# COLOUR REMOVAL AND CHEMICAL OXYGEN DEMAND (COD) REDUCTION OF PALM OIL MILL EFFLUENT (POME) USING FRESHWATER GREEN MICROALGAE: A PRELIMINARY STUDY

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## ABSTRACT

*Raw POME was collected from a local palm oil mill. The raw POME had been treated with anaerobic and aerobic process with 50 days and 16 days of hydraulic retention time (HRT), respectively. 3 different species of microalgae (Ankistrodesmus falcatus, Scenedesmus sp. and Chlorella sp.) were inoculated in a culture media which contained 20%, 40% and 60% dilution of POME. The pH of the treated POME sample was not adjusted and fixed at the original pH of about pH 8-9. The growth of the microalgae was determined every 2 days based on their chlorophyll concentration. Chlorella sp. showed the best adaptation and it grew well in all dilutions of treated POME sample. Chlorella sp. was then chosen and used for remediation of the POME sample (without dilution). Chemical oxygen demand (COD) and colour removal of POME were determined every 2 days. Chlorella sp. performed well with COD reduction and colour removal of 67.87% and 53.26%, respectively.*

**Keywords:** *Ankistrodesmus falcatus*, *Chlorella* sp., green microalgae, palm oil mill effluent, *Scenedesmus* sp.

## INTRODUCTION

*Elaeis guineensis* or also named as oil palm is widely planted in Malaysia since the 1960s [1]. Many types of waste either in solid form or liquid form are generated during the palm oil extraction process [2]. Palm oil mill effluent (POME) is one of the liquid waste by-product which contains a high concentration of chemical oxygen demand (COD) of 45,500-65,000 mg/L, biochemical oxygen demand (BOD) of 21,500-28,500 mg/L, total nitrogen (TN) of 500-800 mg/L, total phosphates (TP) of (94-131 mg/L), oil and grease (OG) of 1077-7582 mg/L and about 40,500 mg/L of total solid (TS) [3-5]. If the POME is discharged without any treatment, it will cause a very serious environmental pollution.

A wide range of research work have been published for POME treatment. Anaerobic-aerobic treatment using ponding system is one of the most common treatment process in treating POME [6]. Over 85% of Malaysia's palm oil mills applied this traditional treatment method for POME treatment [7]. However, some of the common conventional treatment facilities are unable to meet the regulations standards which were set by the Department of Environment (DOE), Malaysia [8]. Therefore, further treatment or post-treatment is needed to polish the effluent.

Microalgae are microscopic photosynthetic organisms. They can be found either in freshwater or marine environment. Generally, microalgae play an important role in self-purification of natural waters. Because of this benefit, it offers an alternative as post-treatment of organic wastewater [9]. Since year 1950, microalgae have been used for wastewater treatment [10]. Microalgae have gained attention of researchers for various types of wastewater treatment due to their capability of removing a wide range of pollutants such as heavy metal, phosphorus, nitrogen etc. from wastewater [11-14]. Agriculture wastewater such as POME is rich in degradable organic matter and the two fundamental components, nitrogen and phosphorus which are needed for microalgae growth [15, 16]. Microalgae are able to utilize the carbon and nutrient sources in POME for their growth and thus removing these contaminations [16].

This study was aimed to investigate the growth of three different species of microalgae (*Ankistrodemus falcatus*, *Chlorella* sp. and *Scenedesmus* sp.) in aeration treated POME. The efficiency of colour removal and COD reduction of POME will also be determined in this study.

## METHODOLOGY

### POME Collection

Raw POME was collected from a palm oil mill which is located in Penang. The raw POME was treated with anaerobic and aerobic process as a pre-treatment before applying microalgae. Both anaerobic and aerobic processes were carried out with 50 days and 16 days of hydraulic retention time (HRT) respectively to reduce the dark colour of POME. About 450 mL of treated effluent was collected every day and stored in a refrigerator at 4 $^{o}$C to limit the activity of biodegradation process of the microorganisms. The characteristics of the raw POME and treated POME were analyzed and listed in Table 1.

