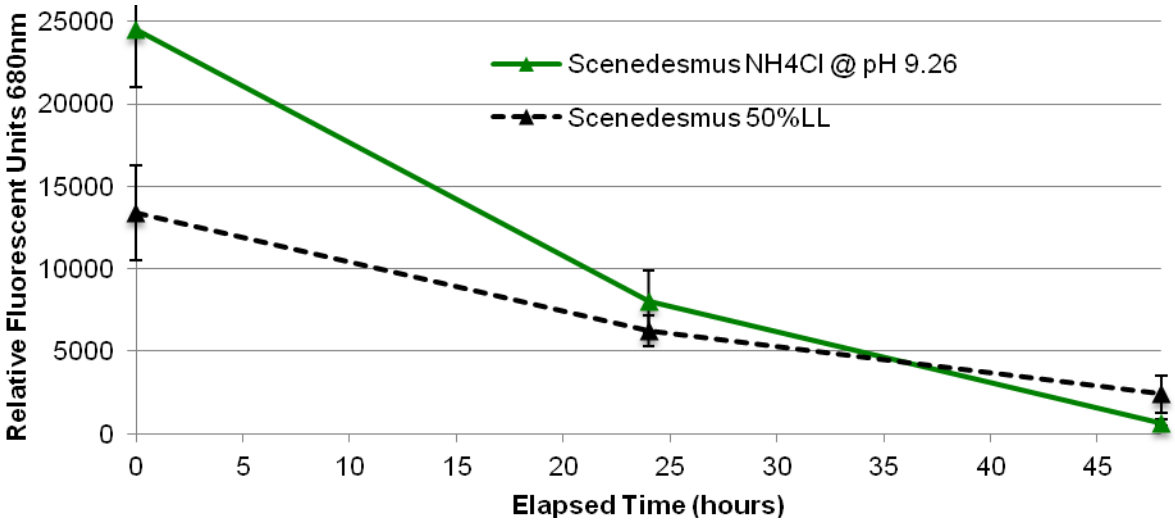


Figure 4-14. Effects of Ammonium Chloride (3.82g/L, 1000ppm-N) at pH 9.26 on *Scenedesmus* sp., error bars represent standard deviation of triplicate cultures.

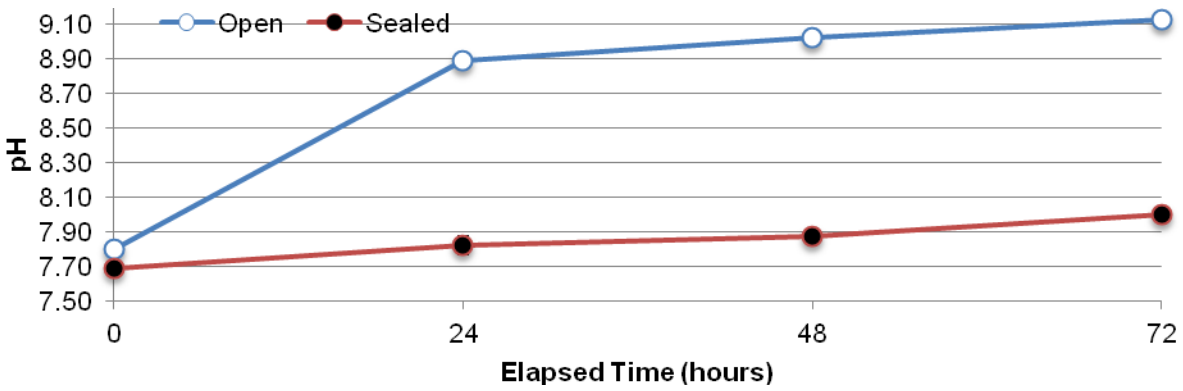


Figure 4-15. Change in pH within Sealed and Open Flasks

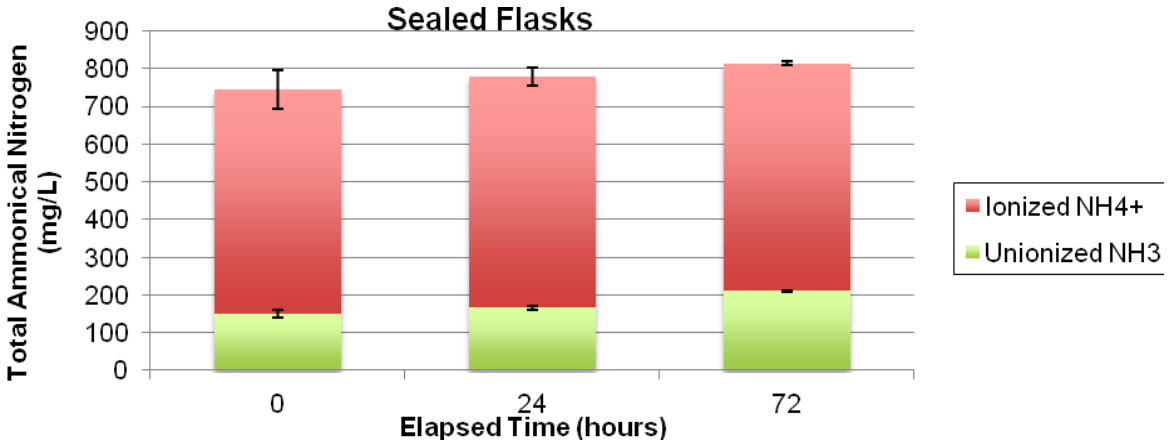


Figure 4-16. Ionized and Unionized Ammoniacal Nitrogen in Sealed Flasks, error bars represent standard deviation of quintuplicates

