Landfill leachate – a water and nutrient resource for algae-based biofuels

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(Received 25 April 2013; accepted 3 July 2013)

There is a pressing need for sustainable renewable fuels that do not negatively impact food and water resources. Algae have great potential for the production of renewable biofuels but require significant water and fertilizer resources for large-scale production. Municipal solid waste (MSW) landfill leachate (LL) was evaluated as a cultivation medium to reduce both water and elemental fertilizer demands of algae cultivation. Daily growth rate and cell yield of two isolated species of algae (Scenedesmus cf. rubescens and Chlorella cf. ellipsoidea) were cultivated in MSW LL and compared with Bold’s Basal Medium (BBM). Results suggest that LL can be used as a nutrient resource and medium for the cultivation of algae biomass. S. cf. rubescens grew well in 100% LL, when pH was regulated, with a mean growth rate and cell yield 91.2% and 92.8% of those observed in BBM, respectively. S. cf. rubescens was more adaptable than C. cf. ellipsoidea to the LL tested. The LL used in this study supported a maximum volumetric productivity of 0.55 g/L/day of S. cf. rubescens biomass. The leachate had sufficient nitrogen to supply 17.8 g/L of algae biomass, but was limited by total phosphorus. Cultivation of algae on LL offsets both water and fertilizer consumption, reducing the environmental footprint and increasing the potential sustainability of algae-based biofuels.

Keywords: algae cultivation; landfill leachate; algal biofuels; bioremediation; Scenedesmus

Introduction

Algae have the potential to substantially contribute to the production of renewable fuels [1–3]; they can potentially meet 48% of US petroleum imports for transportation.[4] Recent analyses of algae-based biofuels, however, have shown that current production methods face significant challenges before a sustainable algal biofuel industry can develop. Among the most critical factors in the development of photosynthetic algae-based biofuels are satisfying the water and nutrient requirements for cultivation.[4–7] Utilizing waste nutrient and water resources for algae cultivation may alleviate economic constraints on large-scale algae cultivation. Exploiting previously fixed nitrogen within wastes avoids the energy-intensive Haber–Bosch process for nitrogen fixation, thereby increasing the efficiency and sustainability of algal cultivation. Many studies suggest using municipal sewage wastewater for the cultivation of algae.[8–10] However, the nitrogen from sewage wastewater alone, typically 15–20 mg/L, is insufficient to support large-scale algae biomass production for biofuels.[11] We present data that support the use of high ammoniacal-nitrogen leachates generated from municipal solid waste (MSW) landfills as an alternative waste nutrient resource to meet the water and fertilizer needs of algae-based biofuel production.

Landfill leachate (LL), the liquid waste generated by landfill sites, is a societal burden due to its high nutrient content and other constituents but may have intrinsic value as an algal culturing medium. LLs can potentially provide algal cultivation with copious amounts of high-nitrogen aqueous media.[12,13] Landfills continue to produce leachates throughout the lifespan of the landfill, even after closure. In Florida, MSW landfills generate an average of 7200 L/ha/day of leachate.[14] These leachates must be managed to prevent environmental contamination. Algae cultivation on LL fulfils a dual role of leachate remediation and biomass production,[15,16] reducing the societal and environmental burden imposed by these pervasive waste disposal centres.

MSW is inherently variable on a spatio-temporal basis and therefore the leachate generated by the MSW landfill is also variable and is contingent on the type of refuse, local climate, precipitation, management techniques, and age of the landfill.[12] Landfills progress through distinct maturation phases as they age.[17] The concentration of total ammoniacal-nitrogen within LLs increases with landfill age and is identified as a long-term issue for the management of MSW LL.[17] Additionally, the pH of LL increases over time as organic acids from decomposing refuse are microbially converted into methane and carbon dioxide gases.

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In comparison, leachates from younger landfills often have high chemical oxygen demand (COD), acidic pH, and relatively low ammonia content.[12] This study focused on the utilization of mature, high ammoniacal-nitrogen MSW LL.

