QUARTERLY PROGRESS REPORT

December 1, 2011 – February 29, 2012

PROJECT TITLE: Bioremediation of Landfill Leachate and Co-Production of Biodiesel

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Background:

A sustainable solution for dealing with landfill leachate has yet to be devised and implemented, although landfills continue to be used as a primary means of waste disposal. The long-term management of landfill leachate remains a major concern in the assurance of environmental quality, even after the closure of a landfill site. All current methods of leachate treatment are energy and cost intensive. The most common method of leachate treatment involves the transportation of leachate to off-site water treatment facilities; incurring transportation and tipping fees, while consuming fossil resources and emitting CO_2. Algal bioremediation may provide a means for effective on-site leachate treatment with major benefits over current methods. These benefits include the reduction of environmentally noxious compounds (e.g. ammonia), oxidation of organic compounds, and the co-production of biodiesel.

The purpose of this research project is to identify algae that can effectively remediate landfill leachate. The study will characterize native Floridian algae for their tolerance of landfill leachate, effectiveness at remediating the landfill leachate, and the potential of the algal biomass as a feedstock for biodiesel production. The research conducted under this project will lay the biological foundation for implementing algal bioremediation of landfill leachate in Florida.

Work Accomplished During this Reporting Period:

Summary

Results obtained in the previous quarter showed that Floridian algal isolates exhibited toxicity responses when initially cultivated on landfill leachate in concentrations greater than 10%, which agrees with the available current literature on the cultivation of algae on raw landfill leachate (Lin *et al.* 2007). During this quarter, empirical testing was conducted to elucidate the *algal toxicity factor* within landfill leachate, in order to avoid dilution of landfill leachate with groundwater. Landfill leachate was analyzed and compared with a standard algae growth medium. Components of the landfill leachate that appeared in relatively high concentrations were targeted as suspected toxicants and tested under controlled conditions. To date, we have increased the tolerance of the Floridian alga *Chlorella cf. ellipsoidea* to 50% raw landfill leachate, a value not reported in the literature. This was accomplished by decreasing the level of unionized ammonia through pH control. The pH control mechanism used in this study was CO_2, a gas that is plentiful at landfill sites.

Objective 1: *Characterize algae tolerant to landfill leachate.*

Determination of landfill leachate toxicity

Landfill leachate is generally considered toxic. A wide variety of constituents can contribute to biological toxicity; high concentrations of salts, heavy metals, xenobiotic organics, and ammonia nitrogen are the most probable toxic agents (Kjeldsen *et al.* 2002). Toxicity is largely dependent on the organism tested, concentration and duration of exposure (Plotkin and Ram 1984; Ward *et al.* 2002). Cultivating algae on landfill leachate requires the resolution of the cause of toxicity. Once the primary toxicant is determined, developing methods for cultivation to avoid the biological impacts can be devised.

Elemental constituents within the Alachua County Southwest landfill leachate (LL) were compared to those found within a standard algae culture medium, Bold's Basal Medium (BBM), to assess potential toxicants (Table 1). Potential inhibitors were identified as: total ammoniacal nitrogen (TAN), sodium, and chloride. High concentrations of TAN, sodium, and chloride characteristic of landfill leachate may be inhibitory depending on specific tolerances of the organism under cultivation.

Table 1. Chemical Composition of ACSW landfill leachate and algae medium (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

Methods of toxicity testing

All algal growth tests in this quarter were conducted in 250 mL Erlenmeyer flasks under light levels of 150 $\mu E/m^2/s$. Tests were carried out either on orbital shakers operated at 140 rpm or in the case of pH control by CO_2, under aeration. Algal growth was measured by chlorophyll fluorescence (Relative Fluorescent Units at 680 nm). All experiments were executed in triplicate and were paired with leachate controls under identical conditions. The larger volumes employed in these experiments were used to assess scale-up from the previously used rapid toxicity test (2 mL) and additionally to allow the introduction of pH control mechanisms, specifically potentiometric CO_2 addition. This quarterly report describes the characterization of the isolated alga *Chlorella cf. ellipsoidea* (AB3).

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 of 81.6 mg/L and chloride levels of 126 mg/L. In contrast, the sodium content of LL measured approximately 2,713 mg/L and the chloride content measured 1,933 mg/L, a 33-fold and a 15-fold difference, respectively. 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 LL.

