2011 International Conference on Environment and Industrial Innovation
IPCBEE vol.12 (2011) © (2011) IACSIT Press, Singapore

Investigation of Microalgae for High Lipid Content using Palm Oil Mill Effluent (Pome) as Carbon Source

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Abstract. Microalgae are reported as the potential resources to produce lipid from their biomass cell. This study is emphasize on the effect of palm oil mill effluent (POME) concentration as carbon source to increase the lipid content by limiting growth rate. The chosen of POME is important as it has enormous organic and nutrient content, even favorable for several types of microalgae species. The experiments were performed at room temperature under continuous illumination at intensity of ± 15 μmol/m^2/s for 20 days. An investigation of five selected strains of green microalgae is applied to compare at the certain biomass growth rate and maximum lipid content. This study showed that Chlorella sorokiniana is the predominant species because it has highest biomass and lipid productivity in diluted POME (250 mg/L) compared each other, with the value 8.0 mg/l/day, 28.27% respectively. The maximum chlorophyll production will be achieved at sixth days with value 0.586 mg/l.day for Chlorella sorokiniana. This paper propose that lipid production can be optimum was reached after sixth days.

Keywords: microalgae, POME, lipid

1. Introduction

Palm oil production is one of the major industries in Malaysia and which currently is the largest production in the world. The number of palm oil mills in Malaysia has increased tremendously; the total productions of crude palm oil in 2008 and 2009 are 17,734,441 and 16,044,874 tones, respectively (Malaysian Palm Oil Board., 2008, 2009). In the year 2008, at least 44 million tones of Palm Oil Mill Effluent (POME) was generated and it is expected to rise every year (Wu et al., 2010). Ponding system is still the most common POME treatment system, used by more than 85% of the palm oil mills in Malaysia (Ma and Ong, 1985). This may be due to the fact that large area of lands in Malaysia could be used as ponds area for POME treatment (Wu et al., 2010) and It can be observed that ponding system is more economically viable and have the capacity to tolerate a wider range of OLR (Poh and Chong, 2009). However, one problem was uncontrollable release of large amounts of CH_4 and CO_2 into atmosphere from open ponds, which may worsen the effect of global warming.

Within this context, a number of researchers proposed that a wastewater management based on the promotion of environmentally sound biotechnologies could be included as a part of the POME management in Malaysia in order to attain a sustainable development. Microalgae, is the only source that can be

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sustainably developed in the future (Ahmad et al., 2011). Microalgae have emerged as one of the most promising alternative sources of lipid for use in biodiesel production compared the conventional crops (Minowa et al., 1995, Singh and Gu, 2010, Ahmad et al., 2011, Huang et al., 2010).

First, the cultivation of microalgae does not need much land as compared to that of terraneous plants (Chisti, 2007). Second, microalgae grow extremely rapidly and many algal species are rich in oils (Xu et al., 2006). Oil levels typically in range of 20 -50% (Chisti, 2007). Third, the entire production process ranging from the cultivation of high lipid microalgae to the production of biodiesel from the microalgae oils has also been explored(Huang et al., 2010). This study is a first attempt to increase the lipid content in microalgae as a result of variations in substrate of POME, then to investigate five different species of microalgae: *Chlorella vulgaris, Chlorella pyrenoidosa, Chlorella sorokiniana, Botryococcus sudeticus*, and *Tetraselmis sp* cultivated in diluted POME for higher lipid production.

2. Material and Methods

2.1 Experimental procedure

POME was added as carbon source from existing facultative pond. The POME used in the batch experiments was previously settled for 1 hour and further diluted with BBM with selected COD concentrations in 250 mg/l (data not shown). The most favorable COD concentration will be investigated with different strains of microalgae like C*hlorella pyrenoidosa* (POME), *Chlorella sorokiniana* (UTEX 1602*), Botryococcus sudeticus* (UTEX 2629), and *Tetraselmis sp* (UTEX 2767). 1 ml chlorampenicol was added into each medium consist of POME as antibiotic and ensure that the culture would be free of contaminants. They were grown in the appropriate medium at room temperature under continuous illumination at intensity of ± 15 μmol/m^2/s for 20 days.

