Polishing of POME by Chlorella sp. in suspended and immobilized system

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Abstract. The effect of using suspended and immobilized growth of Chlorella sp. to treat POME was studied. Cotton and nylon ropes were used as the immobilization material in a rotating microalgae biofilm reactor. The result showed that POME treated in suspended growth system was able to remove 81.9% and 55.5% of the total nitrogen (TN) and total phosphorus (TP) respectively. Whereas the immobilized system showed lower removal of 77.22% and 53.02% for TN and TP. Lower performance of immobilized microalgae is due to the limited light penetration and supply of CO₂ inside the immobilization materials. The rotating microalgae biofilm reactor was able to reduce the biochemical oxygen demand (BOD) to 90 mg/L and chemical oxygen demand (COD) to 720 mg/L. Higher BOD and COD reading were obtained in suspended growth due to the presence of small number of microalgae cell in the samples. This study shows that suspended growth system is able to remove higher percentages of nitrogen and phosphorus. However, an efficient separation method such as membrane filtration is required to harvest the cultivated microalgae cell to avoid organic matter release into water bodies.

1. Introduction

1.1. Palm Oil Mill Effluent (POME)
Palm oil mill effluent (POME) has been recorded as the largest contributors for water pollutants discharged into water bodies in Malaysia. It is a brownish colored voluminous liquid that contains high turbidity, chemical oxygen demand (COD), biochemical oxygen demand (BOD), oil and grease and suspended solids [1,2]. POME is generated from the oil extraction, washing and cleaning processes in the mill [3]. It is estimated that for each ton of crude palm oil (CPO) produced, 5-7.5 tons of water is required of which 50% will end up as POME [4, 5]. POME is typically high in temperature, 85°C with pH 4.7 and turbidity of 11,000 NTU [6].

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The Malaysian government has enforced regulation to limit and monitor the discharged POME effluent through Environmental Quality (Prescribed Premises) (Crude Palm-Oil) Regulations 1977. Under this regulation it is stated that the limit for water discharge from palm oil mill to be $\text{BOD}_3$ of 100 mg/L, suspended solids of 400 mg/L, oil and grease of 50 mg/L, total nitrogen of 200 mg/l and pH of 5 – 9 [7]. Ponding system is the most common POME treatment system in Malaysia where more than 85% of palm oil mills are implementing it before discharging into rivers [8]. However, this treatment that is mainly depending on biological treatments anaerobically and aerobically is not entirely efficient to treat POME, which leads to environmental pollution issues [9]. It was identified that the treated POME using ponding system sometimes does not conform to the discharge standard and the removal of nitrogen from POME is usually insignificant because nitrification process is not common in the ponding system [10, 11].

1.2. Treatment of wastewater by Microalgae

The potential of wastewater treatment by using algal remediation was discovered as early as the 1940’s where microalgae were proposed to be used for the treatment of a municipal wastewater by Caldwell [12], followed by other researchers [13, 14]. The ability of microalgae to thrive in almost any condition, by only requiring basic necessities such as the supply of $\text{CO}_2$, light illumination and nutrient as well as being able to thrive in has made microalgae a highly versatile organism for wastewater treatment.

Since then, many researchers have explored the potential of microalgae for treatment of various wastewater such as municipal [15-20] Steel plant [21], textile [22], piggery [23], and soybean processing [24, 25] mainly for the assimilation of nitrogen and phosphorus. Development of microalgae based commercial treatment plant has contributed significantly to the reduction of pollutant especially biological oxygen demand (BOD), chemical oxygen demand (COD) as well as nutrients mainly nitrogen and phosphorus.

Other researchers have also looked into the potential of using microalgae as cultivation medium for the purpose of biomass cultivation for biofuel production with a simultaneous mechanism of nutrient uptake [26-30]. The combination of wastewater treatment and microalgae biomass is an economical method for biofuel as wastewater is a free source of abundant nutrients.

Microalgae based wastewater treatment has a number of benefits over the conventional wastewater treatments, namely chemical and biological treatment. Aeration process in biological treatment requires a high cost of mechanical aeration as well as the management of sludge produced at the end of the treatment. As for chemical treatment, the chemical used requires a great amount of cost other than contributing to other environmental impact caused by chemical contamination. On the other hand, the microalgae bioremediation offers a more cost effective approach where the biomass produced at the end of the treatment can be used for various purposes such as biofuel production or as feedstock for many industries. Furthermore, the microalgae treatment can serve a dual purpose of wastewater treatment and $\text{CO}_2$ sequestration at the same time [31].

