An Effective Two-Steps Polyacrylamide-Microalgae System for Flocculation and Bioremediation of Palm Oil Mill Effluent

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***Abstract*—The use of microalgae for effluent treatment has been extensively studied in the past decade. Such system has a dual benefit of preventing pollutants from entering the waterbody while simultaneously produces valuable biomass. However, agricultural wastewater especially palm mill oil effluent (POME), contains a significant amount of suspended solids that may interfere with the growth process of the microalgae. The present study explored strategies to reduce POME turbidity for enhanced bioremediation efficiency. polyacrylamide (PAM) was used as pre-treatment of the POME prior to treatment using microalgae. The optimization result shows that PAM at concentration of 24 ppm able to reduce 46.5% of the turbidity. The pre-treated POME was then used for microalgae cultivation for subsequent nutrient removal. Improved condition of the POME has resulted in higher growth rate of three native microalgae, *Chlorella Sorokiniana* (UKM3), *Chlamydomonas*, sp. (UKM6) and *Scenedesmus* sp. (UKM9) with specific growth rate, μ of 0.29 day^{-1}, 0.25 day^{-1} and 0.23 day^{-1} respectively. After 20 days of cultivation, more than 82.3% of biochemical oxygen demand (BOD) was successfully removed by UKM6. While highest chemical oxygen demand (COD) removal achieved was 56.7% by UKM9. The combined process also proved effective at removing 59.1%, 70.0% and 96.2% of TN, PO_4^{3-} and NH_3-N respectively. Therefore, the proposed two-stage treatment method could offer a promising alternative to conventional POME treatment technologies and increase the effectiveness of microalgae for bioremediation.**

***Index Terms*—Microalgae, polyacrylamide (PAM), palm mill oil effluent (POME), bioremediation**

I. INTRODUCTION

The high demand of palm oil around the world has increased the production of palm oil mill industry in Malaysia. In 2020, Malaysia contributed about 25.8% and 34.3% of world's palm oil production and exports respectively [1]. Palm oil mill industry has been reported to produce 90 million tons of lignocelluloses biomass which consists of empty fruit bunches (EFB), oil palm trunks, oil palm fronds and also palm oil mill effluent (POME). From the total amount of biomass produced, about 50 million tons of POME has been generated each year [2]. This amounts shows that POME is the largest waste product from palm oil mill processing and therefore must be efficiently treated to ensure the sustainability of the industry and the environment.

The main characteristic of POME is commonly associated with high concentration of pollutants such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solid, nitrogen and phosphorus. Thus, if it is released directly into the water bodies without proper treatment, it will threaten the lives of humans and animals that depend on the water sources. Conventional treatment of POME using ponding system has its drawbacks. The main concern is the long hydraulic retention time (HRT). As reviewed by Mohammad *et al*. [3], the overall of 100–120 days is required to achieve compliant with the discharge standard enforced by authority. The open pond system also contributes to global warming due to the release of gases such as carbon dioxide, methane and hydrogen sulfide during the treatment process. Although advance technologies such as closed anaerobic methane digested tank, activated sludge plants with aerobic reactor and membrane bioreactor are able to prevent the gas emission, it require high cost of operation [4].

Recently, the use of microalgae in treating agricultural wastewater has been extensively explored by researchers [5]. The microalgae can consume the nutrient available in the POME for their growth, thus reducing the pollution concentration. Nevertheless, it still has a lot of aspect to go through to further enhance the process. The high amount of suspended solid in particular, often hindered the growth of microalgae thus reducing their effectiveness in adsorbing the nutrient from POME [6]. In this research, the main focus is to improve the growth of microalgae by reducing POME turbidity. Polymers such as polyacrylamide (PAM) are widely used to decrease colloidal stability and initiate flocculation for effective sedimentation. Bridging is considered to be the dominant mechanism in the adsorption of the long chain polymer [7]. PAM has a long-chain molecular structure which can extend its segments (loops and tails) into the solution, at least twice the thickness of the electrical double layer. These long segments of the polymers extruding from one particle can adsorb onto other particles, and therefore able to induce bridging flocculation [8].

The application of PAM for pretreatment in combination with microalgae remediation is scarcely reported in previous studies. Therefore, the present work is focused on investigating the efficiency of the two-stage treatment procedure by applying flocculation process before subsequent removal of nutrient using microalgae. Three native microalgae species were investigated in this study. These species were isolated from POME and have been proved to have high efficiency in POME treatment [9, 10].

