Identification of Hawaiian Algae: Methods for Cultivation and Oil Extraction for Potential Use as an Alternative Fuel

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Introduction

The production of renewable energy sources has increased in popularity over the past three decades as those with global environmental concerns strive to reduce greenhouse gas emissions and alleviate the depletion of fossil fuels reserves. Currently most biofuels are produced from crops that compete for arable land and clean water, which could easily drive up food, and local utility costs (Dincer 2008, Demirbas 2011a, Singh et al. 2011). Beal et al. (2012) found that, algae have the potential to produce a large amount of petroleum fuel substitutes while avoiding the need for large amounts of fresh water and arable land. Compared to other terrestrial crops currently being used, algae have a much faster growth rate and have been estimated to be able to produce 20,000-80,000 liters per acre per year, (Demirbas and Demirbas 2011). While a normal crop cycle may take anywhere from three months to three years for oil production, algae can begin producing oil in as little as three to five days (Demirbas 2011b). Finding local algae for the possible production of biofuel in Hawaii would allow the development of local biofuels. The purpose of this research was to identify freshwater algae found in Hawaii that could be grown effectively and explore methods for cultivation and oil production for the potential use as an alternative bio-fuel.

Methods and Materials

Algae samples were collected in Laie, Hawaii from standing fresh water and cultured in a lab at Brigham Young University Hawaii. The algae sample was then grown outdoors in a 75 gallon photobioreactor. To determine the species of algae a French press was used at 80 MPa to prepare 50 mL of a highly concentrated algae solution. Samples were centrifuged for 15 minutes at 4000 rpm and the remaining algae biomass was removed. A 30 µL sample of the concentrated proteins were prepared by adding 250 mM DTT. Five microliters of trypsin were added and the sample was microwaved for two minutes. The sample was spun through a 0.45 µm centrifuge tube filter before being analyzed by LC/MS following the procedure of MacDougall et al. (2012). The proteins were then identified using the SwissProt online database. The database yielded an ion score which is -10*Log10(P), where P is the probability that the observed match is a random event. Algae were removed from a 75 gallon photobioreactor and were harvested during peak growth as determined by absorbance. Algae samples were dried using a vacuum oven at 80°C for 40 minutes. Lipids were extracted from the dried algae samples using a soxhlet extraction as described by Halmi et al. (2011).

Results

Four Algae species were identified from the field samples, Pseudendoclonium akinetum, Chlamydomonas reinhardtii, Stigeoclonium helveticum and Chlamydomonas moewusii. All four species had ion scores above 50 (Table 1). The four species were cultured as a mixture. Absorbance of the mixture was correlated to cell count demonstrating a significant quadratic relationship (Figure 1). An absorbance of 0.40 equaled 2,222,000 cells/ml. Analysis of absorbance indicated a sigmoidal growth curve with cell counts reaching an asymptote on day eight (Figure 2). Crude oil extracted from dried algae yielded on average 0.056 g of oil recovered per gram of dried algal powder. These yields averaged 5.50% of the overall algal dry mass.

Table 1. The ion score and peptides identified for the four algae species identified.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ion score</th>
<th>Peptides Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudendoclonium akinetum</td>
<td>81</td>
<td>2</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>76</td>
<td>2</td>
</tr>
<tr>
<td>Stigeoclonium helveticum</td>
<td>51</td>
<td>2</td>
</tr>
<tr>
<td>Chlamydomonas moewusii</td>
<td>132</td>
<td>6</td>
</tr>
</tbody>
</table>

Individual ions scores > 49 indicate identity or extensive homology (p<0.05).

Discussion

The concentration of oil extracted from algae in this study was similar to the concentration of oil extracted from Chlorococcum sp. (Halim et al. 2011). He reported 0.058g of lipid extract per gram of dried microalgae using the soxhlet reaction. Gouveia and Oliveira (2008) reported extractable oil concentrations from select algae used in biofuel research between 4.1-29.0%. The algae with higher oil content were halotolerant. These findings suggest that the algal species isolated in this study are good candidates for further biofuel study. Chlamydomonas reinhardtii, one of the species in this study has been identified as a species which shows promise for accumulation of neutral lipids which could be extracted as a biofuel (Siaut et al. 2011). Identifying algae species that are present in Hawaii that are productive oil producers, is necessary for the selection of good candidates for algae oil production in Hawaii’s unique ecosystem. Future studies might attempt to isolate pure strains of algae used in this study to compare oil yields from monoculture systems. Additionally, other extraction methods using algae such as saponification or wet milling may result in higher oil yields.

References:


Keywords: microalgae, biodiesel, wet milling, liquid chromatography, mass spectrometry, Pseudendoclonium akinetum, Chlamydomonas reinhardtii, Stigeoclonium helveticum

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