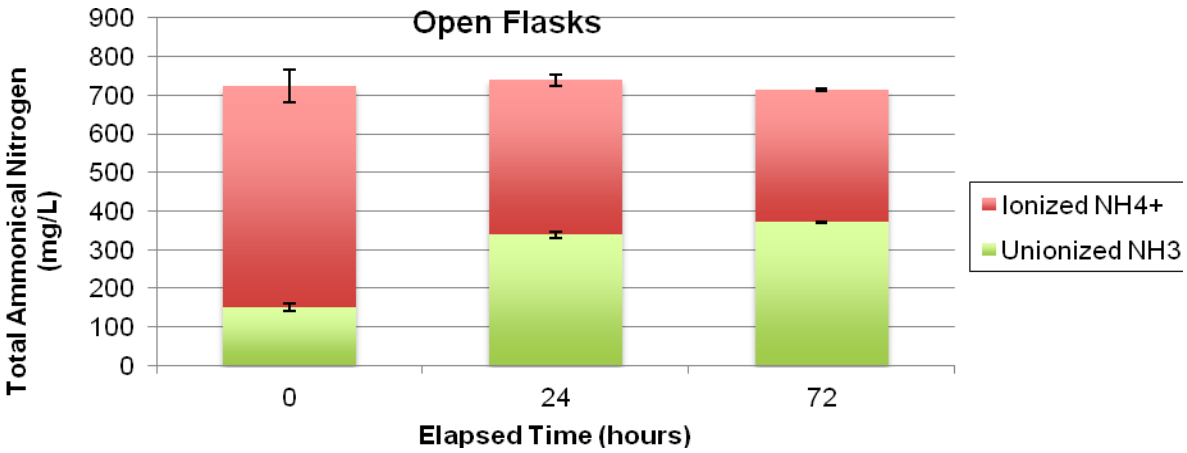


Figure 4-17. Ionized and Unionized Ammoniacal Nitrogen in Open Flasks, error bars represent standard deviation of quintuplicates

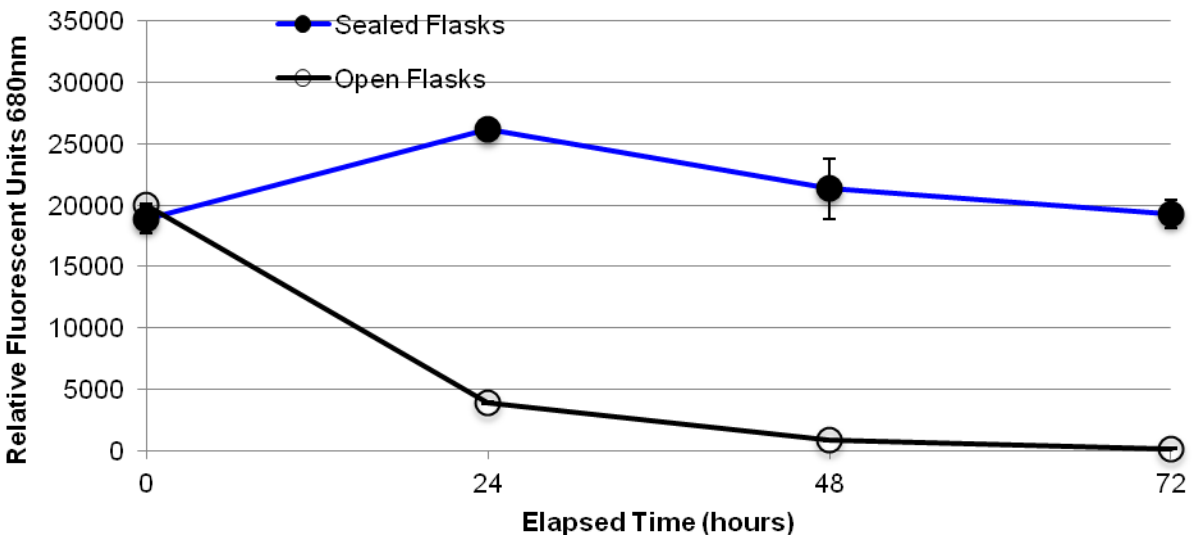


Figure 4-18. Fluorescence in sealed (filled circles) and open (open cirlces) flasks inoculated with *Chlorella cf ellipsoidea,* error bars represent standard deviation of triplicate treatments

