Florida Algae for Biofuel Production and Landfill Leachate Remediation

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Abstract

This study explored the indigenous algae of a municipal solid waste landfill for organisms capable of growing on landfill leachate and simultaneously producing a lipid biofuel feedstock. Utilizing landfill leachate as a growth medium for algae greatly reduces the fertilizer inputs required for the production of algal-based biofuels, especially if this technology is to be implemented on an industrial scale. A closed municipal solid waste landfill in Alachua County, Florida, was bio-prospected for indigenous algae with inherent lipid storage capacities that may provide novel algal strains for biofuel production. Algae were screened via microscopic staining and cultured under laboratory and outdoor conditions. Many algae were found to store oils as an energy reserve, especially under low nitrogen conditions. Algae were further tested for their ability to tolerate landfill leachate as a culture medium. Indigenous algae tolerated all tested dilutions (2.5-100%) of landfill leachate. Certain species of algae (*Chlorella cf. ellipsoidea, Ankistrodesmus* sp.) grew robustly and produced large cellular oil deposits. For the promising species *Chlorella cf. ellipsoidea*, a 10% leachate solution provides growth equivalent to that obtained using 10% Bold's Basal Medium. Coupling algal cultivation with the simultaneous remediation of landfill leachate may provide a sustainable method for algal biomass and biofuel production.

Introduction

Landfilling is currently the most common method for the disposal of anthropogenic solid waste. Landfills must be lined with impermeable membranes, consequently forcing landfill operators to deal with large volumes of liquids percolating through the accumulated waste within a landfill. These liquids, termed landfill leachate (Fig. 1), must be managed for a minimum of 30 years post closure of the landfill. Current methods in leachate remediation involve transfer to publically-owned water treatment facilities or on-site wastewater treatment to meet Groundwater Cleanup Target Levels (GCTLs) (FDEP 2005). Landfills contain large deposits of unutilized nutrient resources. These nutrients include all elemental requirements for photosynthetic growth: nitrogen, phosphorus, potassium, magnesium, calcium, iron, and trace metals. Landfill leachate is commonly considered toxic, and not all organisms can tolerate it as a growth medium. Discovering phototrophic organisms capable of utilizing this nutrient reserve to produce photosynthetically derived fuels reduces the cost of nutrient media and simultaneously remediates the negative environmental impacts of landfill leachate. Organisms found on-site at a municipal solid waste (MSW) landfill are likely to be adapted to utilize nutrients within landfill leachate. Bio-prospecting for native algae with both landfill leachate tolerance and oil production provides a synergistic combination for sustainable biofuel production and bioremediation (Fig. 2).

Landfill Leachate

Figure 1. Landfill leachate is a dark-colored, odiferous liquid rich in ammonium, potassium, and trace metals.