Current literature on algae growth in LL is sparse and suggests that dilution with water and/or extensive pretreatment (15–30 days of mechanical aeration) of the leachate is necessary for algae to survive.[15,16,18] Even at high dilutions (90%), reported growth is only moderate[15] and the diluted leachate would not replace synthetic fertilizers typically used to cultivate algae as a biomass resource. Furthermore, high concentrations (>30%) of LL were shown to be inhibitory or acutely toxic,[15,16] making the use of LL apparently impractical as an algal culture medium. No literature has demonstrated a cultivation strategy to deal with the toxic LL environment, other than dilution [15] or mechanical aeration.[16] This research study aims to evaluate the potential of MSW LL as a cultivation medium for supplying both water and fertilizer nutrients for algae biomass production by comparing algal growth in MSW LL and in a standard growth medium, Bold’s Basal Medium (BBM).

Materials and methods

Algae media

Leachate from a closed, methanogenic-stage landfill (Alachua County Southwest Landfill (ACSWL), Archer, Florida) was used in all experiments. The ACSWL is a 10.9 ha, lined landfill site, which received MSW from 1988 to 1999. The ACSWL was one of the first landfills in the state of Florida to incorporate a bottom liner to mitigate the environmental impact of MSW leachate.[19] After closure, an impermeable top liner was used to seal the landfill and reduce the amount of leachate generated from rainfall events. When active, leachate generated by this landfill averaged 85,000 L/day.[19] Methods employed in leachate management at this site have included research projects on leachate recirculation, including infiltration ponds and horizontal injection; additionally, a major method of management was leachate transport (via tanker truck) to a publically owned treatment works.[19] Current leachate management at the ACSWL involves recirculation by horizontal injection. This mature, closed landfill was chosen as the test facility since all landfills will eventually reach this stage of maturity.

Leachate was routinely collected directly from mainline recirculation plumbing of the ACSWL and stored in airtight containers at 4°C. Leachate from a representative sample was used in cultivation experiments. Basic physico-chemical characterization of the MSW LL is presented in Table 1. Sample pH was measured using an Orion 520A pH meter with an Orion pH electrode (Orion Research Inc., Boston, MA) in accordance with standard method 4500-H+. [20] Conductivity was measured with an Accumet Model 30 conductivity metre (Thermo Fisher Scientific, Waltham, MA) in accordance with standard method 2510-B.[20] COD was measured by colorimetry after closed reflux with potassium dichromate, in accordance with standard method 5220-D.[20] Total ammoniacal-nitrogen was measured using an ion-selective electrode (Orion 95–12) to a publically owned treatment works.[19] Current leachate management at the ACSWL involves recirculation by horizontal injection. This mature, closed landfill was chosen as the test facility since all landfills will eventually reach this stage of maturity.

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<table>
<thead>
<tr>
<th>Component</th>
<th>Average ± std. dev. (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.52 ± 0.101</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>2107.3 ± 39.5</td>
</tr>
<tr>
<td>Conductivity (mS/cm)</td>
<td>17.19 ± 0.07</td>
</tr>
<tr>
<td>Total solids (g/L)</td>
<td>8.24 ± 0.04</td>
</tr>
</tbody>
</table>

Note: LL, landfill leachate.

Table 2. Elemental composition of LL compared with BBM.

