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Oil yield of green microalgae isolated from ponds around Banda Aceh City

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Abstract. Study on CO_2 fixation for the growth of oil-produced green microalgae by cultivating the algae in illuminated glass containers was conducted. Green algae were isolated from some water pond samples around Banda Aceh. The samples contain mixed biomasses. The types of microalgae, as the results of the isolation was identified, and then cultivated in the CO_2 bubbled containers. The algae growth and oil yield were observed under different mediums (modified Detmer and modified CHU-13) and illumination (2 x 8 watts and 4 x 8 watts). Tanjong Selamat samples were used throughout this research based on initial screening stage. The two medium used led to different growths of the algae; the Detmer medium giving higher growth rate. Faster growth rates were found for cultivation using modified Detmer medium and 4 x 8 watts illumination. The oil yield was determined by solvent extraction method. Oil yield using the CHU-13 medium was 88.5%, much higher than the yield of Detmer medium sample 55.4%. It shows the potential use of local green microalgae to produce alternative bio-oil.

Key words: microalgae, biofuel, CO_2 fixation, oil yield

Introduction

Microalgae has been known very helpful in the prevention of global warming through CO_2 fixation. Like plants, algae require sunlight, carbóndioxide and water to grow. Photosynthesis is an important bio-chemical process in which algae convert the energy of sunlight to chemical energy. They are the most efficient plants in capturing and utilizing solar energy from CO_2 for their photosynthesis. To reduce greenhouse effect, wasted CO_2 from the industrial output is accommodated and utilized for the growth of microalgae. Indonesia has hundreds species of microalgae but not much explored for its potential to capture CO_2 and possible production of biofuel (Atmadja et al.., 1996).

Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. Algae contain anything between 2% and 40% of lipids or oils, depending on strain and culture environment. Producing biodiesel from algae provides the highest net energy because converting oil into biodiesel is much less energy-intensive than methods for conversion to other fuels (such as ethanol or methane). This characteristic has made biodiesel the favourite end-product from algae (Spolaore et al.., 2006). Producing biodiesel from algae requires selecting high-oil content strains, and devising cost effective methods of harvesting, oil extraction and conversion of oil to biodiesel. Cost-effective algal cultivation is a key requisite for success algal biofuel production. However, such cultivation of the right strain of algae, especially microalgae, in the right environment and media is a key challenge facing algae fuel companies (Oilgae, 2008).

In this research, therefore, we were interested to search any indigenous microalgae strain or colony potential to produce oil. Indigenous microalgae is expected more adaptable to the local environment when they are cultured in open water bodies. The growth and oil content of the microalgae were observed under cultivation in two medium, namely modified CHU-13 medium and modified Detmer medium which promote grwowth of two differet oily algae strains *Botryococcus brunni* and *Chlorella vulgaris*, respectively.

Materials and Methods

Liquid Medium

Two types of medium were prepared, namely modified CHU-13 and modified Detmer with additional micronutrients for the growth of microalgae. Table 1 and 2 show chemical composition of the medium and the nutrient, respectively. The pH of the medium is set around 7.5.

Table 1. Chemical composition of medium (dissolved in 1000 ml aqueous solution)

No	CHU-13 (Largeau et al.., 1980)		Detmer (Maeda et al.., 1998)	
	Chemical	Weight (g)	Chemical	Weight (g)
1	$NaNO_3$	4,0	$NaNO_3$	4,0
2	Na_2HPO_4	0,08	Na_2HPO_4	0,08
3	$MgSO_4$ heptahydrate	0,2	$MgSO_4$ heptahydrate	0,1
4	$ZnCl_2$ dihydrate	0,107	$ZnCl_2$ dihydrate	0,01
5	Ferroues sulphate	0,02	Ferroues sulphate	0,002
6	Citrate acid	0,1	NaCl	0,01
7	$CoCl_3$ dihydrate	0,107	Na_2-EDTA	0,01
8	Micronutrien	1 ml	Micronutrien	1 ml

Table 2. Micro-nutrient composition according to Largeau et al.. (1980) (dissolved in 1000 ml aqueous solution)

No	Chemical	Weight (g)
1	H_3BO_3	0,00572
2	$ZnSO_4$ heptahydrate	0,00044
3	$MnCl_2$ tetrahydrate	0,00367
4	Na_2MoO_4	0,000084
5	$CuSO_4$ pentahydrate	0,00016
6	0,072N H_2SO_4	one drop

Algae Culture

The algae samples were collected from different water ponds of Banda Aceh and Aceh Besar and cultured in the modified CHU-13 medium and the modified Detmer medium. The algae were subjected to purification by serial dilution. The colonies were isolated and inoculated into each liquid medium and incubated at room temperature under light illumnation with 24 hrs light photoperiod. The purity of the culture was ensured by repeated dilution and by regular observation under microscope. The shape and size of the microalgae showed different types of microalgae. Samples collected in Tanjong Selamat pond produced a good culture simple, therefore, this isolated culture was used throughout the research. Characteristics of the sample locations were pH about 7.7 to 8.5 and salinity level of 0.01 o/oo - 0.02 o/oo.

