QUARTERLY PROGRESS REPORT

September 1 – November 30, 2011

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

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PROJECT WEB ADDRESS: http://biogas.ifas.ufl.edu/leachate/

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:

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

The first objective in the current research project was progressed with the isolation and the initial characterization of two algal candidates for the remediation of landfill leachate. These algae were selected from samples taken at the Alachua County Southwest Landfill (ACSWL) in Archer, Florida. Algae samples were taken from several locations at the ACSWL (*e.g.* pooled rainwater, damp soils, leachate holding tanks, and drainage areas). Each sampling site was recorded by GPS coordinates. Samples were taken in 40 ml screw cap containers, refrigerated after collection, and identified to genera by direct microscopic observation, following a morphological key. Additionally, raw leachate was centrifuged at 15,000 rpm (Eppendorf 5414, Westbury, NY) to determine the possible presence of algae within the landfill leachate; no algae were detected.

Algae were isolated to unialgal cultures by agar plate cultivation. This technique involved the 1:1 dilution of samples with 10% Bold's Basal Medium (BBM), a standard algal culture medium. This solution was then spread across 2% agar plates and incubated under light (150 $\mu E/m^2/s$) until colonies appeared, approximately 7-10 days (Figure 1). Algae colonies were transferred from agar plates and cultivated in BBM 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) and *Chlorella cf. ellipsoidea* (Figure 3).

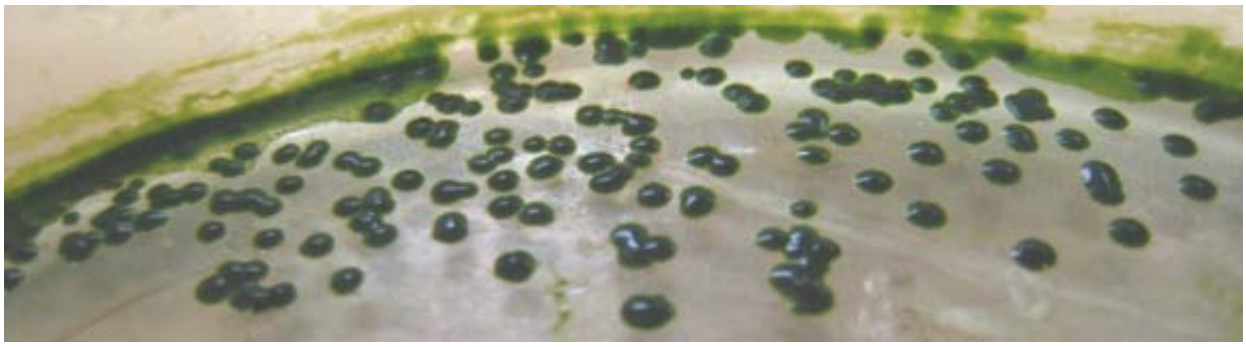

Figure 1. Isolated algae colonies growing on agar. Individual algae colonies (spherical green clusters) are each formed by a single algal cell replicating many times.

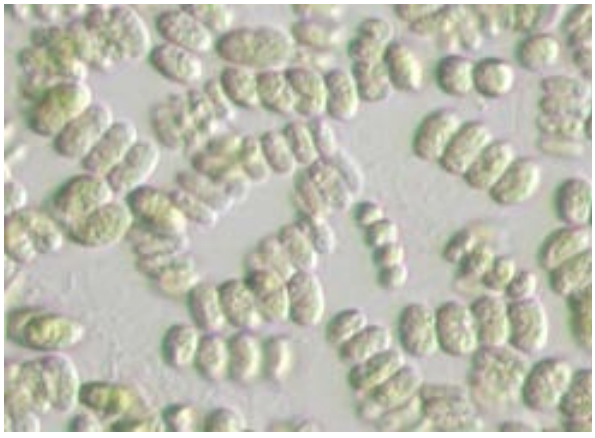

Figure 2. A Floridian alga isolate of the genus *Scenedesmus*.

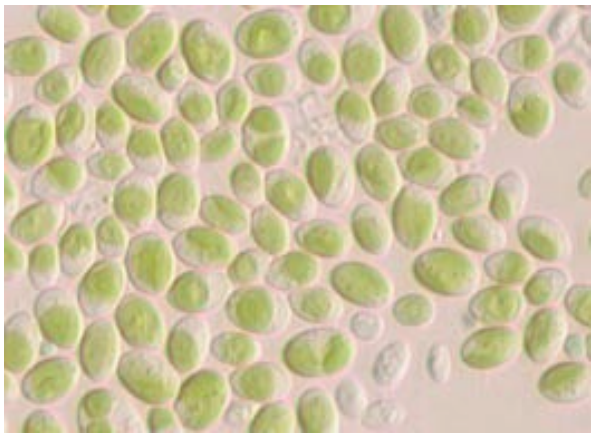

Figure 3. A Floridian alga isolate, *Chlorella cf. ellipsoidea*.

