LIPID PRODUCTION FROM MICROALGAE AS A PROMISING CANDIDATE FOR BIODIESEL PRODUCTION

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Abstract

Recently, several strains of microalgae have been studied as they contain high lipid content capable to be converted to biodiesel. Fresh water microalgae *Chlorella vulgaris* studied in this research was one of the proof as it contained high triacyl glyceride which made it a potential candidate for biodiesel production. Factors responsible for good growing of microalgae such as CO_2 and nitrogen concentration were investigated. It was found that total lipid content was increased after exposing to media with not enough nitrogen concentration. However, under this nitrogen depletion media, the growth rate was very slow leading to lower lipid productivity. The productivity could be increased by increasing CO_2 concentration. The lipid content was found to be affected by drying temperature during lipid extraction of algal biomass. Drying at very low temperature under vacuum gave the best result but drying at 60^oC slightly decreased the total lipid content.

Keywords: biodiesel, lipid, microalgae, nitrogen concentration, productivity

1. Introduction

Microalga is a photosynthetic microorganism that is able to use the solar energy to combine water with carbon dioxide to create biomass. Because the cells grow in aqueous suspension, they have more efficient access to water, CO_2, and other nutrients. Microalgae, growing in water, have fewer and more predictable process variables (sunlight, temperature) than higher plant systems, allowing easier extrapolation from one site, even climatic condition, to others. Thus, fewer site-specific studies are required for microalgae than, for example, tree farming. Also, microalgae grow much faster than higher plants and require much less land areas. However, the utilization of microalgae to overcome global warming is not enough without utilizing an algal biomass before degradation.

There are several ways to make biodiesel, and the most common way is transesterification as the biodiesel from transesterification can be used directly or as blends with diesel fuel in diesel engine [1-2].

Fatty acid methyl esters originating from vegetable oils and animal fats are known as biodiesel. Biodiesel fuel has received considerable attention in recent years, as it is a biodegradable, renewable and non-toxic fuel. It contributes no net carbon dioxide or sulfur to the atmosphere and emits less gaseous pollutants than normal diesel [3-5]. High dependence on foreign oil, especially transportation sector, gives rise to the importance of producing biodiesel for the sake of national energy security.

Microalgae have been suggested as very good candidates for fuel production because of their advantages of higher photosynthetic eficiency, higher biomass production and faster growth compared to other

Table 1. Several Lipid Producing Microalgae

Strain	Spesies	Triolein equivalents ($mg \cdot L^{-1}$) exponential growth	Triolein equivalents ($mg \cdot L^{-1}$) N deficient growth
NITZS54	Nitzschia Bacillariophyceae	8	1003
ASU3004	Amphora Bacillariophyceae	9	593
FRAGI2	Fragilaria Bacillariophyceae	6	304
AMPHO27	Amphora Bacillariophyceae	38	235

energy crops [6-7]. Microalgae systems also use far less water than traditional oilseed crops. For these reasons, microalgae are capable of producing more oil per unit area of land, compared to terrestrial oilseed crops. Microalgae are very efficient biomass capable of taking a waste (zero energy) form of carbon (CO_2) and converting it into a high density liquid form of energy (natural oil). Table 1 gives several lipid producing microalgae capable to produce biodiesel [8].

The present research aimed to produce lipid contained in fresh water microalgae *C. vulgaris* in a closed fermentor. The effect of CO_2 concentration and nitrogen concentration on lipid content were investigated as well effect of drying temperature during lipid extraction.

2. Methods

Materials
A microalgal strain of *C. vulgaris* was kindly provided by Prof. Hong-Nong Chou of The Institute of Fisheries Science, National Taiwan University, Taiwan. All solvents and reagents were either of HPLC grade or AR grade. All other chemicals used were obtained from commercial sources.

