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KINETIC STUDY OF MICROALGAE BIOMASS GROWTH FOR TERTIARY TREATMENT OF PALM OIL MILL EFFLUENT (POME)

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ABSTRACT

In recent years, microalgae have been used to polish Palm Oil Mill Effluent (POME) from the conventional ponding treatment system. The substantial amount of inorganic nutrient and trace metal in POME is believed to encourage the growth of microalgae. The present research studied the kinetic of microalgae mixed culture in treating POME. 10% v/v of microalgae mixed culture was cultured in 10-100 %v/v autoclaved and centrifuged POME in conical flask under light irradiance of 6000 Lux. The supernatant of microalgae mixed culture was collected for BOD and colour (ADMI) determination. The results revealed that the mixed microalgae achieved the highest growth rate of 1.39 d^{-1} at 30 %v/v of POME at the minimum light penetration of approximately 2000 Lux. The corresponding biomass productivity was 0.61 g/L.d. A significant removal of BOD with efficiency of 50%-55% was obtained. Nevertheless, the colour removal efficiency was less than 20%, indicating that the microalgae are not effective in removing colour compound in POME.

Key words: Palm Oil Mill Effluent; Microalgae; Light Penetration; BOD; Colour.

1. INTRODUCTION

The palm oil industry is one of the world's most rapidly expanding industries in the world, especially in Malaysia. According to the statistic of world palm oil production in 2011, total world palm oil production reached 48.99 Million ton where the Malaysia was the second biggest palm oil produced country with total palm oil production 18.00 Million ton. Based on the statistic of Malaysian Palm Oil Board (MPOB), total 423 of palm oil mills in Malaysia have processed 99.436 million tonne of Fresh Fruit Bunches (FFB) in 2011. Yacob *et al.* (2005) estimated that approximately 0.50 - 0.75 tonnes of POME will be discharged from the mill for every tonne of FFB. Thus, total POME discharged to the river in 2011 was estimated in the range of 49.72-74.58 million tonne. The huge amount of palm oil production will results in the generation of substantial amount of polluting wastewater commonly known as Palm Oil Mill Effluent (POME). If this significant amount of the POME with high pollutant was discharged untreated, it will become one of the major sources of aquatic pollution in Malaysia.

According to Ma (2000), fresh POME which is generated by Palm Oil Mill is hot, acidic (pH between 4.0 to 5.0) and brownish colloidal suspension containing high concentration of organic matter, BOD (25,000 mg/L), COD (50,000 mg/L), total suspended solids (40,500 mg/L) and oil & grease (4,000 mg/L). Dark brownish colour is probably from polymerization of tannins and low molecular weight phenolic compound (Limkhuansuwan and Chaiprasert, 2010). Furthermore, discharge of colour effluent into river will inhibit the growth of aqua organism by reducing the penetration of sunlight and affect the photosynthetic activity (Neoh *et al.*2012).

In recent years, there were several studies reported on the improvement of POME treatment by adding microalgae treatment (Neoh *et al.*2012; Habib *et al.*, 1997; Phang and Ong, 1988) after the conventional ponding treatment system. Microalgae had been used for the POME treatment study mainly due to the substantial amount of inorganic nutrient and trace metal in POME which encouraged the growth of microalgae (Ma, 1999) and the ability of nitrogen and phosphorus removal (Shi *et al.*, 2007) by them. Neoh *et al.* (2012) had showed that the growing fungus cultures, *Aspergillus fumigatus* is effective in POME decolourisation. Limkhuansuwan and Chaiprasert (2010) had proved that the lactic

acid bacteria able to removed total phenolic compound where the compounds were directly proportional to the colour intensity of POME.

The present work has performed the kinetic study of the microalgae growth for the tertiary treatment of POME. The minimum light penetration of microalgae mixed culture in POME was determined. The BOD and colour removal efficiency were evaluate.