1.3. Design of Suspended Culture Cultivation

Microalgae are typically cultured in a suspended growth system, which mimics the nature of microalgae natural growth mechanism in the environment. The method of cultivation are usually divided into two main systems; open and closed. Open system may utilize either big shallow ponds, raceway system or circular ponds. The choice of a specific system is influenced by the basic properties of the microalgae, the local climactic conditions and the costs of water and land. These factors means that pond volume and area should be minimized and the cell density in the culture should be maximized to have an economical process [32]. However, the main disadvantages of the open pond are the $\text{CO}_2$ emission to the atmosphere, evaporation losses, risk of contamination and the large area required.
Potential contamination to microalgae culture can be minimized by utilizing closed system for cultivation. The concept of closed system has been explored since a few decades ago [33, 34]. Many advances in the design and operation of closed photo-bioreactors in culturing microalgae such as the flat plate reactor [35, 36] or the tubular reactor [37-40]. The fundamental principle of these designs is to reduce the light path so as to increase the amount of light available to each cell.

These reactors have advantages: ‘clean’ microalgae culture, high light utilisation efficiency which leads to high productivities, temperature control and the ability to be used outdoors in natural sunlight. The wider range of species can be cultivated since contamination is avoided and the reactors can be operated over a much wider climatic range than open system [32]. However, the closed system may require a higher cost in construction compared to an open system.

1.4. Immobilized culture of microalgae
Immobilized microalgae cell is defined as a cell that is prevented from independently moving from its location to all parts of an aqueous system either naturally or artificially [41]. Advantages available by immobilization of microalgae cell are the high biomass concentrates, avoidance of any further utilization of filtration process, and the ability to entrap more than one microorganism as well as being a simple process that is able to be conducted even by non-professionals [42]. However, the major issues in microalgae immobilization are the efficiency of the system to remove pollutants, cost of materials, and cost of immobilization process itself.

The wastewater industry is already familiar to large scale biofilm processes [43]. A scalable microalgae biofilm system could be incorporated into wastewater treatment process to achieve a dual benefit of inexpensive nutrient supply and treated water. Given enough surface area, microalgae biofilm can potentially achieve higher growth compared to suspended growth system [44]. It can contribute to increased density and lower land and water requirement of matrix-immobilized culture [45] without the associated costs of the matrix.

By using biofilm system, it is expected that little or no separation of microalgae and water is required before effluent discharge [46, 47]. Study by Christenson and Sims [48] involved designing and testing a rotating microalgae biofilm reactor for sewage treatment. They were able to achieve effective nutrient reduction as well as biomass production by using cotton substratum. Comparison between immobilized culture submerged in polystyrene and suspended culture in the same condition was done by Johnson and Wen [49]. The immobilized culture showed a greater yield of biomass and lipid content compared to microalgae cultured in the suspended growth system. However, photoinhibition and diffusion limitation of nutrients or CO₂ may limit the performance of immobilized microalgae system [50, 51]. This research was focused on the comparison between application of immobilized and suspended growth microalgae in nutrient uptake in POME and simultaneous cultivation of microalgae biomass for further use.

2. Materials and methods

2.1. Wastewater samples
POME samples was collected from Lumadan Palm Oil Mill (5°15'19"N and 115°39'24"E). All collected POME was filtered to remove large solid and stored at 4°C after sampling before utilized for experiment.

2.2. Microalgae species
*Chlorella* sp. were isolated locally by Borneo Marine Research Institute, University Malaysia Sabah from the lake located in Likas Stadium, Kota Kinabalu. A 20 mL sample was acquired from the culturing chamber of Borneo Marine Research Institute and directly sent to the lab for culturing. *Chlorella* sp. was chosen due to its variety in post–harvest application [52-55]. The microalgae was cultivated in an adjusted
Jaworski Medium (JM). The medium contained 0.02 gl⁻¹ Ca(NO₃)₂·4H₂O, 0.0124 gl⁻¹ KH₂PO₄, 0.05 gl⁻¹ MgSO₄·H₂O, 0.00225 gl⁻¹ EDTAFeNa, 0.00225 gl⁻¹ EDTANa₂, 0.00248 gl⁻¹ H₂BO₃, 0.00139 gl⁻¹ MnCl₂·4H₂O, 0.001 gl⁻¹ (NH₄)₆Mo₇O₂₄·4H₂O, 0.00004 gl⁻¹ cyanocobalamin, 0.00004 gl⁻¹ thiamine HCl, 0.00004 gl⁻¹ biotin, 0.08 gl⁻¹ NaNO₃ and 0.036 gl⁻¹ Na₂HPO₄·12H₂O.

