Full Length Research Paper

Determination of optimum growth conditions and biodiesel production from filamentous algae

Hesham R. Lotfy*, Martha N. Amputu and Celestine Raidron

Department of Chemistry and Biochemistry, Faculty of Science, University of Namibia, P. Bag 13301, Windhoek, Namibia.

Received 7 November, 2011; Accepted 1 August, 2013

Petroleum diesel combustion is a major source of greenhouse gas (GHG). It is also a major source of other air contaminants including NOx, SOx, CO and volatile organic compounds. Algae have emerged as one of the most promising sources for biodiesel production. In this study, a higher algae growth rate was observed in the experiments with excess Na2SiO4, trace metals, Na2EDTA and excess vitamin solution, the increase was above 300%. It was also observed that the experiment that was supplied with CO2 (without simultaneous sunlight exposure) for one hour, for 25 days and the beaker with excess NaH2PO4 solution, have shown a slower growth rate than the control. The results of the experiment on the effect of sunlight exposure for certain times daily for 25 days show that the growth rate is directly proportional to increase of sunlight exposure time (during the 90 min). The results of the experiment on the effect of simultaneous exposure to sunlight and CO2 for certain times daily, for 25 days, show that the growth rate is directly proportional to the increase of sunlight and CO2 exposure time.

Key words: Algal oil, biodiesel, transesterification, glycerine.

INTRODUCTION

The need of energy is increasing continuously due to the increase in population and industrialization. The continued use of petroleum sourced fuels is now widely recognized as unsustainable because of the depletion of supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment leading to increase of global warming. The combustion of fossil fuels is responsible for 73% of the CO2 production (Narendra et al., 2010). With regards to global warming and as dependence on fossil fuels grows, the search for renewable energy sources that reduce CO2 emissions becomes a matter of widespread attention (Ragauskas et al., 2006; Demirbas and Demirbas, 2007). In recent years, cultivation of microalgae has received renewed attention on account of their utility as a feasible CO2 sequestration technology (Ono and Cuello, 2006; Hsueh et al., 2007; Jacob-Lopes et al., 2008).

In the last ten years, many studies have been conducted on biofuels for substituting fossil fuels and reduce the greenhouse gas emission (Bastianoni et al., 2008). Algae, especially microalgae, were found to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels (Chisti, 2007, 2008).

The idea of using algae as a source of fuel is not new (Chisti, 1980; Chisti 1981; Nagle and Lemke, 1990;
Sawayama et al., 1995), but it is now being taken seriously because of the increasing price of petroleum and more significantly, the emerging concern about global warming that is associated with burning fossil fuels (Gavrilescu and Chisti, 2005). Microalgae can provide several types of renewable biofuels which include, methane, biodiesel (methyl esters) and biohydrogen (Gavrilescu and Chisti, 2005; Kapdan and Kargi, 2006; Spolaore et al., 2006). Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops (Shay, 1993).

Bioenergy is one of the most important components to mitigate greenhouse gas emissions and substitute of fossil fuels (Goeldemberg, 2000). Petroleum diesel combustion is a major source of greenhouse gas (GHG). Apart from these emissions, petroleum diesel is also a major source of other air contaminants including NOx, SOx, CO, particulate matter and volatile organic compounds (Klass, 1998). Biomass is one of the better sources of energy (Kulkarni and Dalai, 2006). Large-scale introduction of biomass energy could contribute to sustainable development on several fronts, environmentally, socially and economically (Turkenburg, 2000; UNDP, 2008). Biodiesel is a nontoxic and biodegradable alternative fuel that is obtained from renewable sources. It is reported that algae were one of the best sources of biodiesel and are the highest yielding feedstock for biodiesel. It can produce up to 250 times the amount of oil per acre from soybeans (Hossain and Salleh, 2008). In fact, producing biodiesel from algae may be the only way to produce enough automotive fuel to replace current gasoline usage. The best algae for biodiesel would be microalgae. Microalgae have much more oil than macroalgae and it is much faster and easier to grow (Shay, 1993).

Algae contain anything between 2 and 40% of lipids/oils by weight (Wagner, 2007). Microalgae have much faster growth-rates than terrestrial crops. The per unit area yield of oil from algae is estimated to be between 18,927 and 75,708 L per acre, per year; this is 7 to 31 times greater than the next best crop, palm oil (around 2,404 L) (Wagner, 2007). In addition, the question “Food or Fuel?” is not raised.