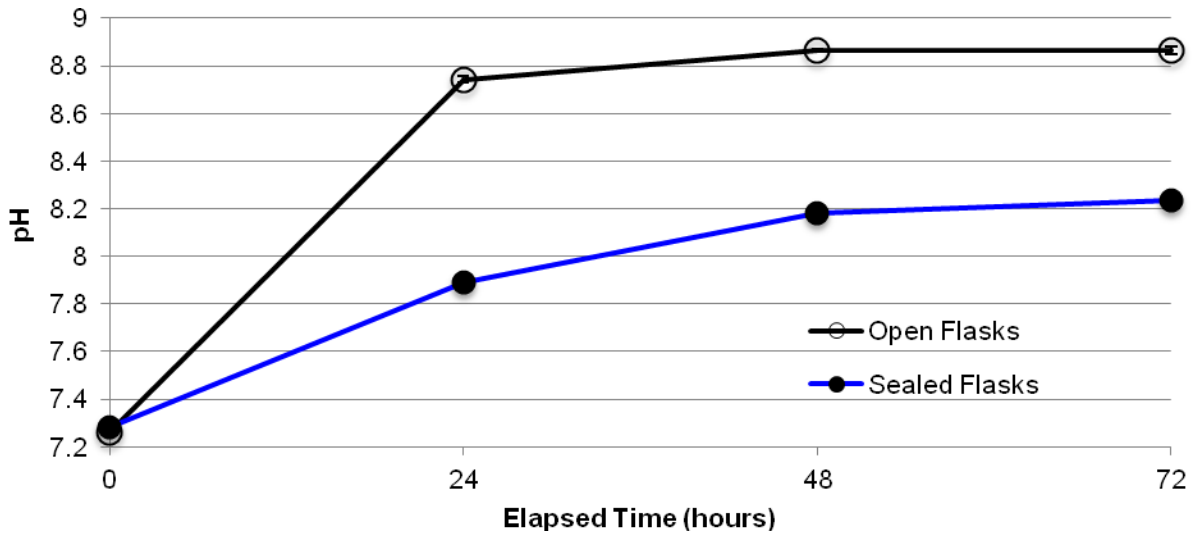


Figure 4-19. Change in pH in sealed (filled circles) and open (open cirlces) flasks inoculated with *Chlorella cf ellipsoidea,* error bars represent standard deviation of triplicate treatments

Figure 4-20. Triplicate sealed (black stopper) and open (white foam) flasks of *Chlorella cf. ellipsoidea* cultivated in 50%LL, green color correlates to chlorophyll fluorescence at 680nm

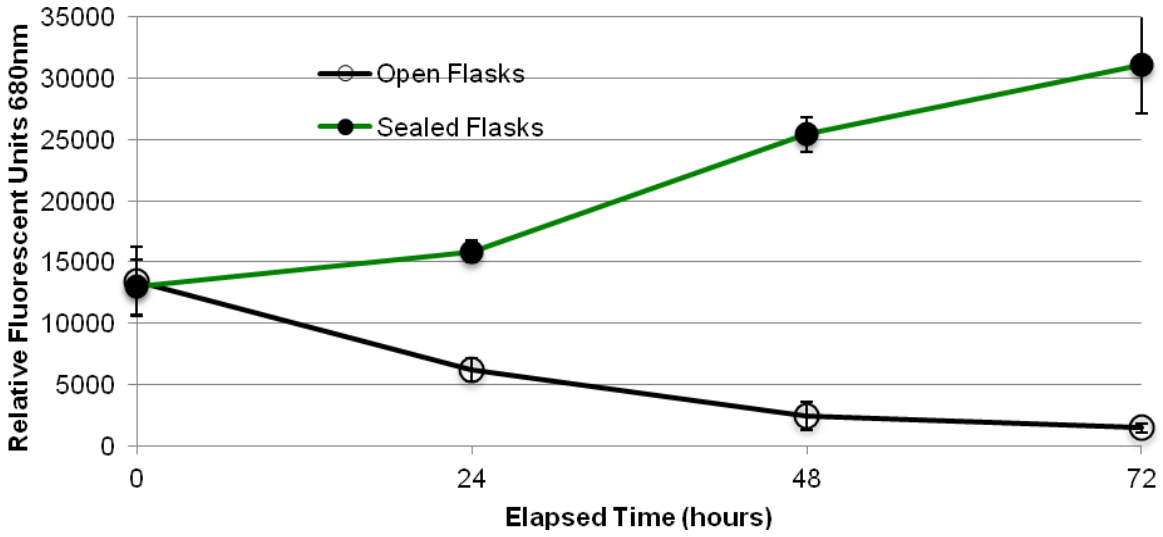


Figure 4-21. Fluorescence in sealed (filled circles) and open (open cirlces) flasks Inoculated with *Scenedesmus* sp., error bars represent standard deviation of triplicate treatments