<table>
<thead>
<tr>
<th>Component</th>
<th>LL</th>
<th>BBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macronutrients (mg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>980</td>
<td>41.2</td>
</tr>
<tr>
<td>Ammonia-N</td>
<td>980</td>
<td>—</td>
</tr>
<tr>
<td>Nitrate-N</td>
<td>—</td>
<td>41.2</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>13.2</td>
<td>53.2</td>
</tr>
<tr>
<td>Potassium</td>
<td>980</td>
<td>106.0</td>
</tr>
<tr>
<td>Magnesium</td>
<td>88.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Calcium</td>
<td>110</td>
<td>6.8</td>
</tr>
<tr>
<td>Iron</td>
<td>16</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>3700</td>
<td>86.3</td>
</tr>
<tr>
<td>Chloride</td>
<td>1800</td>
<td>27.5</td>
</tr>
<tr>
<td>Micronutrients (mg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>0.11</td>
<td>0.5</td>
</tr>
<tr>
<td>Copper</td>
<td>0.17</td>
<td>0.4</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.06</td>
<td>2.0</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.07</td>
<td>0.1</td>
</tr>
<tr>
<td>Toxic metals (mg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.13</td>
<td>—</td>
</tr>
<tr>
<td>Antimony</td>
<td>&lt;0.06a</td>
<td>—</td>
</tr>
<tr>
<td>Cadmium</td>
<td>&lt;0.0032a</td>
<td>—</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.12</td>
<td>—</td>
</tr>
<tr>
<td>Lead</td>
<td>&lt;0.013a</td>
<td>—</td>
</tr>
<tr>
<td>Selenium</td>
<td>&lt;0.068a</td>
<td>—</td>
</tr>
</tbody>
</table>

Notes: LL, landfill leachate; BBM, Bold’s Basal Medium. *Minimum detection limit.
Algae isolation and cultivation

Algae collected from the ACSWL site were diluted with sterilized BBM and incubated on agar plates (2% BD Bacto™ Agar in deionized water) under 150 μmol photons/m²/s illumination. Individual colonies were lifted and cultured in sterilized BBM. Single plating isolations yielded two unialgal isolates, tentatively identified by morphological characteristics as Chlorella cf. ellipsoidea and Scenedesmus cf. rubescens, respectively. A modified BBM was formulated by adding 2% LL to a stock solution of BBM and this was used in maintaining mother cultures of the isolates. The photon flux density of photosynthetically active radiation was measured using a Li-Cor LI-189 instrument (Li-Cor Biosciences, Lincoln, NE) equipped with a quantum sensor. Illumination in laboratory experiments was provided by T5 fluorescent lamps to give 150 μmol photons/m²/s lighting, provided on a 24:0 lighting, provided on a 24:0 photoperiod, at 25 ± 1.5°C.

Algal growth experiments were conducted in 125 mL Erlenmeyer flasks (75 mL active volume). LL was inoculated with exponentially growing mother cultures at a volumetric ratio of 20% (v/v). Mixing was provided by aerating (0.45 μm filtered air at a rate of ~0.065 L/min/flask). Deionized water was added to cultures daily to replace volume lost by evaporation. Algal growth was monitored using absorbance at 680 nm ($A_{680}$) with a spectrophotometer (Genesys 10vis, Thermo-spectronic, Rochester, NY). Mean growth rate ($\mu$) was estimated as follows:

$$\mu = \frac{\ln A_{680n} - \ln A_{680t}}{n},$$

where $A_{680t}$ is the initial absorbance, $A_{680n}$ is the final absorbance, and $n$ is the number of days elapsed. Cell population was estimated from $A_{680}$ after standardization with a haemocytometer (American Optical Co., Buffalo, NY). Dry weights were calculated from $A_{680}$ after standardization with algal biomass dried to constant weight at 105°C.

The content of reduced nitrogen within ACSWL leachate indicated that it may be necessary to regulate the pH of the medium to avoid the known inhibitory effects of free ammonia.[24] It was, therefore, presumed and confirmed by preliminary testing (data not shown) that pH regulation of free ammonia toxicity in ACSWL leachate would be necessary. In pH-adjusted cultures, LL was neutralized by titrating with 1M HCl to an initial pH of 7.0 ± 0.1 (<1%v/v). For both algal isolates, growth medium treatments included a negative control of 100% LL without pH neutralization (LL-Control), a positive control (BBM), and an experimental treatment using neutralized 100% LL (HCl-adjusted LL). All treatments were replicated in triplicate.