2. MATERIALS AND METHODS

2.1 Cultivation of Microalgae

a) 5-Liters of aerobic pond water mix with algae samples were collected from a private oil mill and transported to laboratory for isolation and cultivation.
b) 6 centrifuges tubes were filled with volume of 40 ml microalgae sample each and centrifuged at 5000 rpm and 25^0C for 15 minutes.
c) After removing the supernatant in centrifuge tubes, the microalgae cells were washed with autoclaved (at 121^0C for 20 minutes) ultrapure water.
d) Then, the cell in centrifuges tubes were suspended by using vortex mixer (rotated at 1000 rpm to homogenous suspension). The centrifuge-washing process had been repeated for six times to expel most of the microorganism presented in microalgae sample before the cultivation of algae sample.
e) Bold's Basal Medium (BBM) (recipe from Daphnia Research Group at University of Reading) had been used for microalgae cultivation.
f) Microalgae cultures were grown in 1L Erlenmeyer flaks with silicone stopper, and sparging with Air Pump Zone Z-80 in the wooden incubator box with fluorescent lighting source with light intensity at approximately 6000 Lux, and photoperiod 12:12 hour light/dark cycle.

2.2 POME Preparation

The POME sample was collected from Facultative Pond 3 and autoclaved at 121^0C for 15 minutes and centrifuged at 10,000 rpm

for 15 minutes to eliminate indigenous microbes and suspended solid material. After cooling down, the autoclaved POME was stored in the refrigerator at 4^0C.

2.3 Experiment

A microalgae mix culture cell density of 1 x 10^5 cells/ml was cultured in 0 to 100 % v/v POME and ultrapure water in 250 mL conical flask that was covered with silicone stopper, sparging with 4 L/min air loading Air Pump Zone Z-80 and placed in the wooden incubator box (as shown in Figure 1) with 6000 Lux and photoperiod 12:12 hour light/dark cycle. For each samples, the degree of light irradiance after transmitting through microalgae culture chamber was measured using lux meter. Sampling was done at the beginning and the end of seven days to observe microalgae growth.

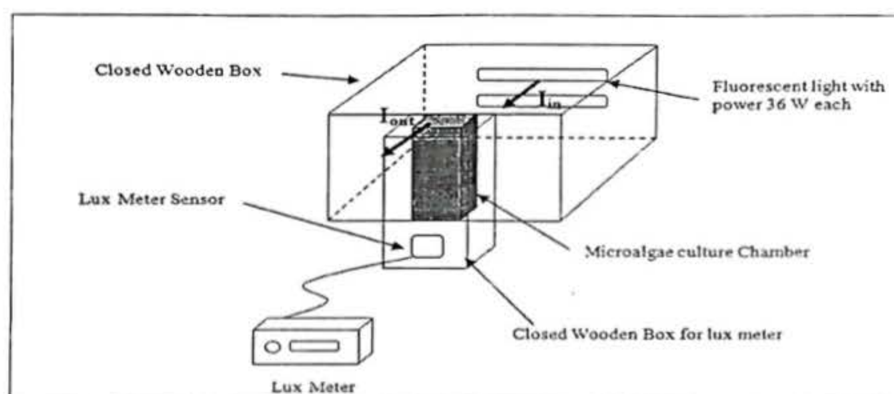

Figure 1: Experimental rig for light penetration

2.4 Kinetic Study on Microalgae Growth Rate

Kinetic study was carried out on the sample with the highest growth rate of microalgae to observe the growth profile of microalgae mix culture within seven days at time interval of 24 hours. For every 24 hours, 1 ml sample was being used for the determination of cell density by using Neubauer Haemocytometer. At day 7, 35ml of sample was centrifuged at 10,000 rpm, 25^0C for 15 minutes. The dry cell weight was determined by oven-drying of a sample at 100^0C for 2 days.