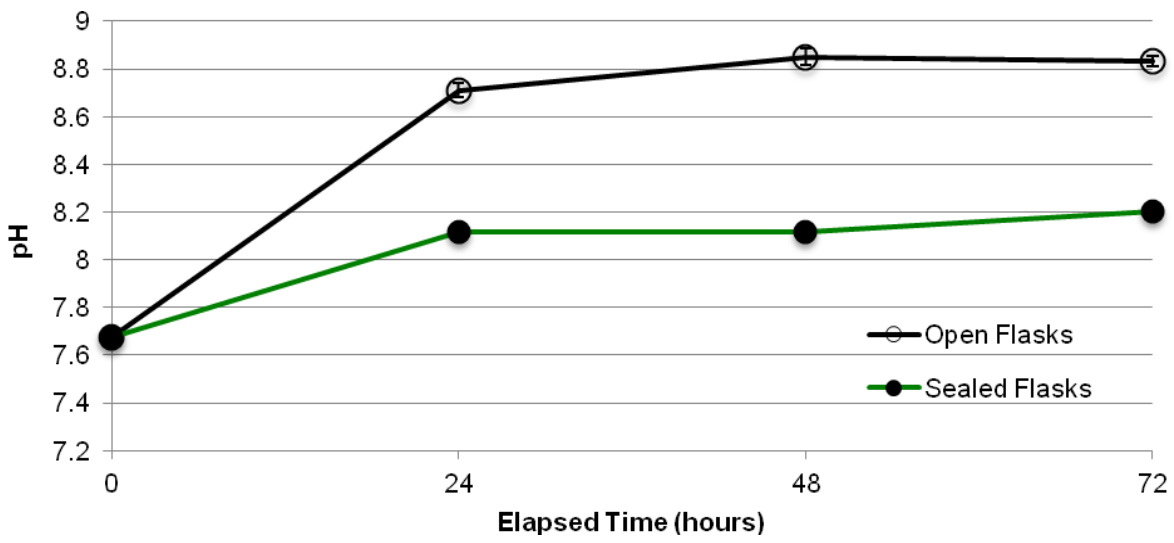


Figure 4-22. pH Dynamics in sealed (filled circles) and open (open cirlces) flasks Inoculated with with *Scenedesmus* sp.; error bars represent standard deviation of triplicate treatments

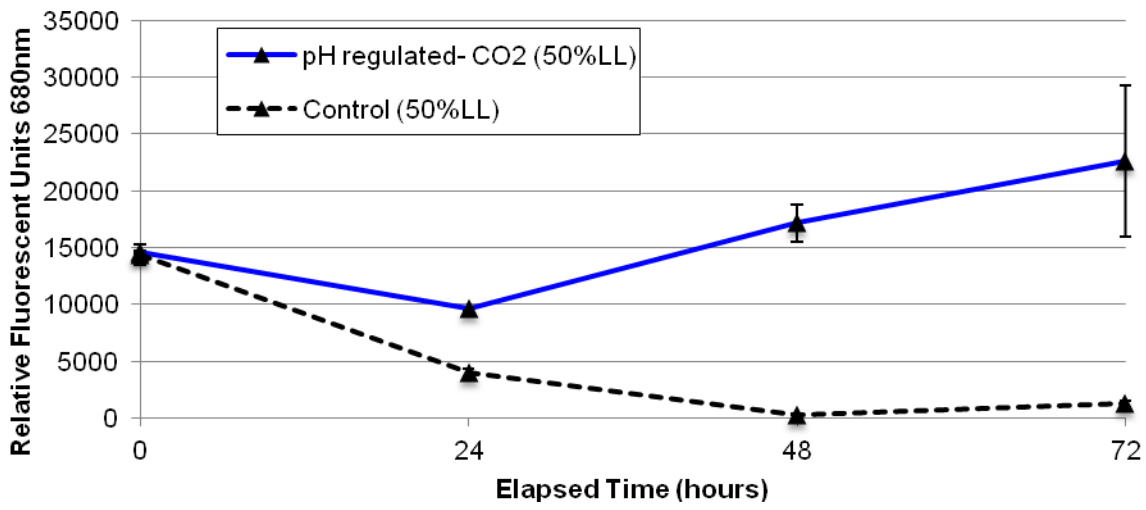


Figure 4-23. Effect of pH control via CO_2 on the cultivation of *Chlorella cf. ellipsoidea* (AB3) in 50% ACSW landfill leachate; error bars represent standard deviation of triplicate cultures

Figure 4-24. Cultivation of *Chlorella cf. ellipsoidea* (AB3) in 50% ACSW landfill leachate; triplicate cultures with pH regulation via CO2 (left) and controls (right); green color correlates with chlorophyll fluorescence at 680nm

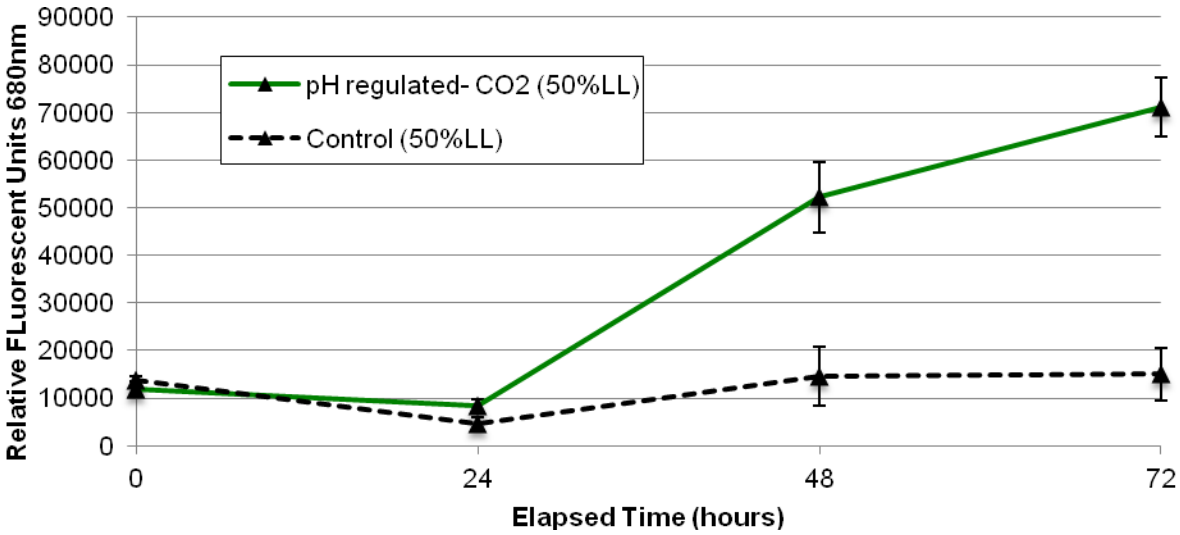


Figure 4-25. Effect of pH control via CO_2 on the cultivation of *Scenedesmus sp.* (ISO2) in 50% ACSW landfill leachate; error bars represent standard deviation of triplicate cultures

