academicJournals

Vol. 8(6), pp. 344-349, June 2014
DOI: 10.5897/AJEST11.339
Article Number: 910213645958
ISSN 1996-0794

http://www.academicjournals.org/AJEST

African Journal of Environmental Science and Technology

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 Na_2SiO_4, trace metals, Na_2EDTA and excess vitamin solution, the increase was above 300%. It was also observed that the experiment that was supplied with CO_2 (without simultaneous sunlight exposure) for one hour, for 25 days and the beaker with excess NaH_2PO_4 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 CO_2 for certain times daily, for 25 days, show that the growth rate is directly proportional to the increase of sunlight and CO_2 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 CO_2 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 CO_2 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 CO_2 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;

*Corresponding author. E-mail: hrlotfy@unam.na, craidron@unam.na.

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 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; 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 biofuel is highly bio-degradable, and algae consume carbon dioxide as they grow, so they could be used to capture CO_2 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 $NaNO_3$ solution (150.008 g in 1 L distilled water), 3.00 ml of trace metal solution, 3.00 ml of $NaSiO_3.5H_2O$ solution (10 ml in 1 L distilled water), 3.00 ml of iron citrate solution (9.0008 g $FeCl_3$ 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 NaH_2PO_4 (11.31 g in 1 L distilled water) and 1.50 ml of $Na_2EDTA.2H_2O$ 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 $NaNO_3$ solution, 1.00 ml of trace metal solution, 1.00 ml of $NaSiO_3.5H_2O$ solution, 1.00 ml of iron citrate solution, 1.00 ml of vitamin solution, 0.50 ml of NaH_2PO_4 solution and 0.50 ml of $Na_2EDTA.2H_2O$ solution were added to each of nine beakers filled with 100.00 ml tap water. In the first beaker, 1.00 ml of $NaNO_3$ 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 $NaSiO_3.5H_2O$ 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 NaH_2PO_4 solution was added in excess, the seventh beaker, 0.50 ml of $Na_2EDTA.2H_2O$ 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_2SiO_4	1.00	4.68	368 ↑↑
Iron citrate	1.00	3.74	274 ↑
Vitamin	1.00	4.04	304 ↑↑
NaH_2PO_4	1.0020	2.75	175 ↓
Na_2EDTA	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 CO_2 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, CO_2 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 CO_2 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 $NaNO_3$ solution, 1.00 ml of trace metal solution, 1.00 ml of $NaSiO_3.5H_2O$ solution, 1.00 ml of iron citrate solution, 1.00 ml ofvitamin solution, 0.50 ml of NaH_2PO_4 and 0.50 ml of $Na_2EDTA.2H_2O$ solutions were added, to each beaker. One gram of algae was added to each beaker and CO_2 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 CO_2 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 $NaNO_3$ solution, 1.00 ml of trace metal solution, 1.00 ml of $NaSiO_3.5H_2O$ solution, 1.00 ml of iron citrate solution, 1.00 ml of vitamin solutions and 0.50 ml of NaH_2PO_4 and $Na_2EDTA.2H_2O$ solution were added to each beaker.

One gram of algae was added to the beakers. The algae were supplied with CO_2 supplement and exposed to the sunlight simultaneously, for 30, 60 and 90 min, respectively. The growth rate in each beaker was observed.

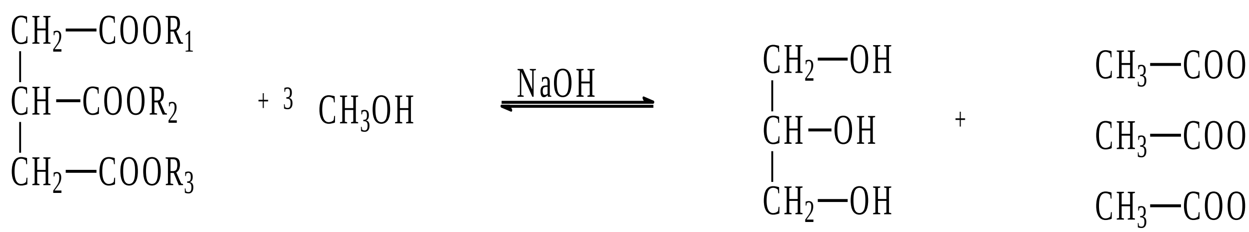
The effect of available space on the growth rate

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 $NaNO_3$ solution, 20.00 ml of trace metal solution, 20.00 ml of $NaSiO_3.5H_2O$ solution, 20.00 ml of iron citrate solution, 20.00 ml of vitamin solution, 5.00 ml NaH_2PO_4 solution and 10.00 ml of $Na_2EDTA.2H_2O$ 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 CO_2 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.



Triglyceride (oil or fat) Methanol Glycerol Methyl Esters

R1–3 are hydrocarbon groups (Biodiesel)

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 bottom layer was drained into a vial and stored. The biodiesel was washed three times with water. The obtained biodiesel was heated at 54°C for 20 min to evaporate all the residual water. Approximately 0.20 ml of biodiesel was obtained and it was then stored in a vial. The extraction and the transesterification processes were repeated several times using the method described by Hossain and Salleh (2008).

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 Na_2SiO_4, trace metals, Na_2EDTA and excess vitamin solution, the increase was above 300%. It was also observed that the beaker that was supplied with CO_2 (without simultaneous sunlight exposure) for one hour and the beaker with excess NaH_2PO_4 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 CO_2 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 CO_2 for certain times daily, for 25 days, showed that the growth rate is directly proportional to the increase of sunlight and CO_2 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.