2.5 Analytical Methods

Analytical determination of BOD_5 and colour for supernatant were carried out in accordance with the Standard Method for the Examination of Water and Wastewater (APHA 1998). BOD_5 was analyzed on sample incubated for 5 days at 20^0C (APHA 5120B) while the colour was analyzed by using ADMI weighted ordinate method with HACH DR 20101 spectrophotometer.

2.6 Kinetic and Yield Parameter

The Monod kinetic model was used to describe the effect of light penetration on the microalgae growth rate. For light limited growth kinetic model, the maximum microalgae growth rate (μ_{max}) was expressed as below:

$$\mu = \frac{\mu_{max} L}{K_L + L} \qquad \text{eq. (1)}$$

where L = the light intensity that passed through the microalgae medium and K_L= the saturation constant for light intensity

For POME limited growth model, the light intensity was replaced by POME concentration in Eq.(1). The calculation of specific growth rate (μ) was shown as below where x is the cell density measured by using Neubauer Haemocytometer (Hadiyanto and Nur, 2012):

$$\mu = \frac{\ln x_t - \ln x_0}{t - t_0} \qquad \text{eq. (2)}$$

The biomass yield based on POME and light intensity (quantum yield) were calculated using Eq. (3) and (4) (Zijffers *et al.*, 2010).

$$\text{Biomass Yield} = \frac{\text{Biomass Concentration (g/L)}}{\text{POME Concentration (g/L)}} \qquad \text{eq. (3)}$$

$$\text{Quantum Yield} = \frac{C_x \times \mu \times V}{PFD_{in} \times A \times 3600 \times 10^{-6}} \text{(g mol/photons)} \qquad \text{eq. (4)}$$

Where C_x = the biomass concentration, V = the volume of culture, PFD_{in}= the photon flux density at inlet and A = the area of culture.

The colour removal efficiency was calculated by using the initial and final concentration of pigment in POME as shown in Eq. (5).

$$\text{Efficiency} = \frac{C_0 - C_f}{C_0} \times 100\% \qquad \text{eq. (5)}$$

3. RESULTS AND DISCUSSION

Light irradiance plays a very important role in determination of microalgae's life and it will affect the growth and photosynthesis of microalgae (Khoeyiz *et al.* 2011; Carvalho *et al.* 2010; Lopes *et al.*2009). Figure 2 shows the average light penetration values measured at various POME concentrations and the result obtained was stated that penetrated light value would be decreasing as the concentration of POME. Lignin, degraded product, tannin and humic acids were the main elements contributed to the colourisation of POME which were able to block the light radiance and affecting the light penetration (Neoh *et al.*, 2012). When POME was diluted, the colour intensity would also reduce and allowing more transmittance of light. Therefore, when the concentration of POME is low, the light penetration is higher.

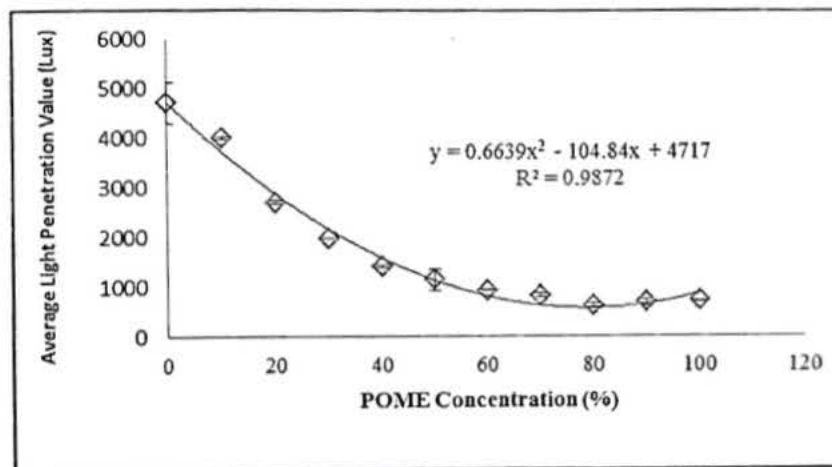

Figure 2: The average light penetration value for each POME concentration.