Figure 4-26. Cultivation of *Chlorella cf. ellipsoidea* (AB3) in 50% ACSW landfill leachate; triplicate cultures with pH regulation via CO_2 (right) and controls (left); green color correlates with chlorophyll fluorescence at 680nm

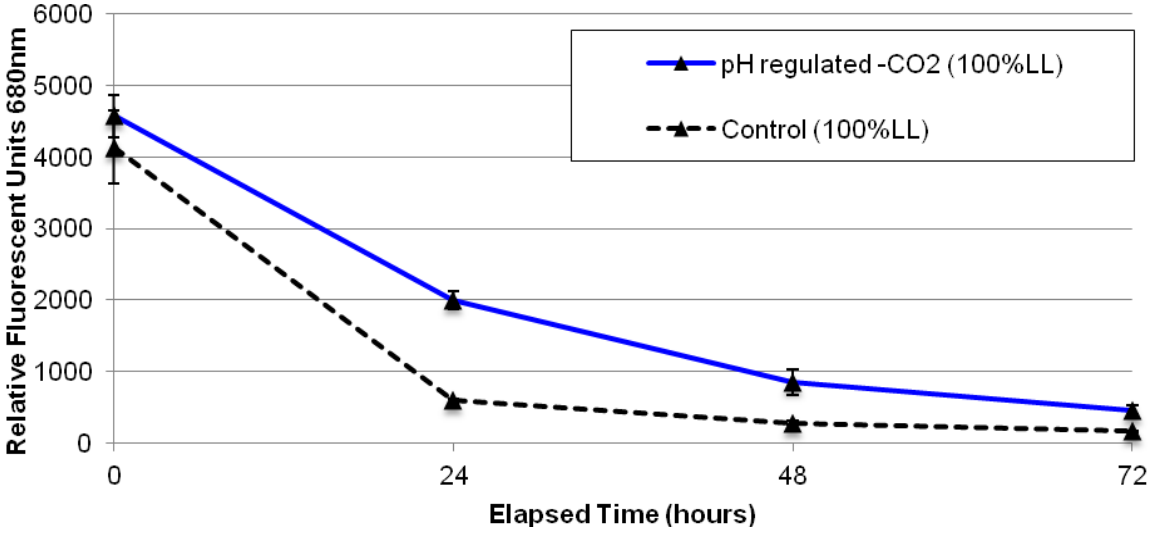


Figure 4-27. Effect of pH control via CO_2 on the cultivation of *Chlorella cf. ellipsoidea* (AB3) in 100% ACSW landfill leachate; error bars represent standard deviation of triplicate cultures

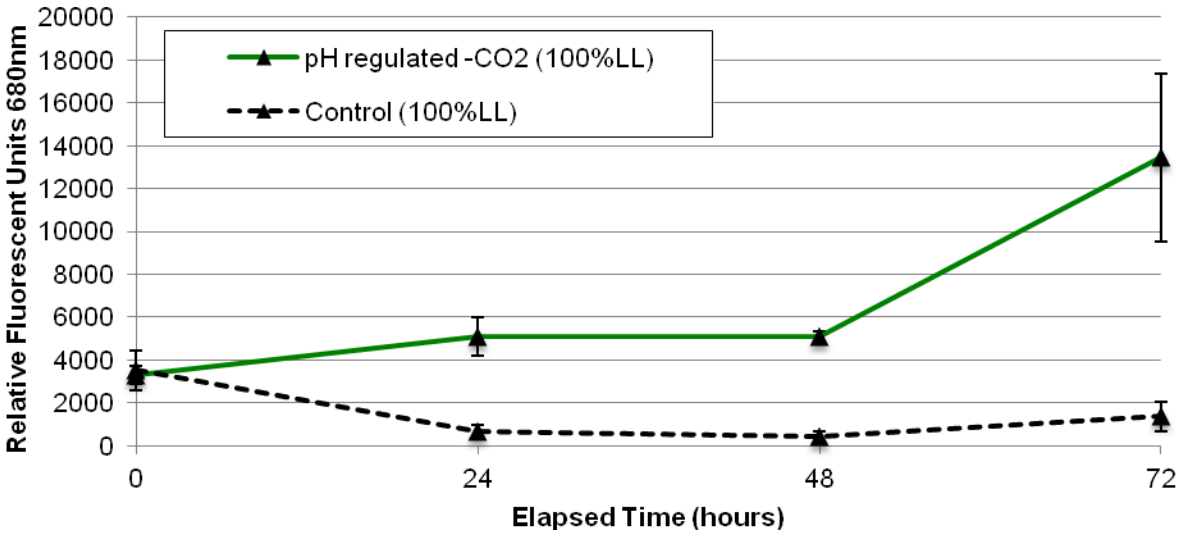


Figure 4-28. Effect of pH control via CO_2 on the cultivation of *Scenedesmus sp.* (ISO2) in 100% ACSW landfill leachate; error bars represent standard deviation of triplicate cultures