### Microalgae and Culture Conditions

The strain of *Ankistrodesmus falcatus* was obtained from School of Biological Science, Universiti Sanis Malaysia. The *Scenedesmus* sp. and *Chlorella* sp. in this study were isolated from Tasik Harapan, Universiti Sains Malaysia. Nutrient agar plates were prepared to cultivate the microalgae sample. A 1% of agar was mixed with Bold's Basal Medium (BBM) and autoclaved at 121 $^{o}$C for 20 minutes to prepare the agar plates. BBM consists of 25 mg/L $NaNO_3$, 7.5 g/L $MgSO_4.7H_2O$, 7.5 g/L $K_2HPO_4$, 2.5 g/L $CaCl_2.H_2O$, 2.5 g/L NaCl, 11.42 g/L $H_3BO_4$, 50 g/L $EDTA.Na_2$, 31 g/L KOH, 4.98 g/L $FeSO_4.7H_2O$, 1.0 mL concentrated HCL and 1.0 mL trace elements solution. The trace elements solution contained 8.82 g/L $ZnSO_4$, 1.44 g/L $MnCl_2.4H_2O$, 1.59 g/L $CuSO_4.5H_2O$, 0.71 g/L $MoO_3$, 0.49 g/L $Co(NO_3).4H_2O$. A series dilution of samples was spread on the BBM agar plates and cultured under 2400±100 Lux of light intensity at room temperature 30±1 $^{o}$C. After one week, the single colonies were streaked onto another agar plate. This procedure was repeated several times until a single strain of microalgae is obtained.

A 10 mL of each single strain of microalgae were inoculated in 250 mL Erlenmeyer flask which contained 100 mL of autoclaved BBM and shaken at 100 rpm. All the cultures were maintained at a temperature of 30±1 ºC with light/dark period: 12 hours: 12 hours at 2400±100 Lux of light intensity.

**Experimental Condition**

The treated POME was diluted into three different dilutions and mixed with autoclaved BBM. The volume ratios of POME in BBM were diluted as 20%, 40%, and 60%. A 10 mL of each microalgae (*Ankistrodesmus falcatus*, *Scenedesmus* sp. and *Chlorella* sp.) were inoculated in every 150 mL of sample (without adjusting the pH value) in a 250 mL of an Erlenmeyer flask. All the samples were shaken at 100 rpm and cultivated at room temperature (30±1 $^{o}$C) with 2400±100 Lux of light intensity for 12 hours of light/dark period. The growth rate of each microalgae was studied every two days based on their chlorophyll concentration. The morphology of the microalgae after being inoculated in treated POME samples was monitored under microscope. The survived microalgae were chosen and proceeded to be used in the POME treatment study.

**Morphological Study**

Microalgae cultures were identified based on the morphological examination of the individual cells under microscope. Both isolated colonies and the morphology of each microalgae species (after cultivated in treated POME sample) were photographed at magnification 40x using Nikon Eclipse (E200) microscope equipped with Q-imaging digital camera and Image-Pro Express 6.0 software.

**Growth Rate Study**

The growth of each microalgae was determined by their chlorophyll concentration. A 1 mL of each sample was withdrawn every two days and centrifuged at 10,000 rpm for 5 minutes to obtain the cells pellet. The supernatant was discarded and added in 1 mL of 90% of methanol to the pellet. The mixture was stored at 4 $^{o}$C in a dark condition for one hour. After one hour, the mixture was centrifuged again for another 5 minutes at 10,000 rpm to separate the supernatant and the cells pellet. The supernatant was then transferred into a cuvette and measured the absorbance reading using Shimadzu UV-spectrophotometer (Model-UV 16001 PC) with wavelength 652 nm and 665 nm [17]. The chlorophyll concentration was calculated using the equation (1) below [17],

$$chlorophyll\ a\ (mg/L) = -8.0962\ \lambda_{652nm} + 16.5169\ \lambda_{665nm} \quad (1)$$

**POME Treatment Study**

The microalgae which was able to tolerate with the low dilution of POME was chosen and used in the POME treatment study without dilution. A 5 mL of each sample was withdrawn every two days and filtered using Watchman Cellulose Nitrate Membrane Filter (0.45 µm of pore size) under vacuum. The filtered liquid sample was collected. The percentage of COD reduction and colour removal of the collected liquid sample were determined according to the standard methods for the examination of water and wastewaters [18]. The removal efficiency of colour removal and the COD reduction were calculated using equation (2) below,

$$removal\ efficiency\ (\%) = \frac{C_i - C_f}{C_i} \times 100\% \quad (2)$$

where, $C_i$ and $C_f$ is the initial and final reading of colour (Pt/Co) and COD (mg/L) respectively.