Statistical analysis

Experiments were conducted in replicates of three. Means, standard deviations, and Student’s t-tests (two-tailed, unpaired, unequal variances, at a 95% confidence level) were calculated with Microsoft Excel (Microsoft, Inc., Redmond, WA).

Results

Growth of algal isolates

Time course of growth and pH of C. cf. ellipsoidea are shown in Figures 1 and 2, respectively. Growth in the positive control (BBM) and in the pH-adjusted treatments was nearly identical over the first 24-h period. From 24 to 72 h, the HCl-adjusted LL treatment plateaued and growth was minimal, whereas the BBM treatment culture continued to grow. LL without pH neutralization (LL-Control) showed little change in algal growth for the duration of the experiment. The BBM treatment showed a gradual increase in pH over the course of the experiment, while the LL-Control treatment had a higher initial pH which rose to 8.9 within the first 24 h and remained alkaline for the duration of the experiment. In contrast, acidification of the culture medium was observed in the HCl-adjusted LL treatments, eventually reaching a pH of 5.2 at 72 h.

Time course of growth and pH of S. cf. rubescens are shown in Figures 3 and 4, respectively. Growth within the BBM and HCl-adjusted LL treatments was nearly identical over the first 24-h period. Growth within the HCl-adjusted LL medium showed less growth than BBM from 24 to 48 h, but nearly equal growth from 48 to 72 h. The Scenedesmus culture continued to grow from 72 to 96 h, whereas the BBM treatment culture plateaued and growth was minimal. Growth within LL without pH neutralization (LL-Control) showed little change over the course of the experiment. General trends observed in pH dynamics during the cultivation of S. cf. rubescens were: the pH of the BBM treatment increased with growth, the pH of the LL-Control rose rapidly in the first 24 h, and the HCl-adjusted LL treatment had acidified rapidly by 48 h.

S. cf. rubescens exhibited significantly ($p = 0.014$) greater growth in the HCl-adjusted LL treatment than C. cf. ellipsoidea (Table 3). Neutralization of LL by acid addition significantly improved the cultivation of both the Chlorella and Scenedesmus isolates compared with the growth in LL controls without acid addition ($p < 0.05$ for both isolates). General pH dynamics followed similar trends in both algal cultures and in respective media. Notably, both the rise in pH in BBM and the acidification within HCl-adjusted LL were more pronounced in the Scenedesmus culture.