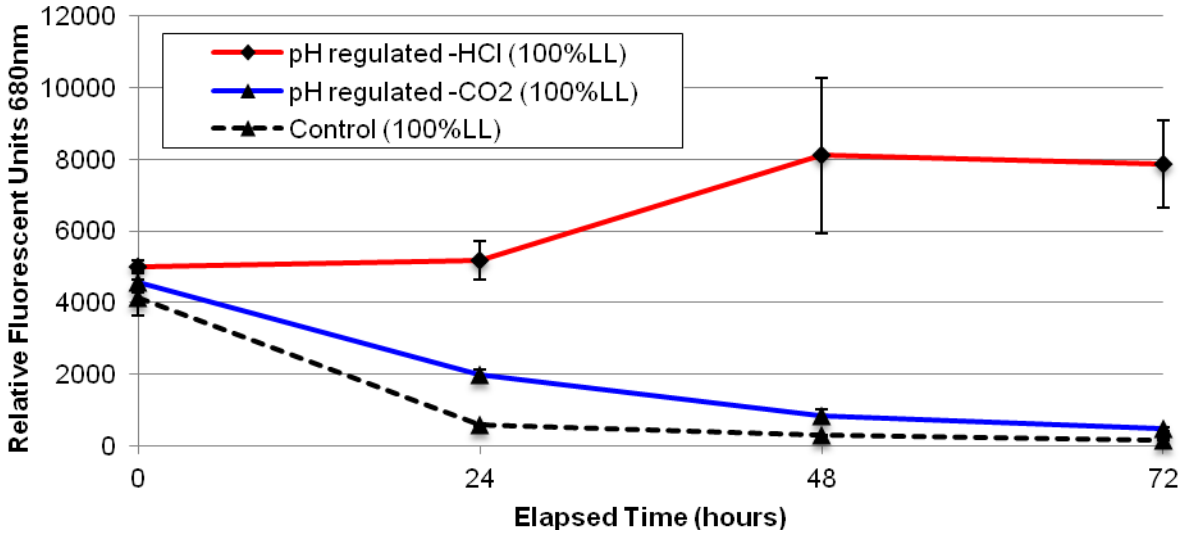


Figure 4-29. Effect of pH control via either CO_2 or HCl on the cultivation of *Chlorella cf. ellipsoidea* (AB3) in 100% ACSW landfill leachate

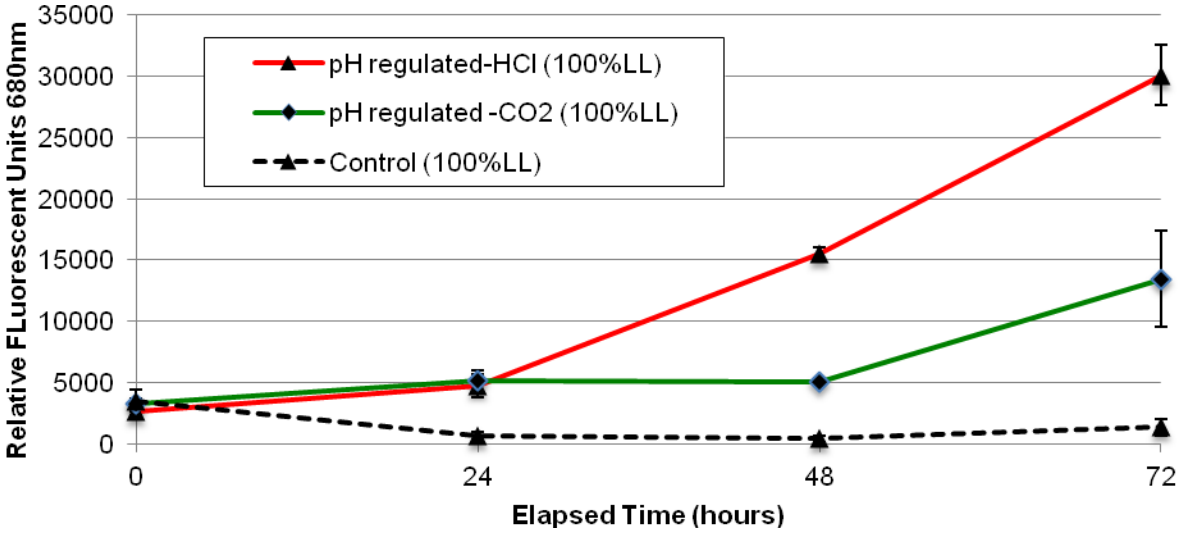


Figure 4-30. Effect of pH control via either CO_2 or HCl on the cultivation of *Scenedesmus sp.* (ISO2) in 100% ACSW landfill leachate

Table 4-4. Comparison of maximum growth rate, TAN, and %Leachate between literature studies

Reference	Cheung *et al.* 1992	Lin *et al.* 2007	Current study
Maximum growth rate	0.43	0.39	0.97
TAN (mg/L)	724	1345	968
% Landfill leachate	5	10	100

CHAPTER 5
CONCLUSIONS

Algae have an unrealized potential in future biofuel production (Sheehan *et al.* 1998, Hu *et al.* 2008; DOE 2010; Stephens *et al.* 2011). Utilizing algae as a feedstock for biofuel production still must overcome significant technological and biological challenges (Sheehan *et al.* 1998, Darzins and Pienkos 2009, Scott *et al*. 2010). Among these challenges, the development of methods for cultivation, harvesting and processing in an ecologically and economically sustainable manner are the most critical. Pittman *et al.* (2011) recently suggested that perhaps to only way that algae biofuels will become economically feasible, in comparison to today's cost of petroleum, is through the combination of algae feedstock production with bioremediation services. Bioremediation services offer both environmental and economic benefits in the cultivation of algae biofuels, allowing a dual role in providing both waste mitigation and resource production (Rawat *et al.* 2011; Wilkie *et al.* 2011).