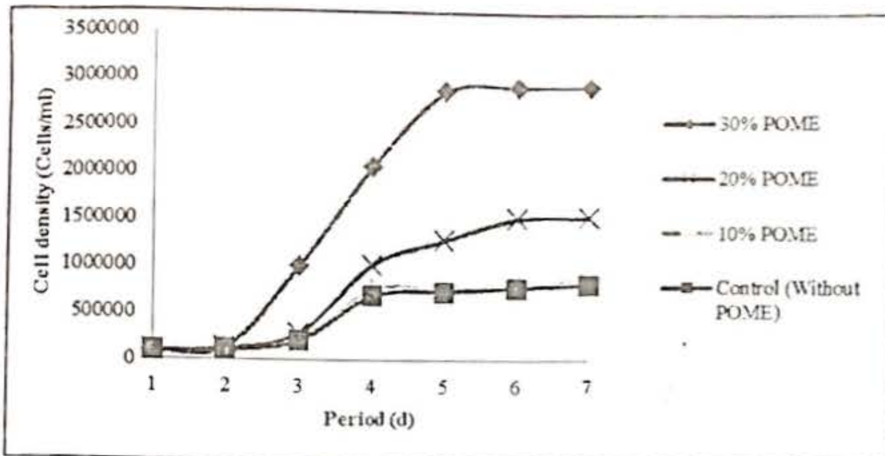

Figure 3: The growth profile of microalgae.

In the present study, the growth of microalgae mix culture was investigated using 0 to 30% v/v of POME and the experimental results are shown in Figure 3. When the POME concentration is more than 30% v/v, there was no significant of microalgae mix culture cell can be observed by microscope due to the darkness of the sample. The mix culture of microalgae experienced the highest growth rate at 30 % v/v POME. As denoted in Figure 1, the minimum light penetration was approximately 2000 Lux which corresponding to the light penetrated in 30% v/v of POME. Then, the kinetic study was performed using 0 to 30% v/v samples and the respective growth profile is portrayed in Figure 3. The lag phase of microalgae is approximately one day and it is followed by the exponential (growth) phase. The microalgae under the cultivation of 30% v/v experienced a longer exponential phase (three days) compare to the others. Eventually, the cells entered the stationary phase after four to five days.

The growth rate was rapid compared to the study of Hadiyanto and Nur (2012) where the microalgae, *Chlorella sp.* entered stationary phase after day 12. The high microalgae growth rate due to the competitive environment created in among the microalgae mix culture. Dauta *et al.* (1990) revealed the optimum light intensity and temperature for the growth of freshwater microalgae was in the range of 740-3700 Lux and 25 – 35^{0}C. However, excess light and heat generated by light irradiance will pushed the cells to death phase in short period of cultivation.

The specific growth rate, biomass yield and productivity of the cells in different POME concentrations were tabulated in Table 1. The microalgae in the POME concentration of 30% v/v have the highest specific growth rate (1.39 d^{-1}), biomass yield (0.50 g cells/g POME and 0.68 g/mol photons) and biomass productivity (0.60 g/L.d) compared to the other POME concentration. A different biomass concentration has led to a different light gradient in the photobioreactor, thus creating different light exposure patterns to the microalgae. Nevertheless, the biomass yield is still able to increase even though the light intensity decrease along the increment of POME concentration. This is in line with the study of Zijffers *et al.* (2010), where a relatively constant yield between 0.6 and 0.8 g/mol photons is obtained by supplying higher photon flux density or light rate.

Table 1: The Kinetic Yield of Microalgae.

POME Concentration (% v/v)	Specific Growth Rate (d^{-1})	Biomass Yield (g cells/g POME)	Biomass Yield (g/mol photons)	Biomass Productivity (g/L.d)
0 (control)	1.21	-*	0.59	0.10
10	1.24	0.22	0.61	0.08
20	1.29	0.46	0.63	0.34
30	1.39	0.50	0.68	0.61

* The biomass yield is based on POME substrate, thus there is no value for control.