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 CO_2 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 CO_2 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 Na_2SiO_4, trace metals, Na_2EDTA and excess vitamin solution, the increase was above 300%. It was also observed that in the experiment which was supplied with CO_2 (without simultaneous sunlight exposure) for one hour and the experiment with excess NaH_2PO_4 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 simultaneous exposure to sunlight and CO_2 for certain times daily, for 25 days, showed that the growth rate is directly proportional to the increase of simultaneous sunlight and CO_2 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

Bastianoni S, Coppola F, Tiezzi E, Colacevich A, Borghini F, Focardi S. 2008. Biofuel potential production from the Orbetello lagoon macroalgae a comparison with Sunflower feedstock. Biomass Bioenergy 10:1-10.

Campbell MN (2008). Biodiesel as a renewable source for liquid fuel. Gueph Eng. J. 1:2-7.

Chisti Y (1980). An unusual hydrocarbon. J. Ramsay Soc. 27-28, 24-6.

Chisti Y (1981). An unusual hydrocarbon. J. Ramsay Soc. pp. 24-26.

Chisti Y (2007). Biodiesel from microalgae. Biotechnol. Adv. 25:294-306.

Chisti Y (2008). Biodiesel from microalgae beats bio-ethanol. Trends Biotechnol. 26:126-31.

Dayananda C, Sarada R, Bhattacharya S, Ravishankar GA (2005). Effect of media and culture conditions on growth and hydrocarbon production by *Botryococcus braunii*. Process Biochem. 40:3125-3131.

Demirbas AH, Demirbas I (2007). Importance of rural bioenergy for developing countries. Energy Convers. Manag. 48: 2386-2398.

Energy Assessment, Preface UNDP (2008). United Nations Development Programme, New York, USA, pp: 219-272. Am. J. Biochem. Biotech. 4 (3):250-254.

Gavrilescu M, Chisti Y (2005). Biotechnology a sustainable alternative for chemical industry. Biotechnol. Adv. 23:471-9.

Goldemberg J (2000). World Energy Assessment, Preface.United Nations Development Programme, New York, NY, USA.

Guillard RRL, Ryther JH (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonudla confervacea* Cleve. Canada. J. Microbiol. 8:229-239.

Haag AL (2007). Algae bloom again. Nature 447(31): 520-521.

Hossain ABMS, Salleh A (2008). Biodiesel fuel production from algae as renewable source of energy. Am. J. Biochem. Biotechnol. 4(3):250-254.

Hsueh HT, Chu H, Yu ST (2007). A batch study on the bio-fixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot spring and marine algae. Chemosphere 66(5):878-886.

Huntley ME, Redalje DG (2007). CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitigat. Adapt. Strat. Global Change. 12:573-608.

Jacob-Lopes E, Lacerda LMCF, Franco TT (2008). Biomass production and carbon dioxide fixation by *Aphanothece microscopica* Nageli in a bubble column photobioreactor. Biochem. Eng. J. 40: 27-34.

Kapdan IK, Kargi F (2006). Bio-hydrogen production from waste materials. Enzyme Microb. Technol. 38:569-82.

Klass LD (1998). Biomass for Renewable Energy, Fuels and Chemicals, Academic Press, New York. pp. 1-2.

Kulkarni MG, Dalai AK (2006). Waste cooking oil-an economical source for biodiesel: A review. Ind. Eng. Chem. Res. 45: 2901-2913.

Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N (2008). Biofuels from microalgae. Biotechnol. Prog. 24:815-820.

Nagle N, Lemke P (1990). Production of methyl ester fuel from microalgae. Appl. Biochem. Biotechnol. 24-5:335-61.

Narendra MV, Shakti M, Amitesh S, Bhartendu NM (2010). Prospective of biodiesel production utilizing microalgae as the cell factories: A comprehensive discussion. Afr. J. Biotechnol. 9(10):1402-1411.

Ono E, Cuello J (2006). Feasibility assessment of microalgal carbon dioxide sequestration technology with photobioreactor and solar collector. Biosyst. Eng. 95(4): 597-606.

Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick Jr WJ, Hallett JP, Leak DJ, Liotta CL, Mielenz JR, Murphy R, Templer R, Tschaplinski T (2006). The path forward for biofuels and biomaterials. Science 31:484-489.

Salisbury FB, Ross CW (1992). Plant physiology (4^{th} Edition). Wadworth, Inc.

Sawayama S, Inoue S, Dote Y, Yokoyama SY (1995). CO_2 fixation and oil production through microalgae. Energy Convers Manag. 36:729-31.

Sazdanoff N (2006). Modeling and simulation of the algae to biodiesel fuel cycle. Report, Department of Mechanical Engineering, The Ohio State University, United States.

Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B (2008). Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenerg. Res. 1:20-43.

Schneider D (2006). Grow your own? Would the wide spread adoption of biomass-derived transportation fuels really help the environment. Am. Sci. 94:408-409.

Shay EG (1993). Diesel fuel from vegetable oils: Status and opportunities. Biomass Bioenergy 4:227-242.

Spolaore P, Joannis-cassan C, Duran E, Isambert A (2006). A commercial application of microalgae. *J. Biosci. Bioeng*. 101, 87-96.

Tan K., Lee K., Mohamed A. and Bhatia S. 2009. Palm Oil: addressing issues and towards sustainable development. Renew. Sustain. Energy Rev. 13(2):420-427.

Turkenburg WC (2000). Renewable energy technologies. In: Goldemberg, J. (Ed). World

Wagner L (2007). Biodiesel from Algae oil. Research report, MORA associates from www.fao.org/uploads.