Experimental Procedure

A time course study was carried out on the algae growth. The experiment was carried out in Erlenmeyer flasks of 150 ml capacity, containing 40 ml liquid medium bubbled with CO_2 for a period of 2 weeks. The culture flasks were inoculated (10% v/v) and incubated at 25 ± 1ºC under two or four 8 watts flouresence lights with 24 hrs light cycle. Cultures were harvested and dry biomass was estimated at 2 days of intervals. The final biomass samples were also analyzed for oil content.

Sample Analysis

Biomass content was determined by gravimetric technique. The cultures were harvested and the cells were washed with distilled water after centrifugation at 5000 rpm. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms

of dry weight. A calibration curve was developed to correlate biomass content and spectroscopic absorbance. The Experiment samples were analyzed using DR/2010 Hach Spectrophotometer at a wavelength of 680 nm. Oil content is defined as solvent extractable fraction in n-hexane by soxhlet technique. Sample separated by soxhlet extraction was dried in rotary evaporator.

Results and Discussion

Effect of CO_2 Aeration

Figure 1 shows that CO_2 and O_2 aeration resulted in significantly different growth pattern with CO_2 aeration giving higher biomass content. On day 6^{th} of cultivation, the biomass content reached the highest value of 1.0263 g-BK/L. It indicates clearly that the fixation of CO_2 produced better growth due to the increase in photosynthesis (Rao et al.., 2007; Moroney and Somanchi**,** 1999). Similar trend is also given using the Detmer medium as shown in Figure 2. At the highest point, biomass content using Detmer medium of 1.2929 g-BK/L is slightly higher than one of the CHU-13 medium.

Based on the microalgae growth pattern there is no phase lag. This occurs because inoculated microalgae were taken from cultures that are in log phase, so they did not experience the phase lag. Microalgae has actively performed cell division in maximum speed and constantly followed the logarithmic curve. This phenomenon was marked by the culture color was greener than at the beginning of the culture.

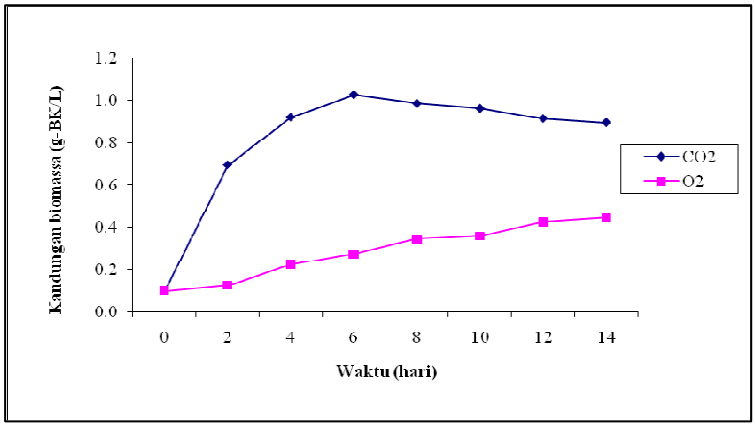

Figure 1. Comparison of the growth of microalgae under different aeration using modified CHU-13 medium (lamps 4 x 8 watts; T = 30°C; pH 7.5).

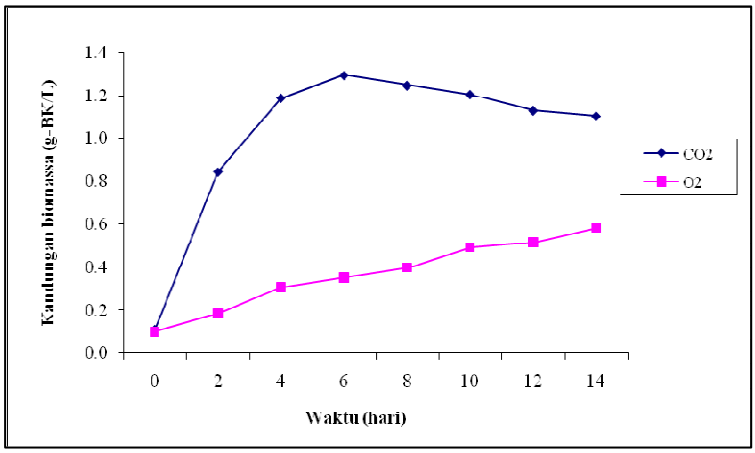

Figure 2. Comparison of the growth of microalgae under different aeration using modified Detmer médium (lights 4 x 8 watts; T = 30°C, and pH 7.5).