Medium and cultivation condition
The normal nutrition medium for cultivation of *C. vulgaris* was made by adding 1 mL of each of IBI (a), IBI (b), IBI (c), IBI (d), and IBI (e) to 1 L distilled water. IBI (a) contained , per 200 mL: $NaNO_3$, 85.0 g; $CaCl_2 \cdot 2H_2O$, 3.70 g. IBI (b) contained, per 200 mL: $MgSO_4 \cdot 7H_2O$, 24.648 g. IBI (c) contained, per 200 mL: KH_2PO_4, 1.36 g; K_2HPO_4, 8.70 g. IBI (d) contained, per 200 mL: $FeSO_4 \cdot 7H_2O$, 1.392 g; EDTA tri Na, 1.864 g. IBI (e) contained , per 200 mL: H_3BO_3, 0.620 g; $MnSO_4 \cdot H_2O$, 0.340 g; $ZnSO_4 \cdot 7H_2O$, 0.057 g; $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$, 0.018 g; $CoCl_2 \cdot 6H_2O$, 0.027 g; KBr, 0.024 g; KI, 0.017 g; $CdCl_2 \cdot 5/2\ H_2O$, 0.023 g; $Al_2(SO_4)_3(NH_4)_2SO_4 \cdot 24H_2O$, 0.091 g; $CuSO_4 \cdot 5H_2O$, 0.00004 g; 97% H2SO4, 0.56 ml. This normal nutrition medium resulted in a nitrogen content of 70.02 mg/L medium. The nitrogen depletion medium was provided by eliminating the addition of IBI (a) to result in a medium with a nitrogen content of 0.02 mg/L medium.

Effect of nitrogen concentration
At first, cells of *C. vulgaris* were cultivated in 4 L normal nutrition medium and incubated batchwisely at 22°C. The system was aerated at an air flow rate of 6 L/min with or without the addition of pure CO_2 gas. The fermentor is agitated at 100 rpm. Four pieces of 18 W cool-white fluorescent lamps are arranged vertically, at a 20 cm distance from the surface of fermentor to provide a continuous light to the system. This gave an average light intensity of 30 $\mu E/m^2 \cdot s$. The optical density of cells was measured at 682 nm every 24 hr using UV-530 JASCO Spectrophotometer, Japan. Cells were harvested at the end of linear phase, i.e. at a cell concentration of about 1.1 x 10^7 cells/mL. To investigate the effect of nitrogen depletion, 1 L of culture from the end of linear phase was diluted by adding 3 L nitrogen depletion medium and the cultivation continued for 7 and 17 days at which time the cells were harvested and the lipid content as well as lipid productivity was measured. Other conditions of incubation such as light intensity, pure CO_2 gas flow rate and temperature were all the same as the corresponding normal nutrition condition.

Effect of CO_2 concentration
The effect of CO_2 concentration on lipid content, lipid composition and productivity was investigated by varying the CO_2 concentration. At first, the culture was aerated under air flow rate of 6 L/min without additional CO_2. By taking into account the CO_2 content in air of about 0.03%, this condition resulted in about 2 mL/min CO_2 as carbon source. The next batch was conducted under the same air flow rate with the addition of 20, 50, 100, and 200 mL/min pure CO_2 gas, or about 0.33, 0.83, 1.67, and 3.33% CO_2, respectively.

Lipid extraction
Dry extraction procedure according to Zhu [9] was used to extract the lipid in microalgal cells. Typically, cells were harvested by centrifugation at 8500 rpm for 5 min and washed once with distilled water. After drying the samples using freeze drier, the samples were pulverized in a mortar and extracted using mixture of chloroform:methanol (2:1 v/v). About 50 mL of solvents were used for every gram of dried sample in each extraction step. After stirring the sample using magnetic stirrer bar for 5 h and ultrasonicated for 30 min, the samples were centrifuged at 3000 rpm for 10 min. The solid phase was separated carefully using filter paper (Advantec filter paper, no. 1, Japan) in which two pieces of filter papers were applied twice to provide complete separation. The solvent phase was evaporated in a rotary evaporator under vacuum at 60°C. The procedure was repeated three times until the entire lipid was extracted. The effect of drying temperature was investigated in this study.

Gas chromatography analysis
Sample was dissolved in ethyl acetate and 0.5 µL of this was injected into a Shimadzu GC-17A (Kyoto, Japan) equipped with flame ionization detector using DB-5HT (5%-phenyl)-methylpolysiloxane non-polar column (15 m x 0.32 mm I.D); Agilent Tech. Palo Alto, California). Injection and detector temperature both were 370°C. Initial column temperature was 240°C, and the temperature was increased to 300°C at a temperature gradient of 15°C/min.

3. Results and Discussion

Effect of CO_2 concentration on growth

Sobczuk *et al*. [10] reported that the yield of biomass increased significantly when the CO_2 molar fraction in the injected gas was reduced. They also showed that with less CO_2 in the injected gas, the O_2 generation rate and the CO_2 consumption rate were greater. Riebesell and his co workers [11] studied the effect of varying CO_2 concentration on lipid composition. They found that increasing CO_2 concentration of up to 1% of air will increase lipid produced by algae.