Testing was undertaken with analytical grade sodium chloride dissolved in deionized water at concentrations in excess of those found in the LL. These tests elucidated the effect of sodium chloride on the alga under laboratory cultivation conditions. Toxicity tests on *Chlorella cf. ellipsoidea* isolated from the ACSW Landfill showed no toxicity symptoms due to NaCl levels of 5.84 g/L, 125% those found in LL (~4.65 g/L) (Figure 1). Sodium and chloride at levels found in the LL are therefore disregarded as primary toxicants to this organism within landfill leachate.

Ammonium chloride toxicity tests

Another point of contrast between LL and BBM was the nitrogen level of the two media. On an elemental basis, LL had 967.8 mg/L of N and BBM had 41.2 mg/L of 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 to the observed toxicity of landfill leachate.

Experimental tests were undertaken to examine the toxicity of nitrogen in the ammoniacal form at concentrations comparable with LL. As chloride had already been disproved as a toxicity factor for the experimental culture, 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* at 1000 mg/L of N, a concentration slightly in excess of ACSW landfill leachate. In fact, growth was observed under these high ammonium conditions (Figure 2).

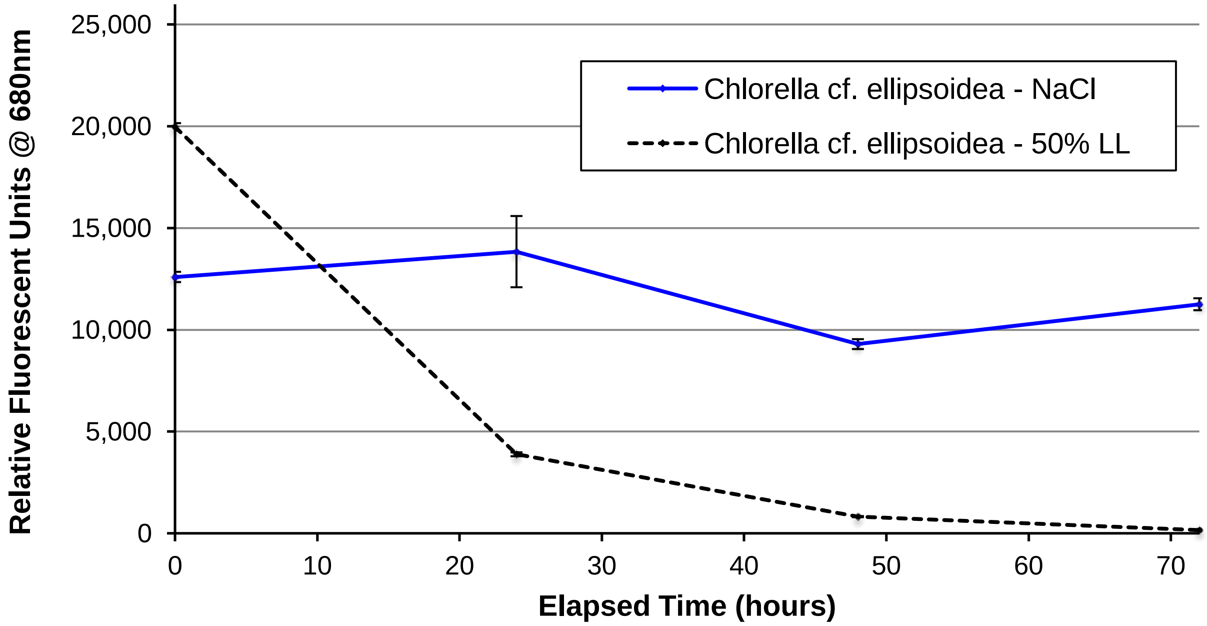

Figure 1. Effects of Sodium Chloride (5.84 g/L) on growth of *Chlorella cf. ellipsoidea* compared with 50% landfill leachate (error bars represent standard deviation of triplicate cultures).