Advantages of microalgal cultivation for biodiesel production over other oleaginous crops are: the former is not only easy to handle and more beneficial from economic point of view but also known for their bioremediation capabilities (Dayananda et al., 2005; Szadanoff, 2006; Chisti, 2007; Huntley and Redalje, 2007; Li et al., 2008; Schenk et al., 2008; Tan et al., 2009). Some positive points of microalgal cultivation are: rapid growth rates, a high per-acre yield (7 to 31 times greater than the next best crop- palm oil), certain species of algae can be harvested daily, short life cycle (approximately 1 - 10 days), ability to synthesize and accumulate large quantities of lipids per dry weight biomass, algae biofuel contains no sulphur, algae biofuel is non-toxic, algae biofuel is highly bio-degradable, and algae consume carbon dioxide as they grow, so they could be used to capture CO2 from power stations and other industrial plants that would otherwise go into the atmosphere, potential to grow in saline water and harsh conditions, less fertilizer and nutrient input requirements and it is the most promising non-food source of biodiesel. After extracting oil from microalgae, the remaining biomass portion can also be used as a high protein feed for livestock (Schneider, 2006; Haag, 2007).

The algae used in this study is filamentous algae also known as "pond moss" or "pond scum" and these threadlike algae often occur in huge greenish masses floating upon the waters' surface. They can form dense mats in static water or long, rope-like strands in flowing water. Its filaments consist of series of cells being joined end to end giving a thread-like appearance.

The main aim of the research was to extract oil from a microalgae species and convert it to biodiesel and to determine the optimum growth conditions of the used algae species.

### MATERIALS AND METHODS

#### Stock solutions preparation

The stock solutions were prepared according to the procedures of Guillard and Ryther (1962).

#### Filamentous algae cultivation

**Cultivation of algae in a synthetic medium**

The medium was prepared by adding 3.00 ml of NaNO3 solution (150.008 g in 1 L distilled water), 3.00 ml of trace metal solution, 3.00 ml of NaSiO3.SiH2O solution (10 ml in 1 L distilled water), 3.00 ml of iron citrate solution (9.0008 g FeCl2 and 9.00 g citric acid in 1 L distilled water), 3.00 ml of vitamin solution (Folic acid 0.015 g and 0.0156 g peptone bacteriological in 100 ml distilled water), 1.50 ml of NaH2PO4 (11.31 g in 1 L distilled water) and 1.50 ml of Na2EDTA.2H2O solution, to a beaker containing 3000 ml of water and 19.62 g of filamentous algae was added.

#### Optimization of growth condition

**The effects of different components concentration of the stock solution on the algae growth (Table 1)**

One milliliter of NaNO3 solution, 1.00 ml of trace metal solution, 1.00 ml of NaSiO3.SiH2O solution, 1.00 ml of iron citrate solution, 1.00 ml of vitamin solution, 0.50 ml of NaH2PO4 solution and 0.50 ml of Na2EDTA.2H2O solution were added to each of nine beakers filled with 100.00 ml tap water. In the first beaker, 1.00 ml of NaNO3 solution was added in excess, the second beaker, 1.00 ml of trace metal solution was added in excess, the third beaker, 1.00 ml of NaSiO3.SiH2O solution was added in excess, the fourth beaker 1.00 ml of iron citrate solution was added in excess, to the fifth beaker, 1.00 ml of vitamin solution was added in excess, the sixth beaker, 0.50 ml of NaH2PO4 solution was added in excess, the seventh beaker, 0.50 ml of Na2EDTA.2H2O solution was added in excess, to
The effect of different components of concentration of stock solution on the algae growth.

<table>
<thead>
<tr>
<th>Stock solution in excess 1.00 ml</th>
<th>Initial mass of algae (g)</th>
<th>Mass of algae after 15 days (g)</th>
<th>Increase in mass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>1.00</td>
<td>3.49</td>
<td>249 ↑</td>
</tr>
<tr>
<td>Trace metals</td>
<td>1.00</td>
<td>4.47</td>
<td>347 ↑↑</td>
</tr>
<tr>
<td>Na₂SiO₃₆.5H₂O</td>
<td>1.00</td>
<td>4.68</td>
<td>368 ↑↑</td>
</tr>
<tr>
<td>Iron citrate</td>
<td>1.00</td>
<td>3.74</td>
<td>274 ↑</td>
</tr>
<tr>
<td>Vitamin</td>
<td>1.00</td>
<td>4.04</td>
<td>304 ↑↑</td>
</tr>
<tr>
<td>Na₂H₂PO₄</td>
<td>1.00</td>
<td>2.75</td>
<td>175 ↓</td>
</tr>
<tr>
<td>Na₂EDTA.2H₂O</td>
<td>1.00</td>
<td>4.29</td>
<td>329 ↑↑</td>
</tr>
<tr>
<td>CO₂</td>
<td>1.00</td>
<td>2.00</td>
<td>100 ↓</td>
</tr>
<tr>
<td>Control</td>
<td>1.00</td>
<td>2.86</td>
<td>186 ─</td>
</tr>
</tbody>
</table>

Table 2. Results of the effect of sunlight exposure on growth after 25 days.