Figure 1 shows the growth of algae under different CO_2 concentration. The figure shows that increasing CO_2 flow rate until 50 mL/min enhanced the growth tremendously. Further increase of CO_2 may result in decreasing the growth rate. Table 2 shows the pH range under different CO_2 concentration. Higher CO_2 flow rate decreased the pH but during nitrogen starvation, the pH was practically stable at around 7. As can be seen from Figure 1, at CO_2 flow rate of 200 mL/min, the growth was once very slow with pH dropped to about 5. But, after two days, the growth increased greatly indicating that the algae recovered from low pH due to exposing at very high CO_2 concentration. At this condition, the pH was monitored to increase from about 5 to 6.4 and constant around this value which was the same pH range as that using lower CO_2 flow rate. As the growth recovered at the same time during the gradual increase of pH, it was evidence from this result that the microalgae *C. vulgaris* could survive under low pH albeit the growth was slow. Iwasaki *et al*. [12] reported the similar behavior of green algae *Chlorococcum littorale* in which under sudden increase of CO_2, activity of algae decreased temporarily and then recovered after several days. The fact that *C. vulgaris* can survive at wide range of pH from 5 to above 8 was beneficial in considering of applying the algae in any conditions such as very low pH under direct flue gas from power plant or higher pH when exposed to not enough CO_2 source.

Effect of nitrogen depletion on lipid content and productivity

Figure 2 shows the lipid content obtained at the end of linear phase during normal nutrition and the results were compared with lipid content obtained during nitrogen starvation. Period of incubation during normal nutrition was also varied to investigate the difference. Figure 2 shows that lipid content obtained after 20 d was higher than that obtained after 15 d. This was due to longer incubation time which led to less nitrogen concentration in the medium. Figure 2 also shows that longer time of nitrogen starvation obviously resulted in higher accumulation of lipid inside the cells.

Figure 3 shows the lipid productivity obtained during this period of time. Typical calculation of productivity was given in Table 3. As shown in this table, cell concentration obtained after 20 days incubation was significantly higher than that obtained after 15 d which led to higher amount of dried algal sample for lipid consequence, lipid productivity obtained after 17 d nitrogen depletion was higher since total time required for incubation was shorter. This 17 d period of normal nutrition was employed for further investigation.

Figure 2 and 3 also reveals that higher lipid productivity can be obtained by varying not only the length of nutrient starvation but also the length of normal nutrition.

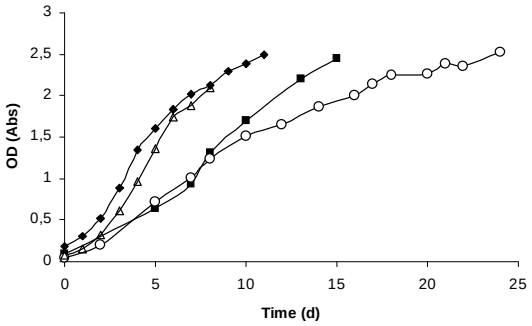

Figure 1. Growth of Microalgae Under Various CO_2 Flow Rrate of (○) 0 mL/min, (■) 20 mL/min, (◆) 50 mL/min and (△) 200 mL/min, all of which Supplied with an Air Flow Rate of 6 L/min

Table 2. Range of pH Measured Under Different CO_2 Concentration

$[CO_2]$ mL/min	pH	
	Normal Nutrition	N depletion
0	6.86 – 8.33	7.49 – 8.30
20	6.74 – 7.15	6.88 – 7.00
50	6.16 – 7.01	6.40 – 6.90
200	5.44 – 6.44	6.01 – 6.30

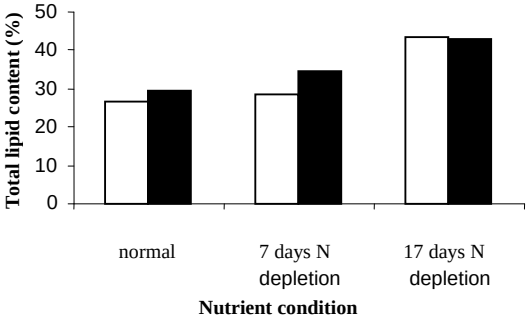

Figure 2. Lipid Content in Microalgae at Various N Condition. Incubation Time Under Normal Nutrition was Conducted for (□) 15 d and (■) 20 d