Estimated growth rates of C. cf. ellipsoidea in BBM and HCl-adjusted LL treatments were only comparable within the first 24 h. Although the HCl-adjusted LL was not toxic to the Chlorella isolate, inhibition of growth for this isolate was apparent after 24 h (Figure 1). In contrast, S. cf. rubescens showed growth in the HCl-adjusted LL (0.83/day) nearly equal to that of growth observed in BBM (0.91/day). Estimated mean growth rate and daily
cell yield of the Scenedesmus isolate within LL were 91.2% and 92.8% that observed in BBM, respectively (Table 3). The mean growth rate of C. cf. ellipsoidea in 100% LL (0.67/day) was also similar to that in BBM (0.73/day). However, cell yield of the Chlorella isolate was severely inhibited, reaching only 44.9% of the cell yield recorded for BBM. Cell density values observed were almost twice as high in BBM and four times as high in HCl-adjusted LL for the Scenedesmus isolate as compared with the Chlorella isolate (Table 3). The LL used in this study supported a maximum volumetric productivity of 0.55 g/L/day of S. cf. rubescens biomass.
Biomass and biofuel production potentials
Potential biomass production was estimated based on the mean elemental composition of algae biomass as reported in the extensive review by Healey.[25] It is recognized that large variations in the nitrogen content of algae biomass exist (1–14%), but 5.5% was taken as a mean estimate. ACSWL leachate can supply enough nitrogen for the production of 17.8 g/L of algae biomass at 5.5% N, roughly 20-fold higher than the potential biomass producible by BBM, 0.8 g/L (Table 4). Phosphorus in the ACSWL
Algae have an unrealized potential in future biofuel production.[2,4,26] Utilization of algae as a feedstock for biofuel production must still overcome significant technological, biological, and resource sustainability challenges.[33,34] Primary concerns in the development of sustainable algae-based biofuels are the high demand for water, nutrients (fertilizers and inorganic carbon), and land resources.[7] The advancement of methods that address resource sustainability issues in producing algae-based biofuels is critical in realizing the potential contribution of algae. Pittman et al. [8] recently suggested that perhaps the 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, leveraging a dual role in providing both waste mitigation and resource production.[9,35] Cultivating algae in LL will undoubtedly provide a tertiary (nutrient) remediation benefit, as described by Lin et al. [15] in the removal of total ammoniacal-nitrogen and orthophosphate in diluted LL. Additionally, there is also evidence to expect that many contaminants within LL may be biodegraded by algae.[36,37] However, previous reports of cultivation of algae within LL required dilution or extensive pretreatment to facilitate algae growth.[15,16] This study provides evidence that the cultivation of algae on high-ammonia LL, without dilution or extensive pretreatment is possible. Remediating environmental wastes provides additional economic incentives to the production of biofuels and additional support for a sustainable society.

The observed growth rates of S. cf. rubescens within BBM and LL were approximately equal, providing evidence that LL may have potential application as an algal growth medium. Observed growth rates of S. cf. rubescens (0.83/day) and C. cf. ellipsoidea (0.67/day) in 100% LL were 113% and 72% greater, respectively, than the growth rate for Chlorella pyrenoidosa (0.39/day) in 10% MSW LL reported by Lin et al.[15] Additionally, growth
rates reported in the present study are significantly higher than the growth rates reported by Mustafa et al. [16] for Scenedesmus quadricauda, Chlorella vulgaris, and Euglena gracilis (0.18, 0.21, and 0.23/day, respectively) in 25% MSW LL which had undergone previous treatment through mechanical aeration in treatment ponds at the landfill site. Furthermore, no tested organisms in either study [15,16] were able to tolerate 100% LL.

Utilizing leachate without dilution or mechanical aeration pretreatment provides an abundant supply of nitrogen, a key fertilizer requirement for producing algae biomass. Ironically, the high nitrogen content in the tested LL is also implicated as the primary inhibitory compound (free ammonia), when pH is not adjusted (Figures 1 and 3). For both isolates, growth on LL was poor without pH adjustment, exhibiting the previously described toxicity impacts of LL.[18] Adjusting the pH with the strong acid, HCl, eliminated the toxicity of the leachate but may have practical drawbacks in application. The operational and environmental costs of utilizing HCl may detract from the benefit of using a waste resource and should be investigated further. Nonetheless, HCl is currently applied for pH control in on-site, full-scale nitrification/denitrification processes for leachate pretreatment at landfill facilities. It is expected that the utilization of an alternative acid for pH regulation would be as effective in regulating ammonia toxicity.