<table>
<thead>
<tr>
<th>Sample</th>
<th>30 min sunlight daily</th>
<th>60 min sunlight daily</th>
<th>90 min sunlight daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mass (g)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Final mass (g)</td>
<td>2.75</td>
<td>2.87</td>
<td>3.01</td>
</tr>
<tr>
<td>Increase in mass (%)</td>
<td>175</td>
<td>187</td>
<td>201</td>
</tr>
</tbody>
</table>

Table 3. The effect of simultaneous CO₂ supplement and sunlight exposure after 25 days.

<table>
<thead>
<tr>
<th>Experimental time</th>
<th>CO₂ and sunlight</th>
<th>Control (sunlight only)</th>
<th>CO₂ and sunlight</th>
<th>Control (sunlight only)</th>
<th>CO₂ and sunlight</th>
<th>Control (sunlight only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mass (g)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Final mass (g)</td>
<td>4.04</td>
<td>2.75</td>
<td>4.72</td>
<td>2.87</td>
<td>4.82</td>
<td>3.01</td>
</tr>
<tr>
<td>Increase in mass %</td>
<td>304</td>
<td>175</td>
<td>372</td>
<td>187</td>
<td>382</td>
<td>201</td>
</tr>
</tbody>
</table>

The effects of sunlight exposure time on the algae growth (Table 2)

Three 250.00 ml beakers were filled with 200.00 ml of water and 1.00 ml of NaNO₃ solution, 1.00 ml of trace metal solution, 1.00 ml of Na₂SiO₃₆.5H₂O solution, 1.00 ml of iron citrate solution, 1.00 ml of vitamin solution, 0.50 ml of NaH₂PO₄ and 0.50 ml of Na₂EDTA.2H₂O solutions were added to each beaker. One gram of algae was added to each beaker and CO₂ was supplied to each for 10 min. The algae were exposed to sunlight at different times, for 30, 60 and 90 min, respectively. The growth rate in each beaker was observed.

The effect of available space on the growth rate

One gram of algae was added to the beakers. The algae were supplied with CO₂ supplement and exposed to the sunlight simultaneously, for 30, 60 and 90 min, respectively. The growth rate in each beaker was observed.

Extraction process

Harvesting

Ninety percent of the algae were collected using a fish net after 10 days of cultivation. The wet algae weighed 56.50 g and they were ground with a pestle in a mortar for 20 min. The ground algae were dried in an oven for 75 min at 80°C to release water. The dried algae weighed 26.85 g.
Triglyceride (oil or fat) Methanol
R1–3 are hydrocarbon groups

**Figure 1.** Transesterification reaction to produce biodiesel.

**Drying time and temperature**

Extraction was done several times on the filamentous algae at different drying times and temperatures in trials to find the best drying time and the optimum temperature. It was found that the best drying time was 75 min at 80°C.

**Oil extraction**

The two methods used are hexane solvent extraction and soxhlet extraction. The soxhlet extraction did not yield satisfactory results. The hexane solvent extraction used 20.00 ml of hexane and 20.00 ml diethyl ether which were mixed with the dried algae to extract the oil. The mixture was allowed to settle for 24 h. The biomass was collected by gravity filtration and weighed 25.58 g. The extracted oil was collected after filtration. The hexane and the diethyl ether were evaporated using a rotary evaporator.

**Transesterification (Figure 1)**

Algal oil (0.80 ml) was mixed with sodium methoxide which was prepared by dissolving 0.0122 g of NaOH in 1.20 ml of methanol. The mixture was shaken for one hour, and thereafter transferred to a separatory funnel and allowed to settle for 12 h.

In the separatory funnel, the layers were clearly formed; biodiesel on top and glycerol at the bottom. The hexane solvent extraction used 20.00 ml of hexane and 20.00 ml diethyl ether which were mixed with the dried algae to extract the oil. The mixture was allowed to settle for 24 h. The biomass was collected by gravity filtration and weighed 25.58 g. The extracted oil was collected after filtration. The hexane and the diethyl ether were evaporated using a rotary evaporator.

**RESULTS**

**Cultivation**

**The effects of different components of stock solution concentration on the algae growth**

A higher growth rate was observed in the beakers with excess Na₂SiO₄, trace metals, Na₂EDTA and excess vitamin solution, the increase was above 300%. It was also observed that the beaker that was supplied with CO₂ (without simultaneous sunlight exposure) for one hour and the beaker with excess NaH₂PO₄ solution showed a slower growth rate than the control.

**The effect of the sunlight exposure time, for 25 days, on algae growth rate**

The results of the experiment studying the effect of sunlight exposure for certain times daily for 25 days, showed that the growth rate is directly proportional to increase of sunlight exposure time (for 90 min).