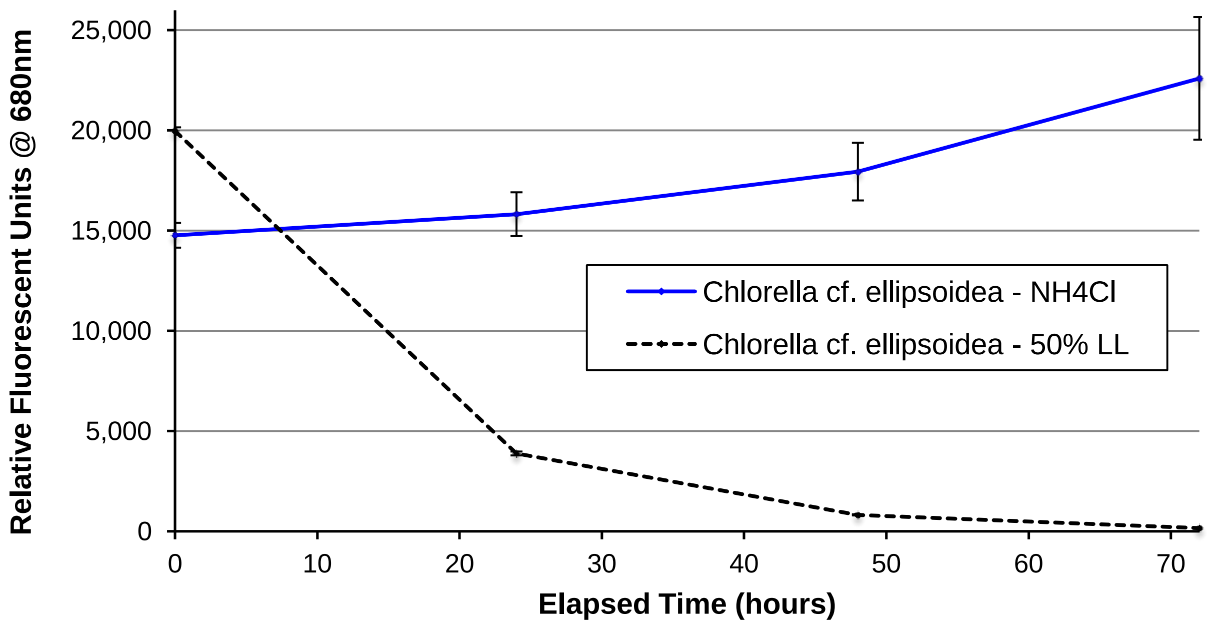

Figure 2. Effects of Ammonium Chloride (3.189 g/L), at pH 7, on growth of *Chlorella cf. ellipsoidea* compared with 50% landfill leachate (error bars represent standard deviation of triplicate cultures).

Effect of pH on the toxicity of ammonium chloride

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 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 the experimental organism even at high concentrations equivalent to those found in 100% LL. Therefore, the Floridian alga was subjected to the same experimental concentration of TAN (1000 mg/L-N); except the pH of the medium was experimentally elevated to the pKa (ionization constant) of ammonium/ammonia (9.26 @ 25 °C). At the pKa value, the ionized and unionized forms of ammoniacal nitrogen are present in equal fractions. The experimental alga culture showed an immediate toxic effect from the increase in the unionized ammonia concentration (Figure 3). The toxicity effect closely resembled the toxic effect observed in the landfill leachate experiments conducted in the previous quarter, in which relative fluorescence decreased significantly within 24 hours. Furthermore, the artificially induced pH of 9.26 closely resembled the pH of LL after aeration 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 TAN. This elucidated the primary toxicity factor within landfill leachate as <u>unionized ammonia</u>. 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.