2.3 Suspended culture
Suspended culture experiment was conducted in a photobioreactor built from transparent materials (Perspex). The laboratory scaled reactor was dimensioned 71 cm long, 31 cm wide and 12 cm deep with a working volume of 20 L. Air diffuser made from 0.84” diameter PVC pipes was installed at the bottom of the photo bioreactor which was connected to an air pump for aeration and also acted as a mixer of the wastewater and microalgae to maximize algae-wastewater contact as well contact with the light supply.

2.4 Rotating Microalgae Biofilm for Immobilized System
The laboratory-scaled microalgae biofilm reactor consisted of cubic tank built using Perspex with dimension 71 cm long, 31 cm wide and 12 cm deep. 2 x 1 in aluminum hollow channel was used to frame out tank and motor parts of the reactor. Rotating drum consisted of two cylinders was set up using 8.9 cm PVC pipe wrapped with cotton and nylon as the growth surface for microalgae. The biofilm was rotated by motors and was partially submerged in POME sample with 40% into aqueous phase and 60% of substrate exposed to gaseous phase. 10 rotation per minute (rpm) ensured that enough time was provided for microalgae exposure to light and nutrients.

![Figure 1. Setup of Rotating Microalgae Biofilm system (not to scale)](image)

2.5 Sampling
Experiments were carried out within a 5-day period and sampling was done daily. All samples were filtered out using a syringe filter (0.45 μm nominal pore size with 25 mm diameter, Sartorius filter) in order to remove any suspended solid.

3. Results and discussion

3.1 Nitrogen and phosphorus removals
Figure 2 shows the rapid removal rate of TN in the first 2 days of experiment and become almost constant after the third day. Nevertheless, all three experiments were not able to remove the TN content completely.
Table 1. Removal of Nitrogen and Phosphorus immobilized and suspended system material using *Chlorella* sp.

| Parameter | Material    | Rpm | Initial Concentration (mg/L) | Final Concentration (mg/L) | Percentage Removal (%) |
|-----------|-------------|-----|------------------------------|----------------------------|------------------------|
| TN        | Cotton      | 10  | 54.8                         | 12.                        | 77.22                  |
|           | Nylon       | 10  | 57.7                         | 15.4                       | 73.33                  |
|           | Suspended Cell | 49.7 | 49.7                       | 9                          | 81.9                   |
| TP        | Cotton      | 10  | 30.43                        | 14.3                       | 53.02                  |
|           | Nylon       | 10  | 41.00                        | 18.1                       | 55.85                  |
|           | Suspended Cells | 22.47 | 22.47                   | 10                         | 55.5                   |

Figure 2. Removal rate of Nitrogen in immobilized and suspended system material using *Chlorella* sp.

Figure 3. Removal rate of Phosphorus in immobilized and suspended system material using *Chlorella* sp.
From the result, the suspended growth treatment showed the most rapid removal of TN most probably due to the free movement of microalgae cell that provided better contact with the pollutants for nutrient assimilation.

The result obtained also showed that the TP removal was slightly lower than that of TN removal. This may be related to the N: P ratio of the POME tested which was implied by Beuckels et al. [56] to have significant effect on the phosphorus uptake by microalgae. Microalgae will accumulate more phosphorus when nitrogen concentrations are high and less when N concentrations are low. This statement suggests that a sufficient concentration of nitrogen is required for an effective phosphorus removal [56]. The same pattern of result was obtained by Gonzalez et al. [23] and Yun et al. [21] where phosphorus removal was low even with the exhaustion of nitrogen. It is also important to note that chemical precipitation is insignificant for the phosphorus removal since the pH was in the range of 7-8 (result not shown) during the entire experiment. Therefore, it can be concluded that TP removal was by the assimilation of microalgae.

The rotating biofilm removed nitrogen and phosphorus via microalgae propagation through the immobilization material. The propagation of microalgae in cotton rope is faster than nylon due to the higher water affinity of cotton. The suspended growth shows higher removal rate of TN and TP due to greater contact between microalgae cell and POME. This is also due to the higher yield of microalgae biomass which is caused by larger light and CO₂ supply of the suspended microalgae. This is a good vindication of reports in literature that imply the difference in nitrogen and phosphorus removal efficiencies depends on the composition of the culture medium such as the concentration, light intensity N:P ratio and light/dark cycle [57, 58].

However, the result found in this study is contrary to the results reported by Gao et al. [59] where biofilm photobioreactor was able to remove higher amounts of nitrogen and phosphorus at >95% and >85% respectively than a conventional photobioreactor. Nevertheless, this result could be affected by the fact that the biofilm photobioreactor was combined with a suspended growth medium. Both of the systems were also assisted by a membrane filtration system before the effluent is discharged.