In order to get the kinetic coefficients of Monod model, linear curve of light intensity and POME concentration was plotted as Figure 4 and 5. The plotted graph based on light intensity (Figure 4) demonstrates a good relationship (R^2 = 0.929) for the specific growth rate and the growth of the microalgae mix culture is satisfied though the amount of light intensity is decreasing. In this case, as long as sufficient amount of light illumination is provided, the microalgae can utilise the light energy for the growth.

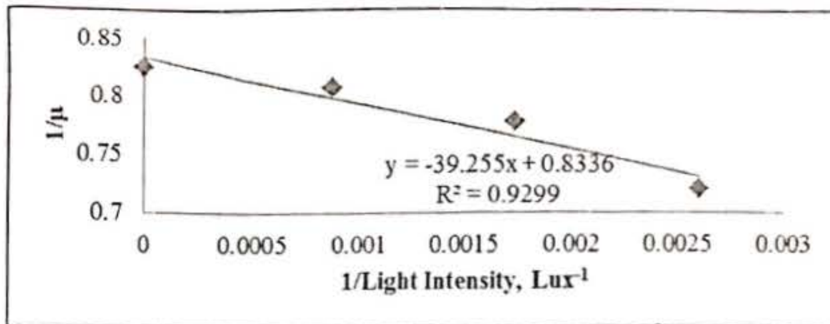

Figure 4 : The Monod Model based on light intensity.

Whereas the plot based on the POME concentration illustrated an acceptable relationship ($R^2 = 0.804$) for the specific growth rate and POME concentration. As the concentration of POME increased, the specific growth rate of microalgae mix culture was also increased. All of the kinetic coefficients that were calculated from Figure 4 and Figure 5 were tabulated in Table 2.

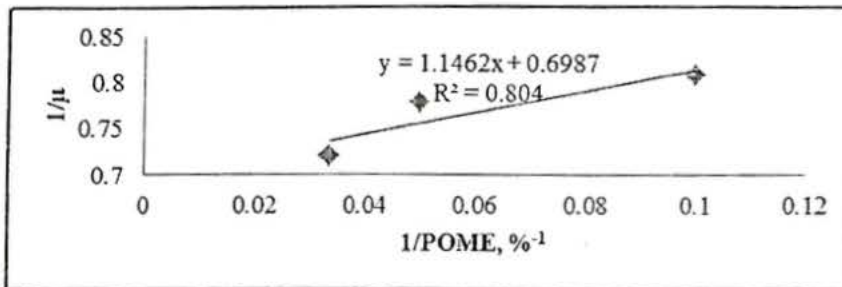

Figure 5 : The Monod Model based on POME concentration.

Table 2: The Maximum Growth Rate and Monod Constant.

Substrate	Equation	μ_{max}	K_s	R^2
Light Intensity	y = 39.25x + 0.833	1.20	47.1	0.929
POME	y = 1.146x + 0.698	1.43	1.64	0.804

From the result, POME was proven to be an alternative source of nutrients to promote the microalgae's growth. While carrying out

the experiment, only the two main variables were considered. However, the fair model related to the microalgae growth rate may due to temperature, pH and other nutrient conditions. The success of a model related to the accuracy of the basic conditions, the determinant factors in physiology and ecology needed to be refined to produce better cell yield (Dauta *et al.* 1990).

In the experiment, the removal efficiency of the BOD and COD of the supernatant were carried out for determined the performance of POME treatment by microalgae mix culture. The analysed results were shown in Table 3 and Figure 6.

Table 3: The BOD_5 and Colour Intensity Values for Undiluted POME and Samples at t = 7.