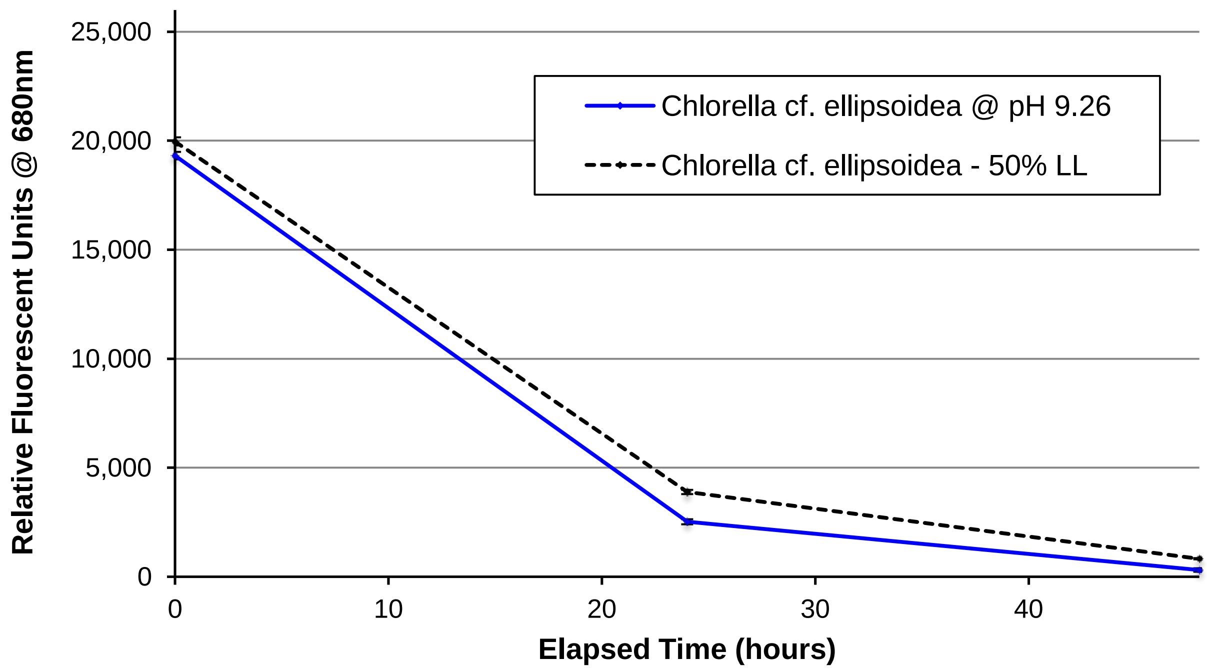

Figure 3. Effects of Ammonium Chloride (3.189 g/L), at pH 9.26, on growth of *Chlorella cf. ellipsoidea* compared with 50% landfill leachate (error bars represent standard deviation of triplicate cultures).

Effect of pH control on algae cultivation in landfill leachate

If free ammonia is the primary toxicant, then pH control should allow the ammoniacal nitrogen within the leachate to remain ionized (NH_4^+) and therefore nontoxic. Controlling the pH of the culture should allow the cultivation of algae in landfill leachate at much higher concentrations than previously observed. Therefore, *Chlorella cf. ellipsoidea* was grown under CO_2-regulated pH conditions in 50% and 100% landfill leachate. Experimental treatments were potentiometrically regulated between pH 6.8 and pH 7.2 by the addition of gaseous CO_2, which dissociated in water to form carbonic acid (H_2CO_3). The pH levels of control cultures were not regulated. Regulation of pH by CO_2 addition in 50% LL showed adaptability and growth in *Chlorella cf. ellipsoidea* (Figure 4). The maximum average fluorescence observed for this species was 22,596 RFUs. *Chlorella cf. ellipsoidea* still exhibited toxicity effects in 100% LL, although the effects were muted compared with the control (Figure 5). All control cultures without pH regulation did poorly, exhibiting the previously observed toxicity effects of LL. The visual difference between algae cultures under pH regulation and the control cultures was striking (Figure 6), clearly showing the removal of toxicity impacts and algae growth.