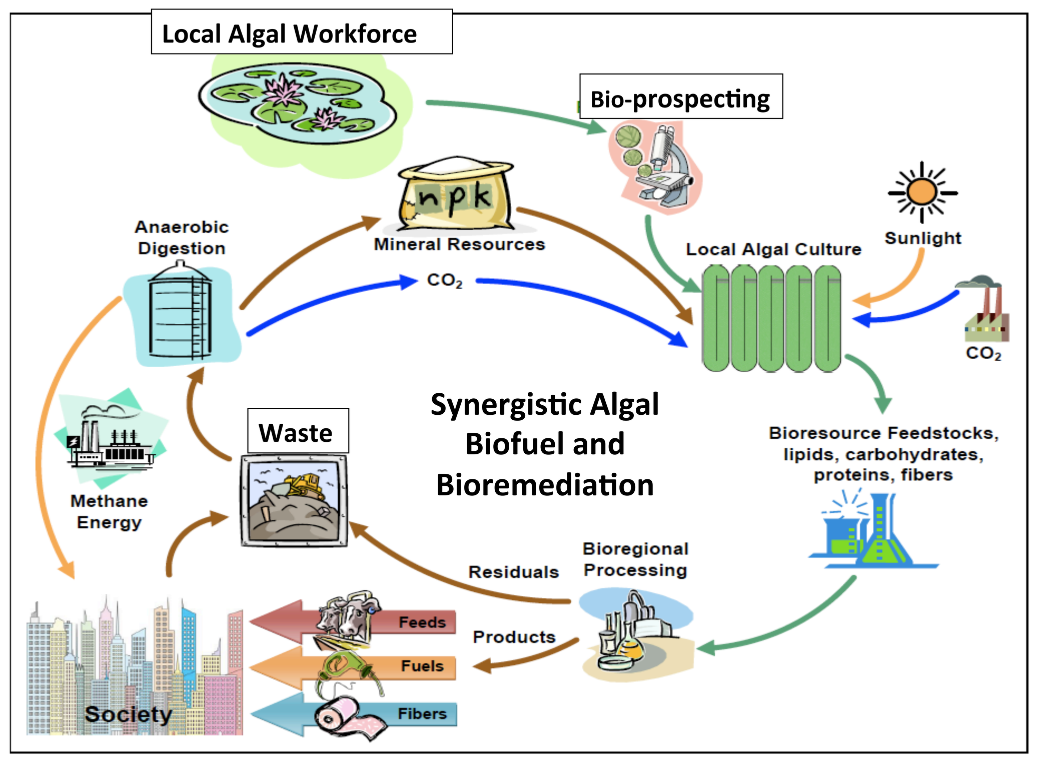

Figure 2. Envisioned sustainable algae biofuel and bio-resource production through recycling and bioremediation of wastes with local algae

Objectives

- Discover novel algae through bio-prospecting algae at the Alachua County South West (ACSW) Landfill
- Determine indigenous algae tolerance to landfill leachate
- Assess oil production potential of bio-prospected algae

Methodology

- *Bio-Prospecting:* Algae samples from the Alachua County Southwest Landfill (Fig. 3) were examined by microscopy. Algae were identified to genus following Prescott 1973.
- *Algal Cultivation*: Native algae, collected on-site, were cultivated in 20L "mini-ponds". The suspension of microalgal cells were mixed by aeration. Leachate was diluted with groundwater (2.5, 25, 50, 75, and 100% landfill leachate).
- *Algal Isolation*: Native algae species were isolated by agar streaking.
- *Algal Growth*: *C. cf. ellipsoidea* cultures were grown in landfill leachate and Bold's Basal Medium (Bold 1942) and monitored by spectro-fluorometry using a Thermo NanoDrop 3300 at 490/680nm, ex/em.
- *Algae oil assessment:* Algal oil potential was assessed with the fluorochrome Nile Red under epi-fluorescent illumination 490/520nm ex/em (Cooksey 1985).
- *Elemental Analysis*: Elemental analyses for N, P, K, Ca, Mg, Fe, Mn, Cu, Zn, and Co were performed by an external certified laboratory.

Results

- *Bio-Prospecting:* Over one dozen genera were discovered to inhabit samples from the ACSWL site (Fig 3). Genera observed included *Chlorella, Scenedesmus, Kirchneriella, Ankistrodesmus, Chlamydomonas, Selenastrum, Pandorina, Pinnularia, Navicula, Rhizoclonium, Oedogonium, Synechocystis, Oscillatoria,* and *Lyngbya (*Fig. 4*)*.
- *Algal Cultivation*: Native algae tolerated all concentrations of landfill leachate (2.5, 25, 50, 75, and 100%), different organisms dominated at all concentrations. *C. cf. ellipsoidea* was present in all concentrations (Fig. 5).
- *Algal Isolation*: *Chlorella cf. elipsoidea* was isolated by agar plate streaking
- *Algal Growth*: Cultures grown on 50 and 100% BBM grew excellently, cultures on either 10% landfill leachate or 10% BBM grew well, cultures on both 50 and 100% landfill leachate had no growth (Fig. 6).
- *Algae oil assessment:* Many indigenous organisms stained positive for oil with Nile Red. *Chlorella cf. ellipsoidea* and *Ankistrodesmus* were exceptionally lipid-rich (Figs. 7 and 8).
- *Elemental Analysis*: Elemental analyses of landfill leachate shows that all required elements for photosynthetic growth are present (Table 1).

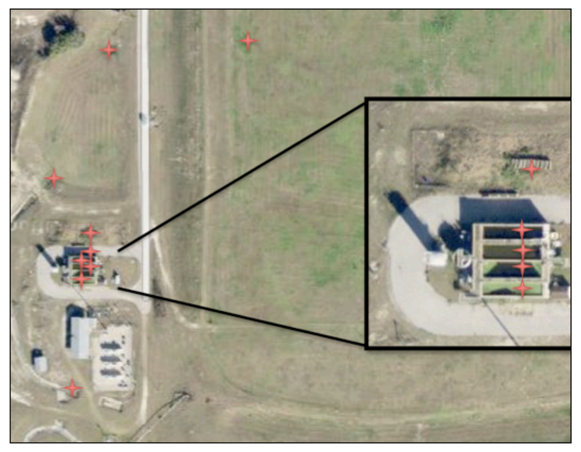

Figure 3. Alachua County Southwest Landfill study site. Red stars indicate locations sampled for algae bio-prospects.