In order to characterize algae for landfill remediation, a rapid toxicity test was developed to quickly assess the tolerances of algae to landfill leachate. The test used in-vivo chlorophyll fluorescence to assess algal survival over a short period of time (24 hours). Fluorescence is a measure of the health of the chlorophyll within the algae cell, and can be used to determine the growth of an algae culture. 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 deionized water only). Each concentration was replicated in triplicate. Fluorescence levels of leachate without algae are taken as background fluorescence and subtracted. Test plates were incubated at 25°C under 150 $\mu E/m^2/s$ illumination on an orbital shaker platform rotating at 140 rpm. Results of the rapid toxicity test demonstrated the rapid toxicity of landfill leachate at concentrations greater

than 10% for both *Scenedesmus* sp. (Figure 4) and *Chlorella cf. ellipsoidea* (Figure 5). Although *Scenedesmus* has a slightly higher tolerance at 30% leachate, the toxicity appears to be universal. However, at present, only two isolates have been tested. A longer term investigation (140 hours) was conducted to evaluate the growth of *Chlorella cf. ellipsoidea* on 10% landfill leachate compared to 10% BBM. It was observed that algae cultivated on 10% landfill leachate had similar levels of growth as the culture on 10% BBM. This demonstrated the potential of landfill leachate as a culture medium for algae growth (Figure 6).

Through the use of this rapid toxicity test, it can be inferred that algae do not tolerate landfill leachate at high concentrations, under the tested conditions. Adjusting culture conditions to support algae growth will most likely be needed for growth of algae on higher concentrations of landfill leachate. Future work will continue the characterization of Floridian algae to landfill leachate.

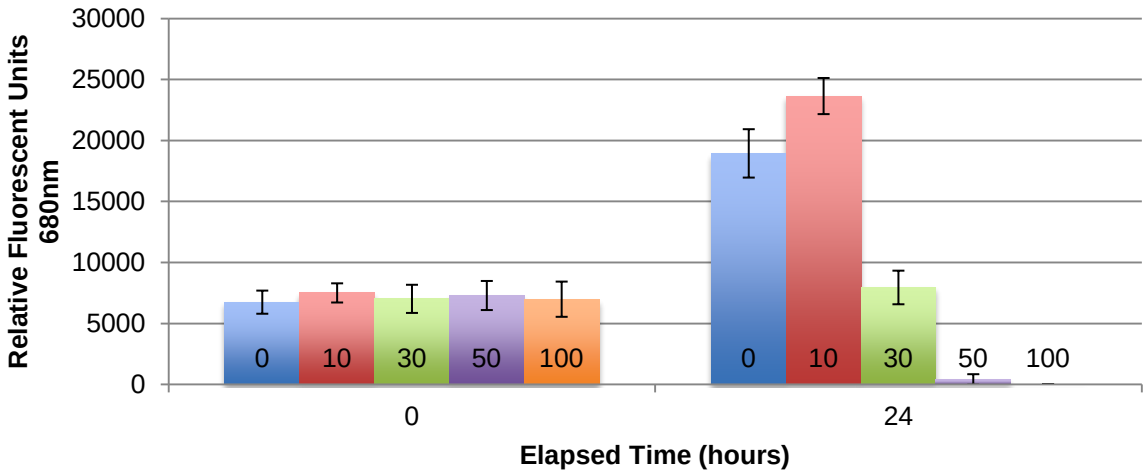

Figure 4. Toxic effect of 24 hour landfill leachate incubation on *Scenedesmus* sp.

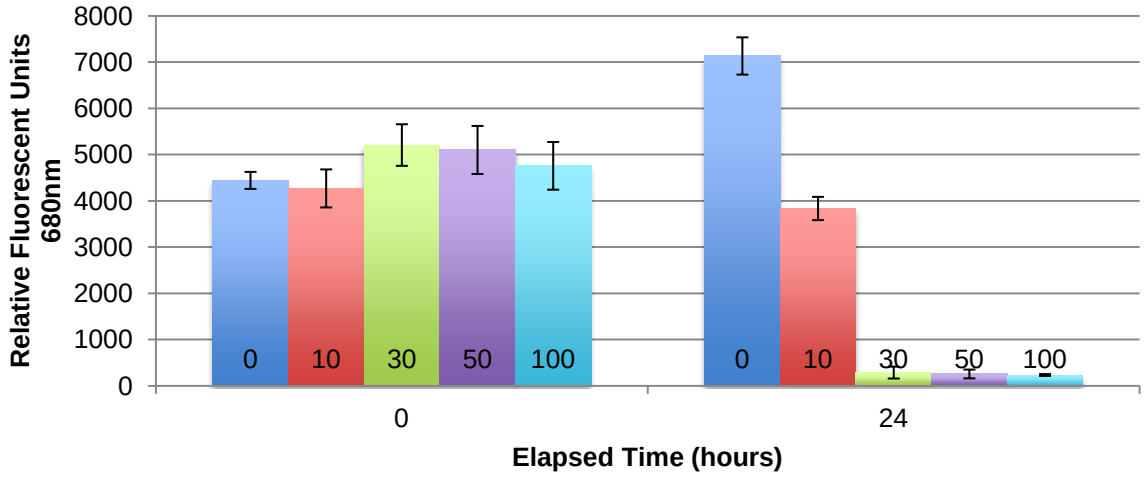

Figure 5. Toxic effect of 24 hour landfill leachate incubation on *Chlorella cf. ellipsoidea*.

