

Full Length Research Paper

Determination of optimum growth conditions and biodiesel production from filamentous algae

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Petroleum diesel combustion is a major source of greenhouse gas (GHG). It is also a major source of other air contaminants including NO_x, SO_x, 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;

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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 (Goldemberg, 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 NO_x, SO_x, 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; Sazdanoff, 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 bio-

fuel 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 NaSiO₃.5H₂O solution (10 ml in 1 L distilled water), 3.00 ml of iron citrate solution (9.0008 g FeCl₃ 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 NaSiO₃.5H₂O 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 NaSiO₃.5H₂O 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

Table 1. The effect of different components of concentration of stock solution on the algae growth.

Stock solution in excess 1.00 ml	Initial mass of algae (g)	Mass of algae after 15 days (g)	Increase in mass (%)
NaNO ₃	1.00	3.49	249 ↑
Trace metals	1.00	4.47	347 ↑↑
Na ₂ SiO ₄	1.00	4.68	368 ↑↑
Iron citrate	1.00	3.74	274 ↑
Vitamin	1.00	4.04	304 ↑↑
NaH ₂ PO ₄	1.0020	2.75	175 ↓
Na ₂ EDTA	1.00	4.29	329 ↑↑
CO ₂	1.00	2.00	100 ↓
Control	1.00	2.86	186 —

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

Sample	30 min sunlight daily	60 min sunlight daily	90 min sunlight daily
Initial mass (g)	1.00	1.00	1.00
Final mass (g)	2.75	2.87	3.01
Increase in mass (%)	175	187	201

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

Experimental time	30 min		60 min		90 min	
	CO ₂ and sunlight	Control (sunlight only)	CO ₂ and sunlight	Control (sunlight only)	CO ₂ and sunlight	Control (sunlight only)
Initial mass (g)	1.00	1.00	1.00	1.00	1.00	1.00
Final mass (g)	4.04	2.75	4.72	2.87	4.82	3.01
Increase in mass %	304	175	372	187	382	201

the eighth beaker, CO₂ was supplied for one hour daily, while the ninth was used as a control, no excess solution was added. One gram of algae was added to each beaker and CO₂ was supplied to each for 10 min.

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 NaSiO₃.5H₂O 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 simultaneous CO₂ supplement and sunlight exposure time (Table 3)

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 NaSiO₃.5H₂O solution, 1.00 ml of iron citrate solution, 1.00 ml of vitamin solutions and 0.50 ml of NaH₂PO₄ and Na₂EDTA.2H₂O solution were added to each beaker.

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.

The effect of available space on the growth rate

Filamentous algae, 34.33 g were collected from a pond and cultivated in two fish tanks. They were filled with 10.00 L of water, 20.00 ml of NaNO₃ solution, 20.00 ml of trace metal solution, 20.00 ml of NaSiO₃.5H₂O solution, 20.00 ml of iron citrate solution, 20.00 ml of vitamin solution, 5.00 ml NaH₂PO₄ solution and 10.00 ml of Na₂EDTA.2H₂O solution. To one tank, 22.88 g of algae were added and 11.45 g of algae was added to the second tank. The algae were exposed to sunlight and CO₂ supplement was bubbled into each tank for 90 min daily.

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.

Table 4. Extraction and transesterification results of filamentous algae oil.

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

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.

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