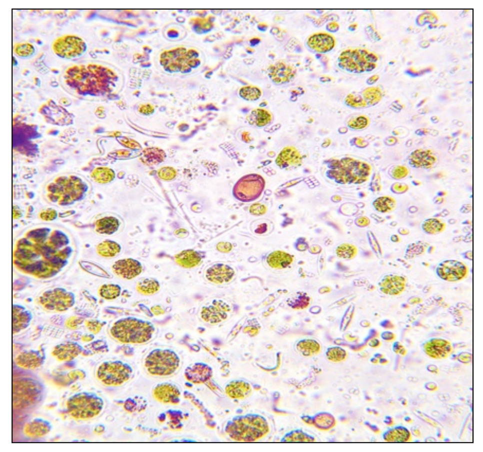

Figure 4. Diversity of algae found in a single sample at the ACSWL study site.

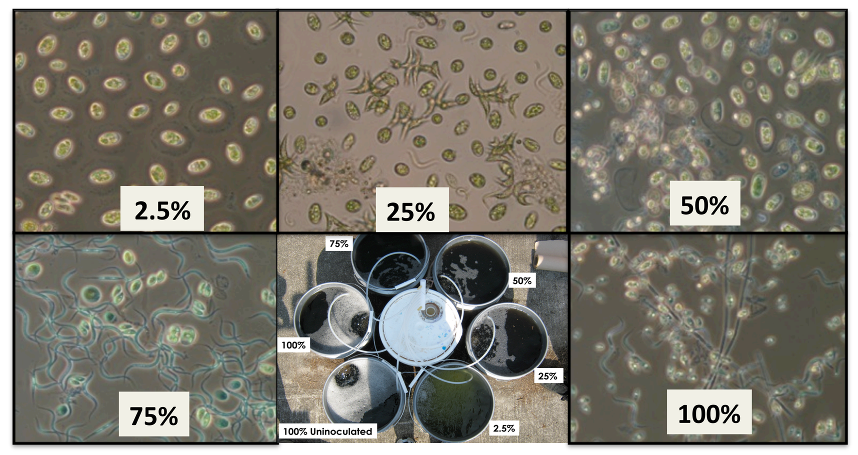

Figure 5. Algae cultures at different leachate concentrations after 3 weeks of growth, 500x. Center bottom: "mini-pond" cultivation.

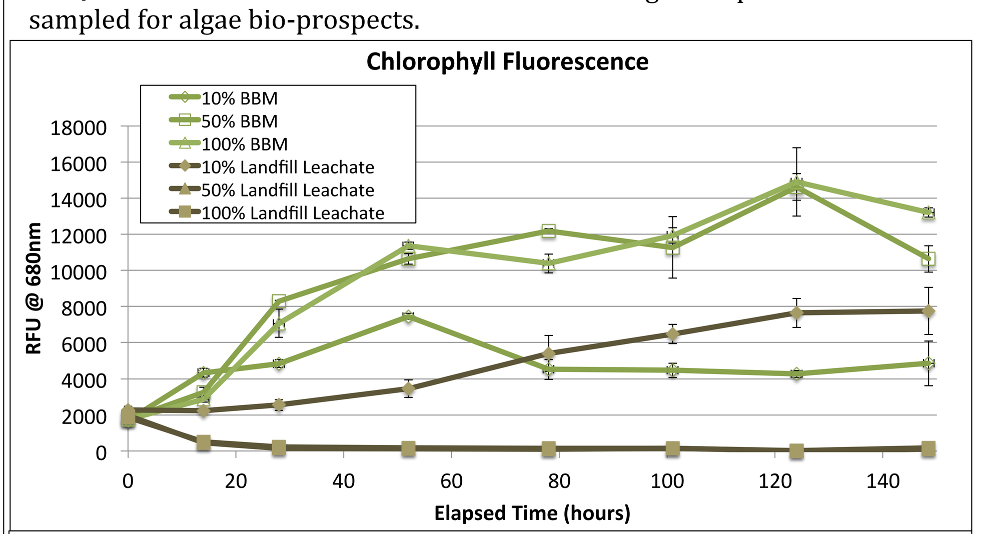

Figure 6. *Chlorella cf. ellipsoidea* isolate cultured on different concentrations of landfill leachate and Bold's Basal Medium (BBM).

Table 1. Elemental Analysis of Landfill Leachate, Reverse Osmosis treated Landfill leachate, and a common algal medium (Bold's Basal Medium).