**RESULTS AND DISCUSSIONS**

**Characteristics of POME**

Raw POME was collected from a local palm oil mill. The characteristics of the raw POME, anaerobic treated POME and aerobic treated POME are listed in Table 1.

**Table 1.** Characteristics of POME

| Parameters | Raw POME | Anaerobic Treated POME | Aerobic Treated POME |
|---|---|---|---|
| **pH** | 4-4.5 | 7-7.5 | 8-9 |
| **Colour (Pt/Co)** | - | - | 3000-4500 |
| **COD (mg/L)** | 70,000-80,000 | 15,000-16,000 | 600-1000 |

**Isolation and Morphology Study**

Two of the green single colonies were selected from the agar plate. Figure 1(a) shows the morphology of *Chlorella* sp. The cells are spherical in shape. The diameter of each cell is about 2-10 µm. The cells of *Chlorella* sp. are without flagella and they are green in colour.

Figure 1(b) shows the morphology of *Scenedesmus* sp. The cells of *Scenedesmus* sp. are arranged either linearly or zigzag pattern. The colonies of the cells are formed in a group and usually 2, 4, 6 or 8 cells will be attached side by side. The cells of *Scenedesmus* sp. are normally elliptical or spindle or crescent in shape with 11-18 µm long and 3.5-7 µm wide.

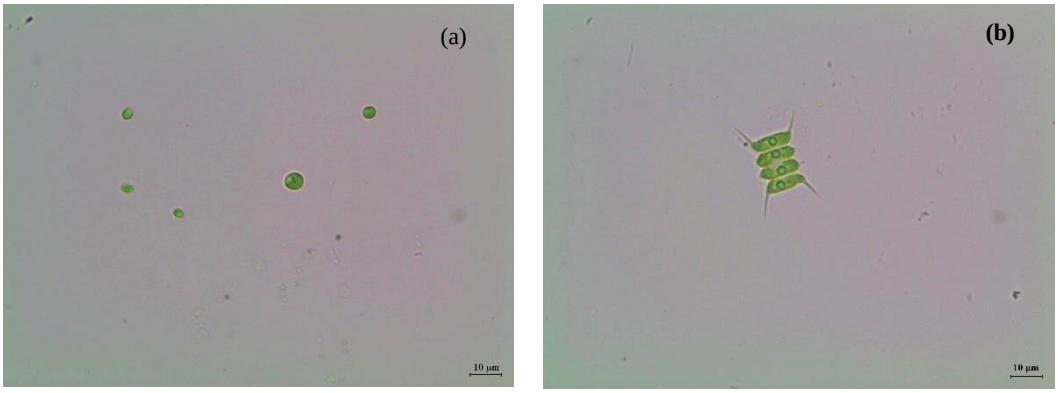


**Figure 1.** Morphology of (a) *Chlorella* sp. and (b) *Scenedesmus* sp.with magnification of 40x

**Microalgae Growth**

Figure 2 (a), (b) and (c) show the growth rate of *Ankistrodesmus falcatus, Chlorella* sp. and *Scenedesmus* sp. in the culture media which contained with 20%, 40% and 60% of treated POME, respectively. All three species could grow well in the media with 20% dilution of treated POME. But, the growth rate of *Ankistrodesmus falcatus* and *Scenedesmus* sp. decrease as the volume of treated POME increases. This is because of the increasing of percentage of POME in media will decrease the light penetration. Light is an essential resource which often limits the growth rate of microalgae. George et al. (2014) had investigated the growth of *Ankistrodesmus falcatus*, and found that it is highly influenced by the light intensity [19]. The results showed *Ankistrodesmus falcatus* grew well as light intensity is increased [19]. Liu et al. (2012) also claimed that the growth of *Scenedesmus* sp. is affected by light intensity [20]. The highest biomass production of *Scenedesmus* sp. was obtained at the highest light intensity [20]. However, *Chlorella* sp. shows good adaptation even in the lowest dilution volume of treated POME. Some of the microalgae species are able to switch between autotrophic and heterotrophic growth regime based on the culture condition. *Chlorella* sp. can tolerate with high concentration of wastewater even in poor light penetration [21]. *Chlorella* sp. can use both metabolic pathways. Under poor light penetration condition, the *Chlorella* sp. will switch to heterotrophic growth regime.

pH is another factor which will affect the growth of microalgae. Different species of microalgae can tolerate different values of pH. In this study, the pH of treated POME was very high at about pH 9. This might cause *Scenedesmus* sp. and *Ankistrodesmus falcatus* to not able to grow well in the treated POME sample.