**The effect of CO₂ supplement and sunlight exposure simultaneously on the growth after 25 days**

The results of the experiment on the effect of simultaneous exposure to sunlight and CO₂ for certain times daily, for 25 days, showed that the growth rate is directly proportional to the increase of sunlight and CO₂ exposure time.

**The effect of available space on the growth rate**

The growth rate in the tank which containing 11.45 g algae was 2.9% after 10 days, while the growth rate in the tank which contained 22.88 g algae was 2.6% after 10 days and under the same experimental conditions. This is in agreement with a different study done by Campbell (2008).

**Extraction**

**Filamentous algae extraction and transesterification results**

Extraction was done several times on the filamentous algae at different drying times and temperatures in trials to find the best drying time and the optimum temperature. Although, the dry mass was not constant but based on the ratio between the dry mass and the amount of oil produced it was found that the best drying time, in our experiments, was 75 min at 80°C. Algal oil was obtained as shown in Table 4. With the transesterification process, only some of the algal oil was converted to biodiesel.
Table 4. Extraction and transesterification results of filamentous algae oil.

<table>
<thead>
<tr>
<th>Dry mass (g)</th>
<th>Biomass (g)</th>
<th>Drying time (min)</th>
<th>Amount of algal oil extracted (ml)</th>
<th>Biodiesel produced (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.23</td>
<td>0.78</td>
<td>60 min at 80°C</td>
<td>Approximately 0.20</td>
<td>No biodiesel produced</td>
</tr>
<tr>
<td>26.85</td>
<td>25.58</td>
<td>85 min at 80°C</td>
<td>0.80</td>
<td>Approximately 0.20 ml of biodiesel produced</td>
</tr>
<tr>
<td>13.97</td>
<td>12.70</td>
<td>75 min at 80°C</td>
<td>1.30</td>
<td>Approximately 0.5ml of oil produced</td>
</tr>
<tr>
<td>31.01</td>
<td>29.93</td>
<td>180 min at 85°C</td>
<td>1.00</td>
<td>No biodiesel produced</td>
</tr>
<tr>
<td>18.83</td>
<td>18.52</td>
<td>170 min at 60°C</td>
<td>0.70</td>
<td>No biodiesel produced</td>
</tr>
<tr>
<td>21.42</td>
<td>18.94</td>
<td>30 min at 80°C</td>
<td>0.50</td>
<td>No biodiesel produced</td>
</tr>
<tr>
<td>14.77</td>
<td>13.21</td>
<td>20 min at 80°C</td>
<td>0.70</td>
<td>No biodiesel extracted</td>
</tr>
<tr>
<td>25.98</td>
<td>21.35</td>
<td>20 min at 80°C</td>
<td>0.50</td>
<td>0.2 ml biodiesel extracted</td>
</tr>
</tbody>
</table>

Some of the oil solidified during the process. Results in Table 4 shows that the best drying time, in our experiments, was 75 min at 80°C.

**DISCUSSION**

In the experiment investigating the effect of the sunlight exposure time only and the effect of simultaneous CO₂ supplement and sunlight exposure in 25 days, it was observed that the growth rate is directly proportional to the length of sunlight exposure time and CO₂ supplement simultaneously. This may be attributed to enhanced photosynthesis which in turn increase the growth rate. Space plays a role in the growth of algae, the results of the experiment on the effect of available space indicated that the algae that were sparsely populated in the growth tank had a higher growth rate. In the experiment on the effect of different components’ concentrations of media solution on the algae growth, it was found that excess of trace metal and silicate solutions have shown the highest growth rates. This is because they play a structural role in the chloroplast membrane, maintains the green colour and assist in the breakdown of purine, which is in accordance with report of Salisbury and Ross (1992).

**Conclusion**

The experiment on the effect of different nutrients on the growth of algae showed a higher growth rate in beakers with excess Na₂SiO₄, trace metals, Na₂EDTA and excess vitamin solution, the increase was above 300%. It was also observed that in the experiment which was supplied with CO₂ (without simultaneous sunlight exposure) for one hour and the experiment with excess NaH₂PO₄ solution, there was a slower growth rate than that of the control.

The results of the experiment on the effect of sunlight exposure for certain times daily, for 25 days, showed that the growth rate is directly proportional to the increase in sunlight exposure time (within 90 min).

The results of the experiment on the effect of simulta-

neous exposure to sunlight and CO₂ for certain times daily, for 25 days, showed that the growth rate is directly proportional to the increase of simultaneous sunlight and CO₂ exposure time (within 90 min).

The results of the experiment on the optimum drying time and temperature, showed that the best drying time was 75 min at 80°C. Algal oil was extracted using hexane and diethyl ether in 1:1 ratio. Through transesterification reaction, some of the algal oil was converted to biodiesel.

**Conflict of Interests**

The author(s) have not declared any conflict of interests.

**REFERENCES**


Lotfy et al.