POME Concentration	BOD_5 (mg/L)	Colour Intensity (ADMI)
Treated POME without dilution	39.5	395
10	17.2	392
20	19.4	350
30	18.6	322

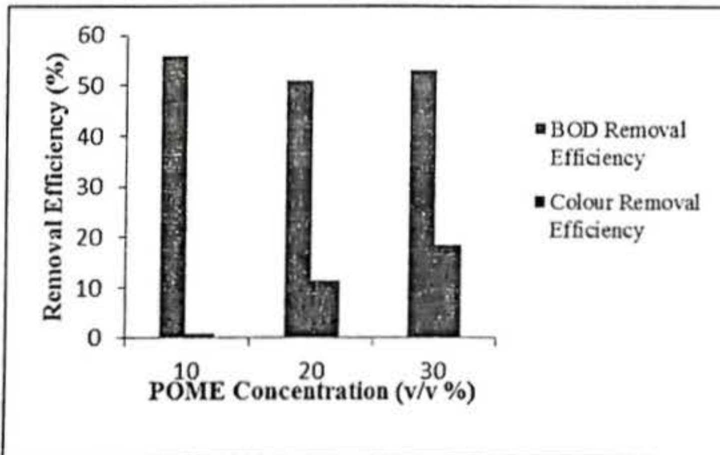

Figure 6: The removal efficiency of BOD and colour after seven days of POME Treatment by microalgae mix culture.

The BOD_5 result after seven days for 30% v/v POME concentration is 18.6 mg/L which corresponding to the removal efficiency of 53%. However, the BOD removal efficiency was considered as low compared to the study of Choi and Lee (2012) which applied the *Chlorella Vulgaris* on the removal of BOD in wastewater and achieved an average of 81%. Surprisingly, the BOD removal efficiency for 10% v/v is slightly higher than those in 30% v/v of POME sample which is 56%. The result indicates that the removal efficiency remain almost the same even though the 30% v/v of POME sample possesses higher microalgae growth rate. According to Choi and Lee (2012), there was no significant change in the BOD removal efficiency with the increment of microalgae dose.

For colour removal efficiency, even the POME sample with 30 %v/v shown the highest colour removal efficiency (18.5%) compared to others at the seventh day, but the decolourisation efficiency indicates that growing microalgae in POME does not give a significant result in up taking of coloured compound in POME whereby shows the colour intensity ADMI of 322 compare to Department of Environment (DOE) discharge standard below ADMI of 200. In this case, several factors need to be considered as Neoh *et al* (2012), stated that coloured compound in POME can be up taken by the fungus pellet where it had a highly porous mycelium matrix and the appearance of hyphae provided a high surface area. While the size and the simple structure of the microalgae has affected the availability of surface area for coloured compound adsorption hence inducing low colour removal efficiency.

From the industrial practicability point of view, several limitations still exist although sufficient illumination, growth nutrient and ideal photoperiod are provided to the microalgae to promote the microalgae growth. One of the main limitations is the depth of aerobic zones where the microalgae can utilise the light as their energy sources. As discussed in previous section, the optimum growth occurred in 30% v/v of POME concentration with the light illumination supplied for the microalgae growth. In a smaller scale, the growth of microalgae in an undiluted sample is proven to be slow due the light irradiance blockage by the colourisation of POME. In addition, the POME can neither be heated to eliminate the growth of heterotrophic organisms such as bacteria nor diluted to provide the optimum condition for microalgae growth.

4. CONCLUSIONS

It can be concluded that microalgae can grow under low light intensity condition and optimum growth was observed once the light penetrated exceeded 2000 Lux. In the 30% v/v POME, the specific growth rate of microalgae was 1.39 d^{-1} with the biomass yield based on POME and light energy of 0.50 g cells/ g POME and 0.68 g/mol photons respectively. The biomass productivity calculated was 0.61 g/L.d. In terms of BOD and colour removal, the removal efficiency in 30 %v/v sample was 52.9% and 18.5% respectively. Even though the highest growth rate of microalgae and significant removal of BOD was observed in 30% v/v of POME, yet the removal efficiency of colour were not significant, indicating that microalgae was not effective in removing coloured compound.

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