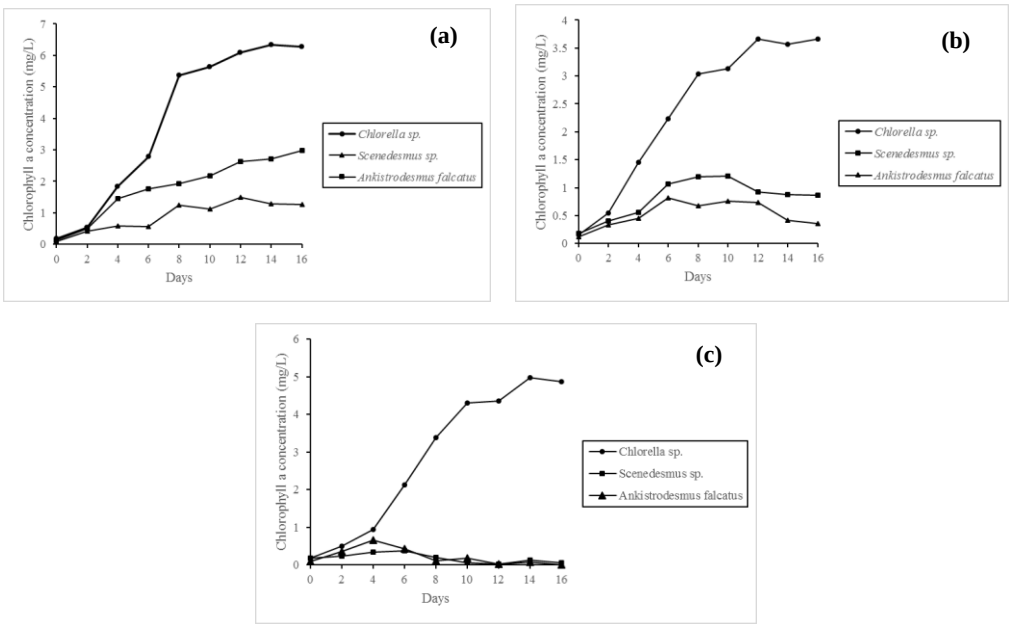


**Figure 2.** The growth of the three species of green microalgae in media which contain of (a) 20%, (b) 40% and (c) 60% of POME

**Morphology of Microalgae**

The morphology of each microalgae species after being cultivated in the media which contained POME was monitored under microscope with magnification 40x. Figure 3(a) displays the morphology of *Chlorella* sp. after 16 days of cultivation period. The cells were clumped together after the 16th day of cultivation. This might due to the cells produced the extracellular polymeric substances (EPS) to protect themselves from the extreme conditions, in this case, POME. Figure 3(b) and 3(c) show the morphology of *Scenedesmus* sp. and *Ankistrodesmus* sp. Many dead cells of *Scenedesmus* sp. and *Ankistrodesmus falcatus* (circled in red) were found within the 16 days of cultivation as both species cannot tolerate the extreme condition of treated POME with high pH and poor light intensity.