Landfills are currently the most common form of municipal waste disposal in the United States (EPA 2009). Leachate from landfills is an abundantly produced anthropogenic waste, which must be actively managed for the protection of environmental quality and human health (Renou *et al.* 2008). Many techniques have been developed for remediating landfill leachate (Table 1-2), but all current methods are energy consumptive, neglect the inherent elemental value within the leachate, and are therefore unsustainable. Landfill leachate is commonly considered biologically toxic (Crawford and Smith 1985; Cheung *et al.* 1992; Jones et al. 2006; Wiszniowski *et al.* 2006; Renou et al. 2008), and current literature shows that algae can only tolerate highly diluted (≤10%) landfill leachate (Lin *et al*. 2007). This research project defined

landfill leachate as a cultivation medium with an inherent value for the cultivation of algae. Landfill leachate tested in this study was rich in water-soluble minerals, including all major and micronutrients required by algae for photosynthetic growth (Table 3-4), with significant potential in the production of algal biomass (Table 3-34). Initial field trials showed an inhibitory effect of landfill leachate, which indicated the presence of a toxicant. Laboratory studies elucidated the primary toxicant as unionized ammonia. A simple cultivation strategy to over come the toxic effect of unionized ammonia is the regulation of medium pH, which ionizes ammonia to ammonium.

Bioprospecting for Culture Sourcing

Bioprospecting indigenous algae of a local landfill site was proposed as an alternative means of culture procurement for bioremediation and biofuels production. The results of bioprospecting demonstrated the remarkable array of algae present in the relatively small physical area of the landfill. Within this fixed area even a small sample can have tremendous biological variability. It is due to the great diversity of algae and microbes in general that bioprospecting has such capacity to find novel organisms. Untapped species await discovery and application in nearly every corner of nature. Industries based on the unique characteristics of undiscovered microorganisms will continue to reshape to the way in which humans live and produce goods. This characteristic is not exclusive to landfills, but can be emulated at any geographical area, as algae are found nearly everywhere. The phycological diversity of most regions has not been explored. Just as with bacteria, insects, plants, and other life forms, novel organisms can provide entirely new industries not previously imagined. As so aptly put by Antoni van Leeuwenhoek in 1679,

All we have yet discovered is but a trifle in comparison of what still lies hid in the great treasury of Nature.

Cultivating native algae, through bioprospecting, may provide a simple method for culture sourcing in innumerable algae based applications (aquaculture, remediation, CO_2 fixation, biofuel production, *etc.*). The considerable phylogenetic diversity of algae is the basis that gives bio-prospecting the capacity to find and apply novel organisms. Seventeen genera of algae were identified from only 15 sample sites taken at the ACSW landfill. Finding novel algae with potential application to regional anthropogenic and ecological needs can allow a paradigm shift in both the extent and the productivity of algae cultivation. Investigating the indigenous algae of the ACSW landfill site has led to the discovery of novel algae, with potential in landfill leachate remediation and resource production.

Landfill leachates have ample elemental resources for photosynthetic biomass production. Elemental nutrients such as nitrogen, phosphorus, potassium as well as other essential macronutrients for photosynthetic growth (i.e. magnesium, sulfur, calcium, and iron) and trace metals (manganese, cobalt, copper, zinc) are present in ample quantities within landfill leachate. These nutrients can be used in photosynthetic cultivation for bioresource production. Tapping photosynthetic microbes is a brilliant way to harness solar energy for the production of carbon-based commodities, which are essential to a thriving human community. Mutually beneficial relationships between microbe and man can emerge by simply looking down the microscope at what is naturally occurring in the bounds of the backyard. Investigating the micro-flora of the ACSW landfill site has led to the discovery of novel algae, which may have great potential in future resource production and biological remediation.