Component (mg/L)	SW Archer Landfill Leachate	Bolds Basal Medium
Macronutrients		
Nitrogen	1,119.60	41.20
__Ammonia-N	1,110.00	_
__Nitrate-N	9.60	41.20
Phosphorus (PO_4)	9.98	163.10
Potassium	980.00	170.30
Magnesium	88.00	7.39
Calcium	110.00	6.82
Iron	16.00	1.00
Micronutrients		
Manganese	0.11	0.50
Copper	0.27	0.02
Zinc	0.06	0.50
Cobalt	0.07	0.01

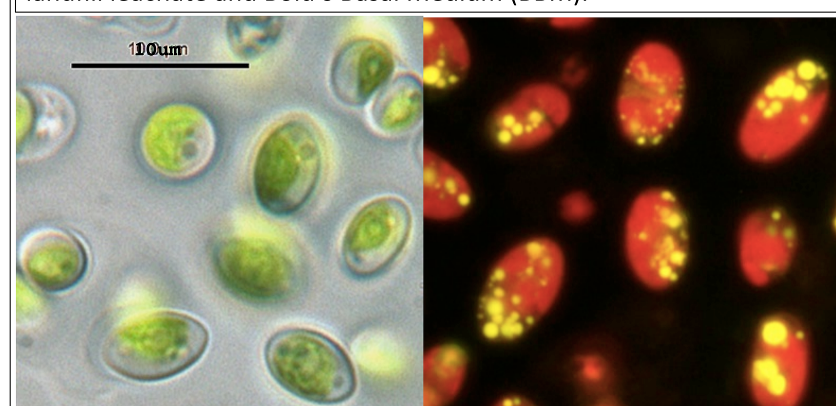

Figure 7. *Chlorella cf. ellipsoidea* under brightfield and fluorescent microscopy. Oil fluoresces yellow when stained with Nile Red

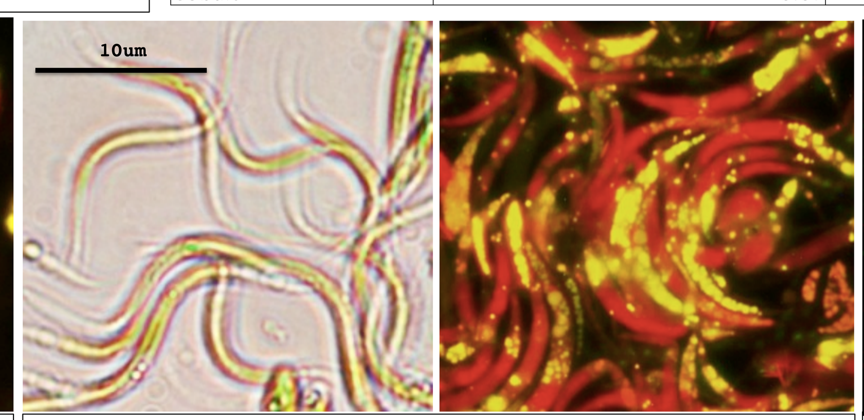

Figure 8 *Ankistrodesmus sp.* under brightfield and fluorescent microscopy. Oil fluoresces yellow when stained with Nile Red.

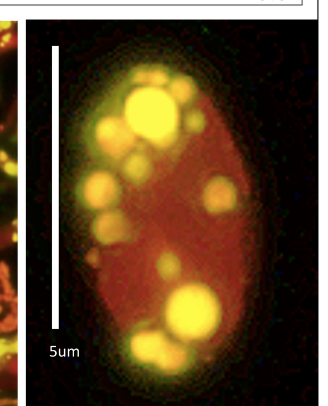

Figure 9. *C. ellipsoidea* accumulating oil

Discussion

Bio-prospecting algae in waste sites, such as MSW landfills, for the dual purpose of bio-remediation and biofuel production can have significant synergistic potential in the sustainable production of bio-resources. Nutrients within the wastes reduce the cost of supplying inorganic culture media for algae, while the utilization and photosynthetically-driven oxidation of the waste reduces its environmental burden. Financial incentives for bio-remediation can mitigate the cost of producing biofuels from algae and develop the technology in a sustainable manner. We ultimately hope not to promote the production of landfill leachate, but look to the future when waste elements are conscientiously recycled for photosynthetic algae biofuel production. Improving waste practices will undoubtedly have a positive effect on the overall potential of efficient resource use. Until this time, there remains countless liters of landfill leachate with which to cultivate algae-derived biofuels. Future work will focus on maximizing growth and lipid productivity of promising strains growing in undiluted landfill leachate and other underutilized, waste resources.

Conclusions

- Landfills are widespread deposits of unutilized nutrients.
- Algae can utilize elemental nutrients held within landfills.
- The bio-prospected species *Chlorella cf. ellipsoidea* and *Ankistrodesmus sp.,* found at the Alachua County Southwest Landfill tolerate landfill leachate.
- Discovered native algae produce large cellular deposits of oil (Fig. 9), which can be used as a feedstock for biofuel production.

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