2.2 Microalgae and Medium

The strains of *Chlorella sorokiniana*, *Botryococcus sudeticus*, and *Tetraselmis sp* were provided by the Culture Condition of Algae at the University of Texas (Austin, Texas, USA). *Chlorella vulgaris* was collected from Korean Collection for Type Cultures (KCTC) Biological Resource Center (BRC) and *Chlorella pyrenoidosa* was obtained by screening local microalgae in POME. Five strains of microalgae were grown in different medium. *Chlorella vulgaris* and *Chlorella pyrenoidosa* were cultivated in Bold Basal Medium (Laura et al., 2005), *Chlorella sorokiniana* in Modified Bold 3N Medium, *Botryococcus sudeticus* and *tetraselmis sp* were grown in Soil Extract Medium as reported by UTEX (1953).

2.3 POME Collection

POME was collected from facultative ponds at FELDA palm oil mill in Bukit Besar, Johor Bahru. The samples were stored in plastic containers with proper label. For preservation, samples were refrigerated at about 4°C in order to any contamination and limit the activity of biodegradation process.

2.4 Antibiotic Preparation

Antibiotic chlorampenicol 99% was used in this experiment. Chlorampenicol 300 mg was dissolved in 10 ml stock solution. Ethanol 70% was prepared and act as solvent for chlorampenicol. Then syringe with 0.2 mm filter membrane was used to filter sterilize chlorampenicol solution. After filter sterilized, the antibiotic was added into synthetic medium to a final concentration of 30μg/ml. the antibiotic stock solution was sealed with parafilm and stored at 4^0C fridge until use (Eik Lee Fang., 2009).

2.5 Physical, Chemical and Biological Analyses

Samples were collected in 20 days for analysis of optical density (OD), chlorophyll content, chemical oxygen demand (COD), total suspended solid (TSS),volatile suspended solid (VSS), cell dry weight (CDW) and lipid content.

2.5.1 Optical Density

Optical density for biomass factor was determined at wavelength 600 nm using shimadzu 160 A.

2.5.2 Chlorophyll Content

8 ml of microalgae cells were harvested by centrifugation at 5000 rpm for 5 min and discarded the supernatant for chlorophyll *a* content. Put pellet in sonicator for 1 min and top up 1 ml 90% aqueous of acetone solution. The mixture of 3 ml with aqueous acetone and steep sample at 4^0C in the dark for 2 h. Next, the settled pellet has been separated by centrifuging in centrifuge for 5 min at 5000 rpm. Then, the pellet was transferred into a Cuvettes bottle and ready to measure. All analysis was followed in a standard method of APHA, (2005).

2.5.3 Chemical Oxygen Demand (COD)

Chemical oxygen demand for determination of organic matter was performed according to the standard methods for the examination of water and wastewaters (APHA, 2005).

2.5.4 Total suspended solid (TSS) and volatile suspended solid (VSS)

Microalgae biomass was obtained by filtering a sample through a pre-dried 0.45 µm Whatman filter paper followed by drying in oven for 1 h at 103 ^{0}C. Suspended volatile solids were obtained by incinerating the dried solids at 550 ^{0}C in furnace for 15 min. All analyses were measured by standard methods (APHA, 2005). Formula for TSS and VSS calculation followed by Metcalf and Eddy (2004).

2.5.5 Cell dry weight

The cultures were harvested by centrifugation at 4000 rpm, 15 min and the cells were washed with distilled water. Then the pellet was freeze dried. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms of dry weight (mg/L) (Rao et al., 2007).

2.5.6 Lipid content

The total lipids were extracted from microalgal biomass using a modified method of Bligh and Dyer (1959). Thereafter, the total lipids were followed by Yoo et al (2010) and measured gravimetrically (Yoo et al., 2010).

2.5.7 Kinetic and Yield Parameter (Converti et al., 2009)

- The specific growth rate was calculated by the equation:

$$\mu = \frac{1}{t}\ln\left(\frac{Xm}{X0}\right) \quad \text{..... (1)}$$

  Where Xm and Xo are the concentrations of biomass at the end and the beginning of a batch run, respectively, and t is the duration of the run.

- The lipid productivity was calculated by the equation:

$$v = \frac{Cl}{t} \quad \text{..... (2)}$$

  Where Cl in the concentration of lipids at the end of the batch run and t is the duration of the run.

- The yield of the microalgae lipids was calculated by the equation:

$$Y(\%) = \frac{W_L}{W_{DA}} \quad \text{.....(3)}$$

  Where W_L and W_{DA} are the weights of the extracted lipids and of the dry algae biomass, respectively.