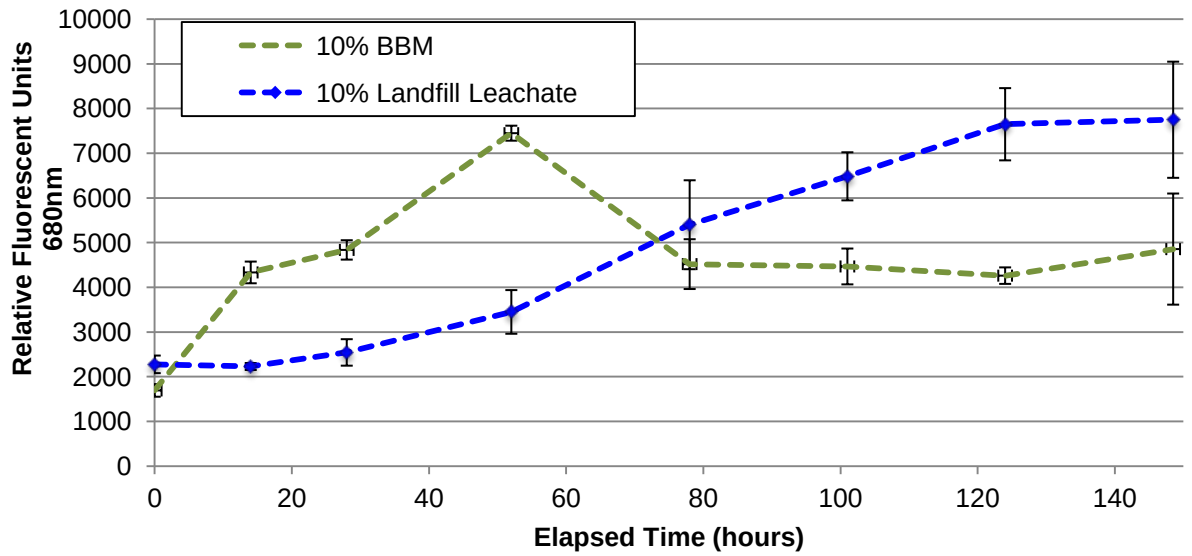

Figure 6. Comparative growth of *Chlorella cf. ellipsoidea* on 10% landfill leachate and 10% Bold's Basal Medium.

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

Laboratory cultivation methods that will allow for the assessment of algal growth, remediation capacity, and oil content are in the process of being developed. With the development of cultivation methods, progress will be made towards reaching objectives 2 and 3. Evaluation of remediation using developed laboratory cultivation methods (Figure 7) will foster future experimental trials for remediation testing and algal biomass generation for lipid extractions.

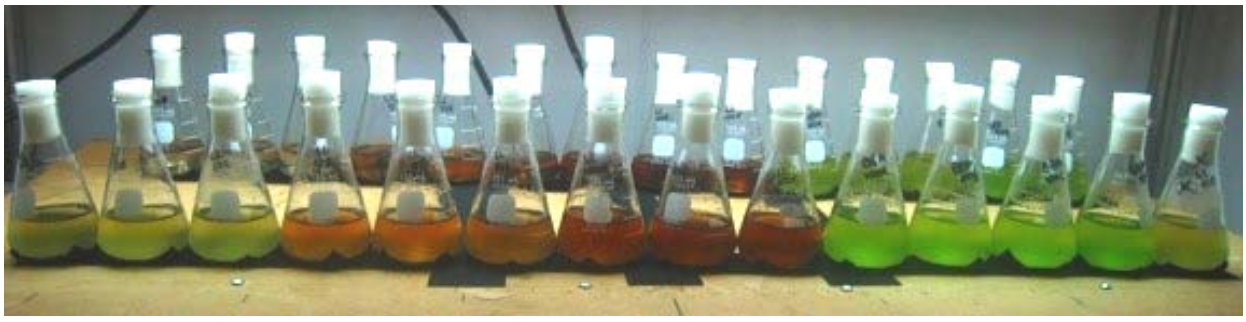

Figure 7. Algae (*Chlorella* sp.) grown in various concentrations of landfill leachate under laboratory conditions.

Information Dissemination Activities:

The project website was published at: http://biogas.ifas.ufl.edu/leachate/

Presentations:
A.C. Wilkie. Utilizing Native Algae for Biofuel Production. Presented at the *Third Annual Florida Energy Systems Consortium Summit*, Gainesville, FL. September 27, 2011.

A.C. Wilkie. Bioenergy and Sustainable Technology. Presented to the *Alpha Epsilon Honor Society*, Agricultural and Biological Engineering Department, University of Florida, Gainesville, FL. October 5, 2011.

Posters:
S.J. Edmundson and A.C. Wilkie. Florida Algae for Biofuel Production and Landfill Leachate Remediation. Presented at the *Third Annual Florida Energy Systems Consortium Summit*, Gainesville, FL. September 27-28, 2011.