Effect of Cultivation Medium

Use of different medium resulted in similar growth profile, but use the Detmer medium yielded higher biomass content as given in Figure 3. It also shows the log phase begins the day 0 to day-to-1, a decline phase in the growth rate started on day 2 to day 4, the stationary phase occurred on day 5 to day-to-6 and the phase toward the deaths occurred in day-to-7. The decrease of growth rate began on day 4 to day 7 because of substrate limitations, population density and availability of CO_2 was lower. In addition, deposits of toxic metabolism can slow down the growth so it faced the stationary phase. During this stationary phase, cell division still occurs despite the decreased nutrient, but cell division could still occur. The culture color was changing during growth. The change occurred from the beginning to the end of cultivation i.e. starting from a clear green color, dark green, translucent green back to the formation of green precipitate contained in the bottom of the culture.

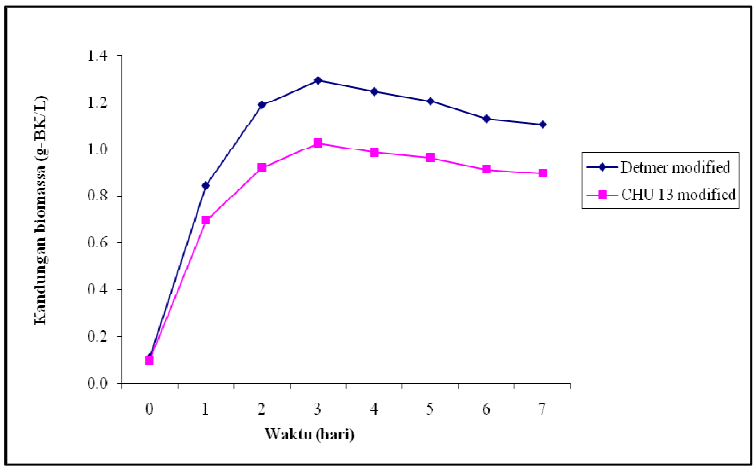

Figure 3. Growth of microalgae using different medium (lights 4 x 8 Watt; CO_2 flow rate 2 L/min; T = 30°C; pH = 7.5)

Light Effect on the Growth of Microalgae

Figure 4 shows the intensity of light affected the growth of green microalgae. Use of high intensity gave faster growth rate; 4 x 8 watts illumination reaching stasionary phase half of times one using 2 x 8 watts. High light intensity has intesified photosynthesis process since presence of light is requisite for the photosynthesis.

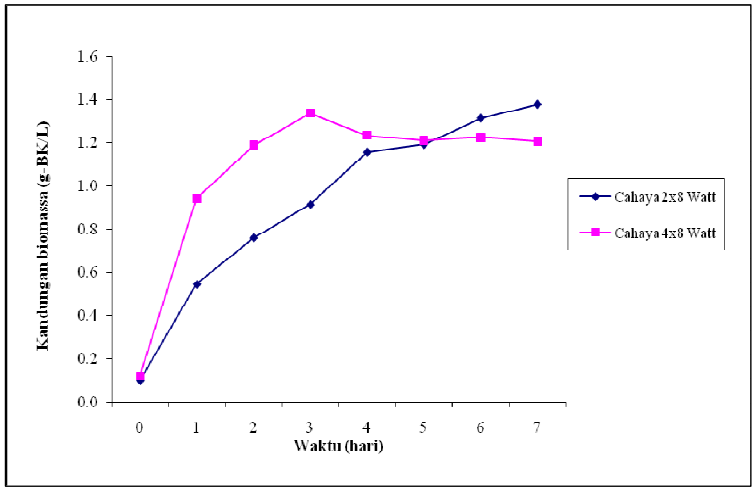

Figure 4. Growth of microalgae with different light intensity (CO_2 flow rate 2 L/min; modified Detmer médium; T = 30°C; pH = 7.5).

Oil Content of the Algae

Effect of medium on oil content of the microalgae is presented in Table 1. The results obtained oil or fat content of microalgae with a solvent n-hexane at 1.372 g/L and oil yield of 55.4% for modified Detmer medium while the modified CHU-13 medium obtained at 1.903 gr/L and oil yield of 88.5%. Oil content of algae was greater in Detmer modified medium than the CHU-13 one.

Table 1. Oil content of microalgae resulting from different cultivation condition.

No	Description	Weight of biomass	Weight of crude oil	Oil content
1	Detmer; CO_2 2 L/min	2,476 gr	1,372 gr	55.4%
2	CHU-13; CO_2 2 L/min	2,149 gr	1,903 gr	88.5%
3	Detmer; light 4x8 Watt	2,232 gr	1,773 gr	79.4%
4	Detmer; light 2x8 Watt	2,274 gr	1,191 gr	52.3%
5	Detmer; CO_2 2 L/min	2,557 gr	1,903 gr	74.4%
6	Detmer; O_2 2 L/min	1,623 gr	0,813 gr	50.1%

Conclusions

Green microalgae was succesully isolated from some water ponds and their growth and oil content were observed. Faster growth rates were found for cultivation using modified Detmer medium and illumination of 4 x 8 watts flourescence light. The oil yield was determined by solvent extraction method. Oil yield using the CHU-13 medium was 88.5%, much higher than the yield of Detmer medium sample 55.4%. It shows the potential use of local green microalgae to produce alternative bio-oil.

Acknowledgements

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