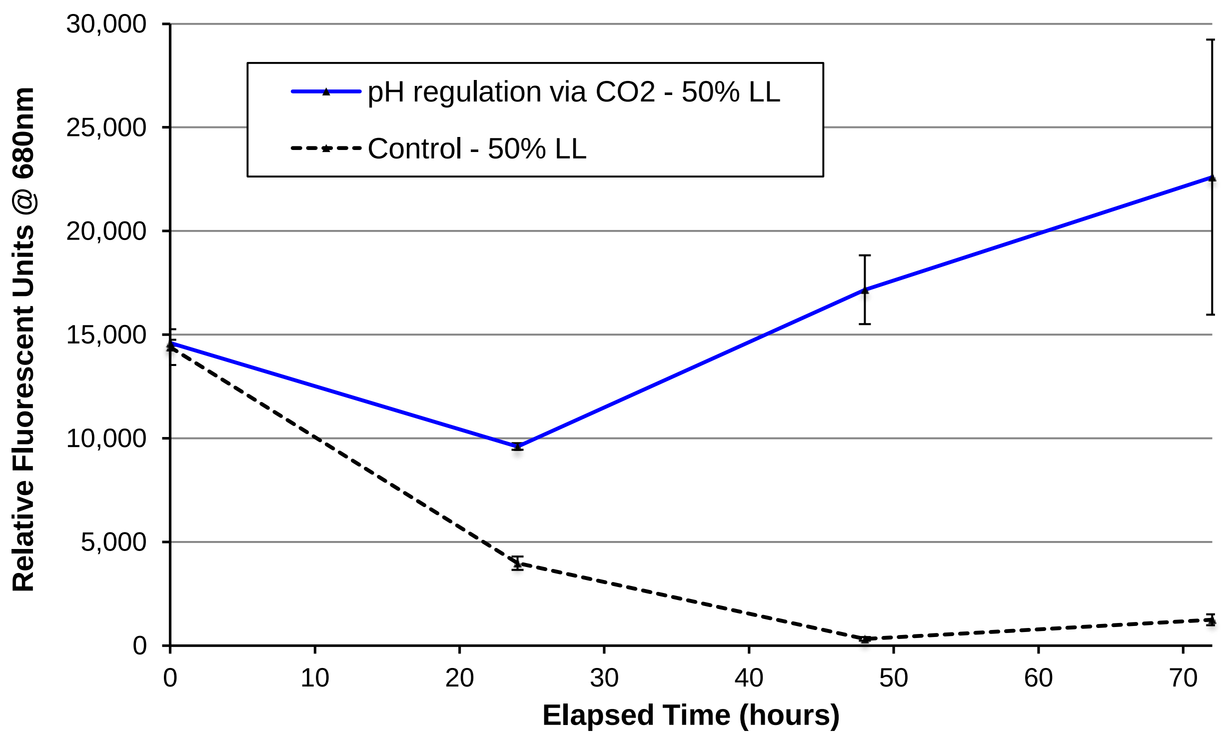

Figure 4. Effect of pH regulation via CO_2 on the cultivation of *Chlorella cf. ellipsoidea* in 50% landfill leachate compared to growth in 50% landfill leachate without pH regulation (error bars represent standard deviation of triplicate cultures).