Landfill Leachate Cultivation Strategies

Existing literature shows poor growth of algae on landfill leachate, especially at concentrations ≥10% (Cheung *et al.* 1992; Lin *et al*. 2007). Initial growth experiments confirmed these findings (Figure 4-2), with the cultivation of *Chlorella cf. ellipsoidea* at 10%LL showing moderate growth and rapid toxic effects at the higher concentrations of 50 and 100%LL. A rapid toxicity test was used to establish that the observed phenomenon was true for a different species (Scenedesmus sp. (ISO2)). The toxic effect was similar, with higher concentrations showing toxic effects within 24hours of exposure (Figures 4-7 and 4-8). The investigation of toxicity factors was initiated by an elemental analysis of the leachate and comparative assessment against a standard algae medium (BBM). Two factors were striking; the NaCl and TAN levels within leachate were an order of magnitude higher than the standard medium. Pure salts of sodium chloride (NaCl) and ammonium chloride (NH_4CL) were sequentially investigated for toxic effects through 72-hour growth studies and compared to toxic effects observed in landfill leachate. Neither of the pure salt solutions showed toxic effects in either of the algae cultures at concentrations comparable to landfill leachate (Figures 4-9, 4-10, 4-11, and 4-12). It is well known that unionized ammonia is a cellular toxin (Warren 1962) and also an uncoupler of photosynthethis (Abeliovich and Azov 1976). The acid dissociation constant of ammonia is 9.26 at 25°C, and thus at neutral pH ~99% of TAN is present as the ionized ammonium form. Pure salt toxicity tests of ammonium chloride (NH_4CL) were revisited at an experimentally raised pH of 9.25. The results showed rapid toxicity effects remarkable similar to those of landfill leachate in both experimental algae cultures (Figures 4-13 and 4-14). During the course of these experiments, it was

observed that the pH of landfill leachate rose under open cultivation conditions even in the absence of algae, but remained relatively constant under sealed conditions (Figure 4-15). "Abiotic" treatments validated the increase in LL medium pH and therefore the relative fraction of unionized ammonia content within the leachate (Figures 4-16 and 4-17). By simply sealing the Erlenmeyer flask and preventing a pH elevation, it was observed that algae would survive in high concentrations of leachate (50%) (Figures 4-18, 4-19, and 4-20). Leachate pH remained relatively constant under sealed conditions, presumably due to the bicarbonate-carbonic acid equilibrium of the leachate, which loses carbonic acid as carbon dioxide to the atmosphere under open conditions.

Despite the well-known fact that landfill leachates are high in total ammoniacal nitrogen (Wiszniowski *et al*. 2006; Renou *et al*. 2008) no literature has proposed a cultivation strategy to deal with the toxic leachate environment other than dilution. Empirical investigations determined that the waste environment was toxic due high concentrations of total ammoniacal nitrogen, which when the pH of the solution rose was increasingly in the unionized ammonia form. When pH is regulated by the addition of acid in this case carbon dioxide or hydrochloric acid, the toxic effect of the leachate is dissolved and growth of algae is significantly increased. Based on toxicity studies of landfill leachate described within the literature it is expected that for other landfills in a late methanogenic stage (*i.e.* low organic acids and high ammoniacal nitrogen), the same cultivation principles will hold true.

The utilization of CO_2 is a prudent use of resources to the cultivation of algae, especially at the landfill site, which generates substantial quantities of CO_2 through the microbial degradation of municipal solid waste, although hydrochloric acid demonstrated

improved results at 100%LL. A combination of HCl, or another readily available acid and CO_2 may prove a more appropriate cultivation method in future applications of phycoremediation of high ammonia wastes. Phycoremediation has much to offer in the sustainable amelioration of human wastes. Appropriate measures to ensure environmental protection are necessary for the protection of future generations of humans and wildlife. This under-researched field can simultaneously mitigate environmental harm and provide human communities with an array of resources (fuels, fibers, feeds, *etc.*). Humans make a plethora of high ammonia wastes (*e.g.* municipal sewage, animal manures, industrial effluents) many of which are considered toxic. The application of phycoremediation may be significantly improved in these wastes if suitable means of culture sourcing and pH regulation are implemented. In the process of phycoremediation the aim must be to provide optimal growing conditions for the organisms, in particular, minimizing the effect of toxic components of waste streams. Under ideal conditions optimized growth will give optimized remediation capacity. Even when heavily diluted, growth of algae on landfill leachate was previously reported to be only moderate. Mediocre growth cannot be expected to produce resources in enough quantity to be used as commodities. It was therefore an objective of this project to understand the biological impact of the waste environment, and provide cultivation techniques for fostering photosynthetic growth.

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BIOGRAPHICAL SKETCH

Scott J. Edmundson is a native Floridian, born in Orlando and raised in Saint Cloud. He attended high school in the regional International Baccalaureate program, and graduated with honors receiving an I.B. diploma. Scott elected to attend the University of Florida and while enrolled as an engineering student was inspired by a professor of Botany and pursued this life science field of study. As an upperclassman, Scott was a student in the inaugural year of the UF/IFAS Bioenergy and Sustainability School (2006), and dove into the applied and philosophical study of growing photosynthetic microbes for fuels and waste treatment. Scott continued this research as an independent study and graduated with a Bachelor of Science degree in botany. In the UF Botany program, Scott met his wife, Haley G. West, in the university herbarium. Together they started a small organic farm and sold produce for several years. Scott currently lives on Comet Farm near the Santa Fe River with his wife and 2.6-year-old son, Kepler West Edmundson. Scott continues to pursue algal research, exploring the holistic integration of photosynthesis for anthropogenic waste re-utilization. Scott's current research is focused on the application of algae for the bioremediation of landfill leachate. Utilizing algal biomass to generate useful co-products, such as petroleum replacements. Scott is a gardener at heart; the fascination of the botanical world is one of his fundamental attributes. Scott is applying his skills with the botanical realm to develop the potential of algae for a variety of uses similar to traditional agricultural crops, but also for widespread bioremediation of impacted waters and the reuse of anthropogenic wastes. Scott is interested in achieving this ecological revolution through both teaching and fundamental research. Scott earned his Master of Science degree in Interdisciplinary Ecology in the spring of 2012.