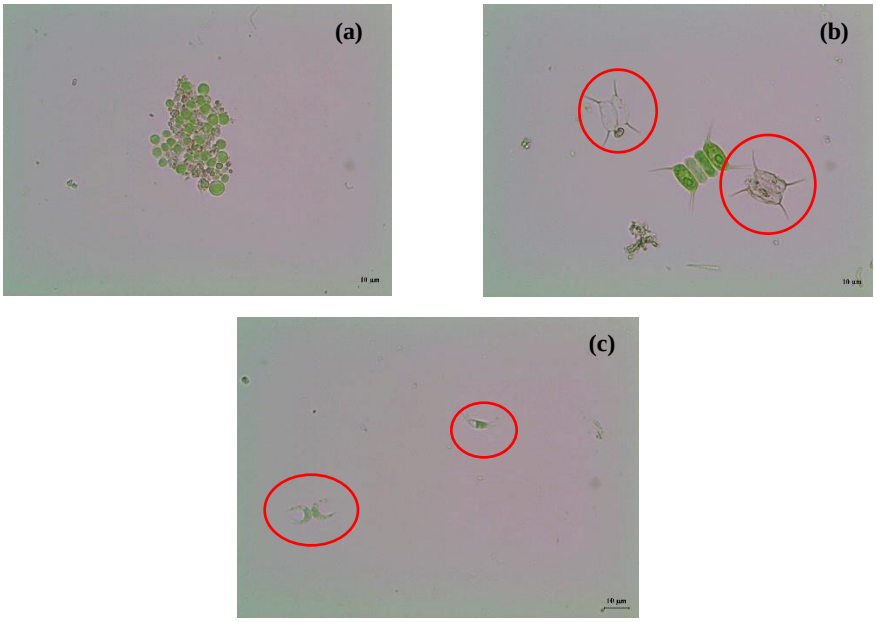


**Figure 3.** The morphology of (a) *Chlorella* sp., (b) *Scenedesmus* sp. and (c) *Ankistrodesmus falcatus* after the 16 days of cultivation

**COD Removal and Colour Removal**

*Chlorella* sp. was chosen in the subsequent study on COD reduction and colour removal of POME (without dilution). Figure 4 shows the growth rate of *Chlorella* sp. in POME. The chlorophyll concentration decreased after day 12$^{th}$ and this indicated the death phase in POME for this strain.

Figure 5(a) and 5(b) show the efficiency of COD reduction and colour removal of *Chlorella* sp. respectively within 16 days. The highest percentage removal of colour removal and COD reduction was obtained at day 10$^{th}$ with 53.26% and 67.87% respectively. There is small removal of colour and COD reduction of the control sample (without autoclave) which might be due to the degradation activity of the bacteria which contained in POME.

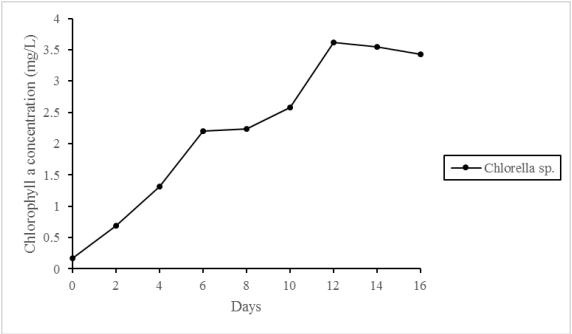


**Figure 4.** The growth of *Chlorella* sp. in POME (without dilution) within 16 days

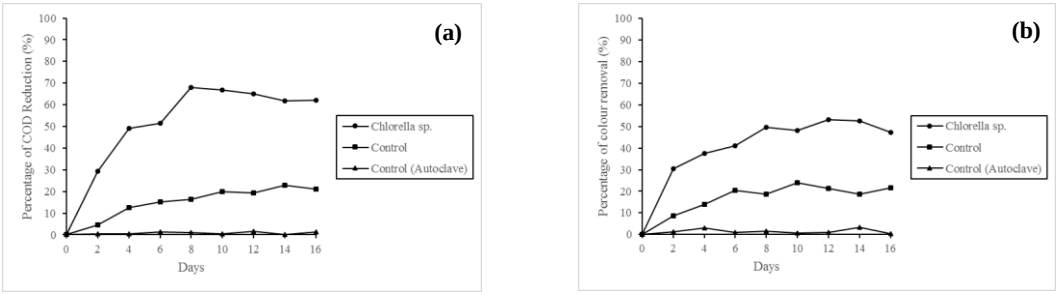


**Figure 5.** The percentage efficiency of (a) COD reduction and (b) colour removal of POME (without dilution) for 16 days

**CONCLUSION**

Compared to another two strains, *Ankistrodesmus falcatus* and *Scenedesmus* sp., *Chlorella* sp. could adapt well in POME even the POME sample was poor in light penetration due to the high turbidity and extremely dark brown. *Chlorella* sp. is suitable to be used for remediation of POME since it showed a good result on COD reduction and colour removal of POME with about 67.87% and 53.26% respectively.

*Ankistrodesmus falcatus* and *Scenedesmus* sp. show good adaptation in media which contained a low concentration of POME. They are also the potential strains to be used in the remediation of POME. *Chlorella* sp. performed well among the three strains. However, there are several parameters such as pH, temperature, light intensity etc. which have to be considered in order to obtain the optimum growth of these microalgae and enhance the good performance of POME remediation process.

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