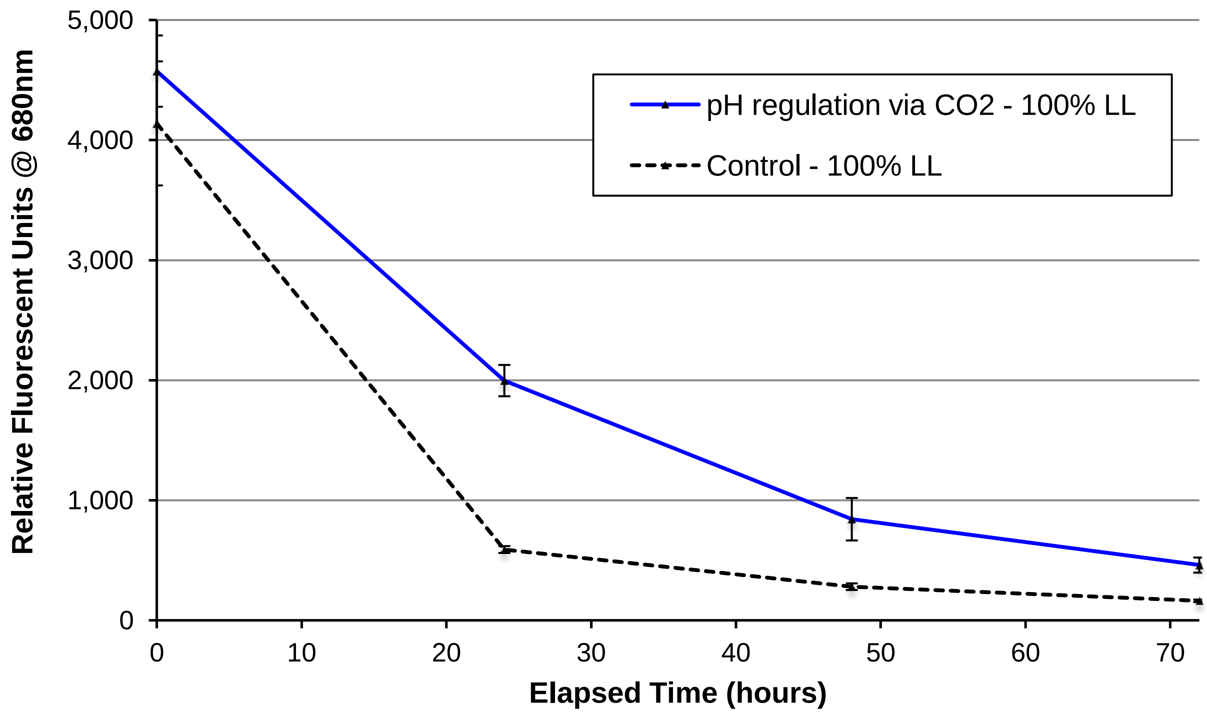

Figure 5. Effect of pH regulation via CO_2 on the cultivation of *Chlorella cf. ellipsoidea* in 100% landfill leachate compared to growth in 100% landfill leachate without pH regulation (error bars represent standard deviation of triplicate cultures).

Figure 6. Cultivation of *Chlorella cf. ellipsoidea* in landfill leachate; triplicate cultures with pH regulation via CO_2 (left) and controls without pH regulation (right); green color correlates with algae growth and chlorophyll fluorescence at 680nm.

Objective 2: *Determine leachate bioremediation potential of algae.*
Objective 3: *Examine the viability of biodiesel production from algal biomass.*

Fundamental cultivation parameters are essential for the application of algae-based bioremediation of landfill leachate. Progress has been made towards realizing both remediation and biofuel production goals through the development of cultivation techniques in higher concentrations of landfill leachate. Laboratory cultivation methods in which a Floridian algal isolate grew in 50% raw landfill leachate were developed (in this quarter) through empirical testing of probable leachate toxicants. Further improvements in leachate tolerance may be possible now that the underlying principles have been established. This will lead to the development of laboratory cultivation methods that will allow for the assessment of algal growth, remediation capacity, and oil content.

Conclusions

The research conducted in this quarter shows a dramatic increase in algal tolerance to a high (50%) concentration of landfill leachate. Through resolving the primary toxicant acting upon the algae, growth of *Chlorella cf. ellipsoidea* was obtained in 50% LL, five-fold the previous maximum concentration for this organism. Although undiluted leachate still exhibited toxicity symptoms, it is clear that we have identified the primary algal toxicant within the leachate as unionized ammonia. This suggests that future application of algae-based bioremediation will require the efficient use of pH regulation by CO_2 or other means. Utilizing CO_2 for pH control is an appropriate solution for a landfill site as landfills produce CO_2 biologically within the landfill gas and after the combustion of methane in flares or generator equipment. The method and application of CO_2 into cultivation flasks and larger pond/bioreactor systems must be optimized, but this approach shows promise in increasing the concentration of landfill leachate tolerable to algae. CO_2 may also have a positive effect on the accumulation of biomass, remediation capacity, and oil content.

Applying CO_2-based pH control may allow cultivation of algae on landfill leachate without groundwater dilution, reducing costs and the total volume of managed landfill leachate. Within undiluted landfill leachate (100%), CO_2 gas was inefficient at regulating the pH, presumably due to a high bicarbonate presence within the leachate and continual off gassing of CO_2 in aeration-mixed cultures. Future testing of alternative mixing techniques, mineral acids and combinations of mineral acids, CO_2, and/or mixing techniques may provide optimal pH regulation for algae growth and bioremediation in undiluted landfill leachate.

References:

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Ward, M.L., Bitton, G., Townsend, T. and Booth, M, (2002). Determining toxicity of leachates from Florida municipal solid waste landfills using a battery-of-tests approach. *Environmental Toxicology* **17**(3):258-266.

Information Dissemination Activities:

We have made updates and additions to the project website, including details of events, news, photos, presentations, and posters. The website is available at: http://biogas.ifas.ufl.edu/leachate/