Growth inhibition of C. cf. ellipsoidea, where the pH of the treatment medium continued to decrease after leachate neutralization with HCl, makes a case for the utilization of a pH controller to regulate the pH instead of initial neutralization. A continued decrease in pH is attributed to both the decreased buffering capacity of the LL after neutralization and the phenomenon of medium acidification due to cellular uptake of ammonium ions.[38] The Chlorella isolate showed less adaptation to LL as a growth medium than the Scenedesmus isolate, suggesting that there may be inhibitory compounds within the LL to which this isolate is more sensitive. Growth of the Chlorella isolate in the pH-adjusted treatment plateaued in correlation with medium acidification. From the observed data, it is not possible to distinguish between the impact of medium pH and possible growth inhibitors within the LL (e.g. refractory organic compounds, metals, etc.). It is expected that the majority of the reported COD for this mature LL is present as refractory organic compounds that are not readily degradable, but which may have significant biological effects. Cultivation of algae tolerant to these unknown compounds would provide conditions of oxygen saturation and may aid in the removal of these recalcitrant organics. The Scenedesmus isolate appeared to be unaffected by the potential inhibitory conditions of LL and was more tolerant of the pH change in the medium, emphasizing the suitability of S. cf. rubescens in favour of C. cf. ellipsoidea, under the described experimental conditions. No impact of acidic pH on growth was observed within the Scenedesmus culture. Indeed, cultures of the Scenedesmus isolate were observed growing at pH values < 4 in preliminary trials (data not shown). Buffering the system may foster the growth of algae sensitive to pH fluctuations. However, cultivating an alga at an extreme pH may be advantageous in reducing culture contamination in outdoor growth systems, analogous to the cultivation of the alkaliphile Spirulina.[39]

The high nitrogen content of LL makes it an attractive N-source for industrial algae biomass production. Using MSW LL has the potential to reduce the nitrogen, water, and greenhouse gas emission footprints of algae biomass production. MSW landfills can supply wastewater, carbon dioxide (combustion of landfill gas), land area (tops of closed MSW landfill sites), and essential nutrients for the cultivation of algae biomass, which can be used for algae-based biofuels. Industrial production of algae biomass sited at landfill facilities can transform landfills from societal burdens to centres of resource generation and nutrient recycling.

Various toxic heavy metals may potentially be found in LL, depending on the site, materials landfilled, and age of the landfill.[17] Algae biomass may accumulate heavy metals, limiting the potential use of the biomass. On the other hand, certain species of algae with high affinities for valuable metal ions may provide a biological means of bio-mining LLs. In the production of biofuels, heavy metals are not expected to transfer from the algae biomass to the oil or gas produced by biofuel processing.

Algae biomass generated from LL cultivation can be used as a feedstock for several biofuels, depending on the species and cultivation conditions. Algae biomass can be converted to biofuels through anaerobic digestion (CH₄), fermentation (e.g. to ethanol, butanol, or hydrogen), or hydrothermal processing. Ammonia released through biomass processing can be recycled to algae cultivation. If high in lipids, the algae biomass can be refined into high-value liquid fuels such as biodiesel or jet fuel. Estimates of available nitrogen, biomass, and biofuel production highlight the potential of utilizing MSW LL for algal biomass production.

Conclusion

Experimental observations indicate that MSW LL may provide both water and nutrient resources for the production of algae-based biofuels. Displacing the use of potable waters and the energy embedded in synthetic fertilizer production can reduce both the cost and the environmental footprint of algae-based biofuels. Utilizing algae in a dual role of waste mitigation and biofuel production can be extended from commonly explored areas such as municipal sewage and agricultural wastes to applications in industrial and solid waste remediation. MSW landfills are currently the most commonly employed means of solid waste management and the liquid leachates generated at these facilities can provide abundant nitrogen for the production of algae biomass. This study provides evidence that the cultivation of algae on high-ammonia LLs, without
dilution or extensive pretreatment, is possible. The LL used in this study supported a maximum volumetric productivity of 0.55 g/L/day of S. cf. rubescens biomass. Once algae biomass is sustainably and cost-effectively generated, several different process trains can be used to produce biofuels, such as anaerobic digestion, transesterification of neutral lipids, hydrothermal treatment, or combustion. The potential biofuel production from the total waste nitrogen available in LLs in Florida was estimated at 3.33 GL of algae-derived biofuel/year.

Acknowledgements
This research was funded by the Hinkley Center for Solid and Hazardous Waste Management, Gainesville, Florida [grant number 97359], and the Waste Management Division, Alachua County Hazardous Waste Management, Gainesville, Florida [grant number 75650].

References