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II. Methodology

A. Collection and Characteristic of POME

The POME used in this study was obtained from anaerobic pond at Sri Ulu Langat Palm Oil Mill in Selangor, Malaysia (3°52′59.34″ N, 101°16′35.87″ E). The samples were collected in 20 L plastic container and filtered using filter cloth to remove visible solids that might interfere with the coagulation-flocculation process. A minimal change in the initial nutrient concentration may not affect the main finding of the study. It is because the flocculation dosage, rather than initial nutrient concentration, is the significant factor in the flocculation process [11]. The sample was then stored at 4 ℃ until analysis. The physicochemical properties of the POME are listed in Table I.

TABLE I: Characteristics of Raw Anaerobic POME

Parameter	Value
pH	7.81 ±0.05
TSS (mg L^{-1})	2789.2 ±208.7
Turbidity (NTU)	1940 ±155.2
BOD (mg L^{-1})	880.6 ±79.3
COD (mg L^{-1})	2480.3 ±121.3
TN (mg L^{-1})	315.0 ±21.0
TP (mg L^{-1})	106.0 ±4.5
K (mg L^{-1})	2419.3 ±237.1
Mg (mg L^{-1})	591.0 ±34.8
Mn (mg L^{-1})	2.89 ±0.1
Zn (mg L^{-1})	0.51 ±0.19

B. Pre-treatment of POME

The optimum dosage of polyacrylamide (PAM) for turbidity removal was determined using standard jar test apparatus. The jar test is a laboratory-scale experiment that resembles the coagulation-flocculation process used in water treatment plant. The main objective is to determine the right amount of coagulants/flocculants for effective sedimentation of suspended solid.

The concentration of PAM used in this study was 300 ppm and added in different ratio into five 1L glass beakers that contains POME. One additional beaker of POME without PAM was used as control. The ratio of POME and PAM in each beaker is shown in Table II. The mixing process involves the use of mechanical flocculator to evenly distribute the PAM in POME. The speed of the stirring process was set up at 100 rpm for 2 min. Then the speed was reduced to 30 rpm for 30 min in order to encourage the flocculation process. The sample was leave for an hour for sedimentation of the flocs. In the present work, the residual sludge was removed through gravity sedimentation process. The concentrations of total solids solid (TSS) and turbidity were determined according to the procedures described in APHA 1995 using a 10ml sample in the 105 ℃ oven and HACH 2100 AN Turbidimeter respectively.

TABLE II: PAM Dosage Used in the Jar Test Experiment

Beaker	PAM (ml)	PAM concentration in mixture (ppm)
1	0	0
2	10	6
3	20	12
4	30	18
5	40	24
6	50	30

C. Microalgae Species and Cultivation

The species of microalgae that were used in this research are *Chlorella sorokiniana* (UKM3), *Chlamydomonas* sp. (UKM6) and *Scenedesmus* sp. (UKM9) with NCBI accession number of KP262477, KP262477 and KP898730 respectively. All species were obtained from POME sources after series of isolation, purification and screening experiments. The native strains produced greater result as it requires less time to adapt in its natural environment. In addition, local strains were also used to avoid biosafety issues [12].

Prior to cultivation in POME, the microalgae were cultured in a standard medium, bold basal medium (BBM) as a control experiment. BBM is the medium of inorganic salts which are widely used in culturing living freshwater plankton algae. The ingredients for BBM was prepared as per described by Ding *et al.* [13].

The experiments were conducted in 1L batch mode under controlled temperature of 25±2 ℃ and exposed to continuous light supply at 8000 Lux intensity. The inoculation was performed using 30% (v/v) inoculum size. The aeration was provided continuously with 5% (v/v) of CO_2 and 95% of atmospheric air. The CO_2 used in the study was of 99.9% purity and obtained from high pressure cylinders supplied by Leeden gases, Malaysia.