### 3.2 BOD and COD removal

**Figure 4.** Removal rate of BOD in immobilized and suspended system using *Chlorella* sp.

**Figure 5.** Removal rate of COD in immobilized and suspended system using *Chlorella* sp.
From the obtained data for BOD and COD removal, the immobilized microalgae cell was able to achieve a steady removal throughout the 5-day operation. At the end of experiment, an average of 86% and 51% of BOD and COD respectively was removed from the samples. The BOD was removed well under the specified limit of 100 mg/L of BOD, stated by Malaysian authority [7]. However, comparing both treatment systems, the removal by suspended microalgae cell presented fluctuating and inconsistent readings as indicated by the large range of errors in the plotted data.

The increase in biomass of microalgae with time has resulted in contamination of microalgae cell in the effluent of suspended solid due to the lack of separation between wastewater sample and microalgae cell. This is mainly caused by the motility of microalgae cell itself and has been reflected by the higher turbidity reading in samples collected for suspended cell system (result not shown). However, other literature has reported a contrary result where the immobilized microalgae presented steady but lower nutrient removal due to the limitation of biomass growth inside the immobilization medium of sodium alginate [60], when compared to a suspended cell treatment system. This result might however be affected by the different immobilization methods applied in the research.

The fluctuation of BOD and COD removal in the suspended treatment system shows that microalgae cell separation before effluent discharge is crucial for the application of microalgae to wastewater treatment. Without a solid-liquid separation, a complete treatment of wastewater will not be achieved where the BOD and COD reading will still be high due to the presence of microalgae cell in the discharge. This fugitive cell will cause an eventual eutrophication in the water bodies. With the increasing amount of biomass with time, the separation of biomass and water was becoming more difficult. The microalgae biomass was not able to be completely separated from the sample and had caused the increase in BOD as well as COD concentration in the water sample.

3.3 Biomass recovery

![Figure 6. Recovered Chlorella sp. biomass from immobilized and suspended cell system](image)

Two different methods were used for the recovery of both immobilized and suspended solids. The biomass yield for suspended microalgae cell was monitored daily whereas the immobilized microalgae was only recovered after the 5-day experiment to avoid any potential interruption to the system.
Comparison between two materials of biofilm shows that cotton rope has a higher yield in microalgal biomass after 5 days of operation compared to nylon rope at 1.9 g/m² and 0.6 g/m² respectively. This could be contributed by the better hydrophilicity attribute of cotton rope that facilitated the attachment of cell and the distribution of wastewater thoroughly within the biofilm. From previous research, it was evident that different materials of biofilm would result in different growth rates of microalgae. Similar result was obtained by Christenson and Sims [61] and Gross et al. [62] found respectively that cotton rope and cotton duct, showed the best microalgae growth among other tested materials. However, the exact reason affecting microalgae growth in an attached growth system is still inconclusive. From the review done by Schnurr and Allen [63], they concluded that many factors affect the growth of microalgae onto biofilm including surface tension, water contact angle/hydrophobicity, surface energy and surface micro patterning.

The effect of biofilm utilization for microalgae growth can be seen based on the comparison between the immobilized and suspended cell system. Figure 6 shows a significant difference between both methods. Microalgae biomass yield in suspended cell is higher when compared to the attached growth in biofilm. Attached growth system within the cotton and nylon rope has limited light penetration that is much needed for microalgae growth. Since the microalgae are deep within the immobilization material, and only in contact with wastewater at certain times due to the rotation of the biofilm, limitation of nutrient occurred which was reflected in the lower yield in biomass. Freely moving cell in suspended solid ensures sufficient light and nutrient supply for photosynthesis and promotes the microalgae growth.

4. Conclusion
The biofilm has shown a high potential in the polishing of POME which showed steady removal for TN, TP, BOD and COD. However, the efficiency of immobilized treatment is limited by insufficient supply of light and nutrient. This study concludes that immobilized microalgae culture has significant limitation for wastewater treatment if compared to suspended cell system. From the suspended cell system operation, it can be concluded that even with the effective removal of TN and TP, the treatment of wastewater will not be completely effective as fluctuation in BOD and COD removal can still be seen which is caused by the existence of residual microalgae biomass in the effluent water. Therefore, it is evident that an efficient biomass separation technique is very important for an effective treatment of wastewater using microalgae. Further study on the combination of suspended treatment system of microalgae with a solid-liquid separation method such as membrane filtration is recommended for higher efficiency of biomass removal from the effluent.

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