3. Results and Discussion

3.1 Specific Growth

Five strains were tested for their ability to produce high lipid content in diluted POME at concentration 250 mg COD/l. Among of them, *chlorella sorokiniana* was the highest in terms of specific growth rate and

biomass productivity (see Table 1), when compared to the other species. The specific growth rate and biomass productivity of *chlorella vulgaris* was similar to that *Botryococcus sudeticus*, then biomass productivity of *Tetraselmis sp* was only 4.0 mg/l/day, which was about half of that of *Chlorella sorokiniana.* However, chlorella *pyrenoidosa* has the smallest one. The maximum biomass was obtained when the cell grown in 20 days, but the significant growth occurred in 6 days.

3.2 Lipid Content

As can be seen in Table 1, the total lipid contents for the microalgae cultured in this study ranged from 21.34% to 30.83% of the dry weight. The lipid productivity for *chlorella vulgaris* was lower than *Botryococcus sudeticus*, but the biomass productivity was higher than 5.3 mg/L/day. The total lipid content of C*hlorella sorokiniana, Chlorella vulgaris, Chlorella pyrenoidosa, Botryococcus sudeticus and Tetraselmis* were 28.27%, 21.34%, 21.51%, 30.83%, 25.69%, respectively. In this case, *Chlorella sorokiniana*, was the superior in terms of lipid content using diluted POME.

In a previous study (Liu et al., 2008), total lipid content representing 20-50% of the dry biomass weight were found to be quite common, and some microalgae even exceeded 80%. An increased lipid content also occurred in many microalgae as a response to different culture condition (Yoo et al., 2010). However, lipid production by microalgae is regulated by environmental factors, and to optimize them, a control is needed (Elsey et al., 2007).

Table 1 Main parameters of growth, biomass productivity and lipid content of microalgae in appropriate medium

Strains of microalgae	Specific growth rate (/day)	Biomass productivity (mg/l/day)	Percentage of Lipid (%)
Chlorella vulgaris	0.084	5.9	21.34
Chlorella pyrenoidosa	0.048	2.9	21.51
Chlorella sorokiniana	0.099	8.0	28.27
Botryococcus sudeticus	0.083	5.3	30.83
Tetraselmis sp	0.065	4.0	25.69

3.3 Substrate Consumption

Figure 1 shows the maximum chlorophyll production on microalgae was obtained at sixth day, and then it was decreased after those days for each microalgae. *Chlorella sorokiniana* could be achieving maximum chlorophyll production compared each other at 6 days, with value 0.586 mg/l.day and substrate consumption 152 mg/l.day. this curve describe that the heterotrophic condition would be reach after sixth days, because of microalgae can growth without supply CO_2 and sunlight more. Next, can decide that microalgae can produce high lipid content when the consumption of substrate increased but decreasing of chlorophyll production. In this case *Chlorella sorokiniana* consume substrate fastest than other strains of microalgae.

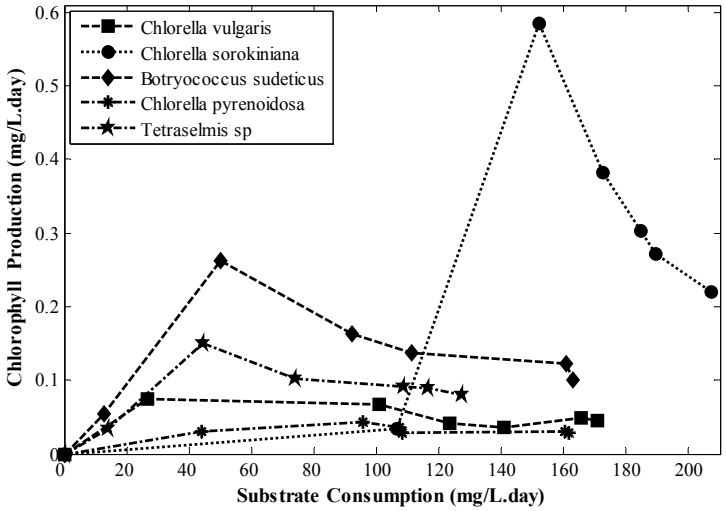

Figure 1. Chlorophyll production of microalgae based on substrate consumption

4. Conclusion

This study examined the growth of microalgae in diluted POME. Five strains of microalgae and different concentrations of substrate were used in this study. In the batch experiment, concentration of POME at 250 mg/l was a better medium for the five of microalgae species. In terms of cell growth, biomass and lipid content, *Chlorella sorokiniana* to be the most suitable species among of the other strains examined.

5. Acknowledgements

This research was supported by the Fundamental Research Grant Scheme (Vot 77520) and E-Science Fund (Vot 79387).

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