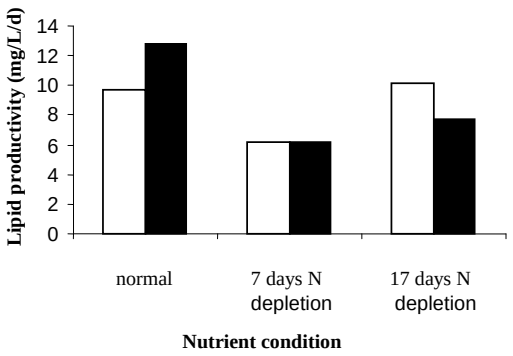

Figure 3. Lipid Productivity by Microalgae at Various N Condition. Incubation Time Under Normal Nutrition was Conducted for (□) 15 d and (■) 20 d

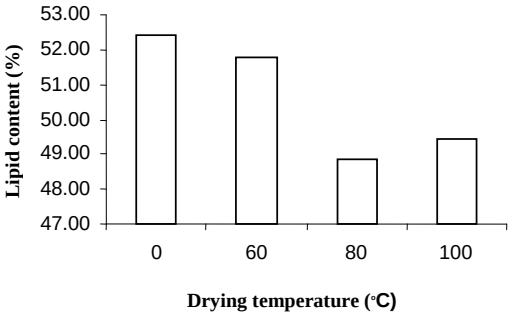

Figure 4. Lipid Content at Various Drying Temperature

Table 3. Typical Information Required to Calculate Lipid Productivity

Parameters	Incubation time	
	15 d	20 d
Cell concentration	1.1 x 10^7 cell·mL^{-1}	1.3 x 10^7 cell· mL^{-1}
Biomass/mL culture	0.55 mg· mL^{-1}	0.86 mg· mL^{-1}
Total lipid content	26.71%	29.53%
Lipid productivity	9.75 mg L^{-1}· d^{-1}	12.77 mg L^{-1}· d^{-1}

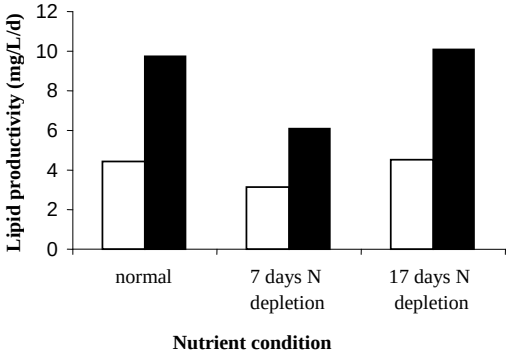

Figure 5. Lipid Production at Various CO_2 Flow Rate of (□) 0 and (■) 20 mL/min

Effect of drying temperature during lipid extraction
Figure 4 shows the effect of drying temperature on the lipid content. Heating at 60°C resulted in a slight decrease of lipid content but when heating was conducted under 80°C or higher temperature, the lipid content decreased significantly.

Effect of CO2 concentrantion on lipid productivity
The effect of CO_2 on growth as given in Figure 1 correlates directly to the lipid productivity since growth was enhanced tremendously by increasing the CO_2 concentration. Effect of CO_2 concentration on lipid productivity was given in Figure 5.

As shown in Figure 5, under all CO_2 concentrations, the lipid content tend to increase when the algae was exposed to nitrogen starvation condition. Similar with the results obtained in Figure 3, exposing at nitrogen starvation condition once resulted in decreasing the lipid productivity. This was caused by the slow growth of algae under nitrogen depletion. However, exposing at longer time of nitrogen depletion (17 days) resulted not only in higher lipid content but also in increasing the lipid productivity at about the same or even higher than lipid productivity at the end of normal nutrient.

4. Conclusion

Fresh water microalgae *C. vulgaris* was a good candidate for Biodiesel production due to its lipid content in addition to its easy growth. It was found that cultivating in nitrogen depletion media will result in the accumulation of lipid in microalgal cells. Although lipid productivity was slow under nitrogen starvation due to slow growth rate of algae, its lipid productivity during nitrogen depletion could be higher than that obtained at the end of linear phase during normal nutrition. The drying temperature during lipid extraction from algal biomass was found to affect the lipid content. Drying at 60°C only slightly decrease the lipid content.

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