D. Determination of Microalgae Growth Parameter in POME

Microalgae growth rates recorded each day by measuring the dry biomass of microalgae. 10 mL of microalgae samples were filtered using pre-weighted 0.45 µm GF/C membrane filter paper, then oven dried at 105 ℃ overnight. The dry weight of the biomass and the specific growth rate, µ of the microalgae were calculated using Eq. (1) and equation (2) respectively.

$$DCW\ (mgL^{-1}) = (a-b) \big/ sample\ volume(L) \quad (1)$$

where a = weight of filter paper and biomass residue; b = weight of filter paper.

$$Specific\ growth\ rate, \mu = \frac{ln\ (x_2 - x_1)}{t_2 - t_1} \quad (2)$$

where x_1 and x_2 is initial and final biomass dry weight respectively; t_2 and t_1= Time (day) at x_2 and x_1 respectively.

E. Nutrient Removal Analysis

The nutrients such as BOD, COD, TN were measured according to HACH DR 2800 spectrophotometer manual [13]. A 10 mL of POME sample was taken from the culture every two days and centrifuged at 8000 rpm for 10 min. The supernatant was appropriately diluted prior to the analysis of respective elements [14]. The PO_4^{3-} and NH_3-N were determined using Ion Chromatography (882 Compact IC plus conductivity detector 1) (Metrohm, Switzerland) using a Metrosep A Supp 5 (150/4.0 mm) column and Metrosep C 4 100/4.0 respectively [15], [16]. Then, the nutrient removal percentage was calculated using Eq. (3):

$$Removal\ (\%) = \frac{C_1 - C_2}{C_1} \times 100 \quad (3)$$

where C_1 and C_2 = BOD, COD, TN, PO_4^{3-} and NH_3-N concentration at the beginning and at the end of cultivation respectively.

F. Statistical Analysis

In order to determine the significant of different in pollution removal analysis, results were statistically analyzed using one-way analysis of variance (ANOVA). Initially, the data was tested for homogeneity and independence (Leven's test) to satisfy the assumption for ANOVA. Then, post-hoc comparison test was done to determine the significant different of means for each growth rate towards the percentage of pollutants removed. The level of significant was set at $p < 0.05$.

III. Result and Discussion

A. Optimum Dosage of PAM for Turbidity Removal

The influence of different dosages of PAM was investigated to determine the optimum dosage. Results showed that the turbidity removal increased with an increase in PAM dosage. However after it reach a certain value, the turbidity removal started to decrease. As shown in Table III, the lowest turbidity value of 207.5 NTU was obtained using 24 ppm PAM. This represents 46.5% removal from the initial turbidity value of the POME which was 387.7 NTU. The POME could be overdosed at 30 ppm as the turbidity value raised to 240.5 NTU. Therefore, 24 ppm was selected as the optimum dosage for the PPM.

TABLE III: Pretreatment of POME Using PAM

Beaker	PAM concentration in mixture (ppm)	Turbidity (NTU)
1	0	348.0
2	6	264.3
3	12	255.7
4	18	241.0
5	24	207.5
6	30	240.5

As can be seen in Fig. 1, only 10% of turbidity removal was obtained without the addition of PAM, which was attributed to gravity settling. Increased volume of PAM enhanced the aggregation of particles, forming larger flocs that are subsequently removed by sedimentation.

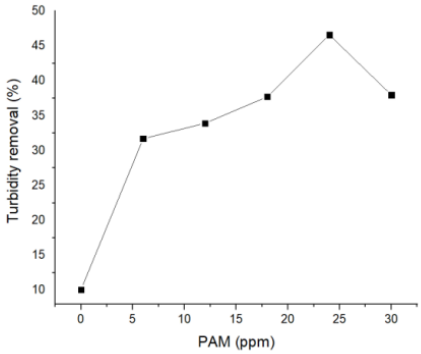

Fig. 1. Turbidity removal percentage using from POME using PAM.

The aggregation of the particles occurred through three major mechanisms: charge neutralization, sweep-floc mechanism and adsorption bridging. The addition of coagulants produces a various form of cationic species, which are adsorbed by negatively charged particles and caused charge neutralization [17]. Sweep coagulation occurs from formation of big amorphous precipitates (sweeping flocs) and the colloidal particles got enmeshed in these precipitates [18]. Whereas for the third mechanism, the polymer chains such as PAM can initiate flocculation by attach to the particle surface on one side, and the extended end on the other side might attach to the surface of other free particles, forming bridges between particles and polymer [19]. However, overdosing can disrupt this phenomenon. Therefore, optimum dosage of the flocculants should be determined in wastewater treatment process. This explains why the increase in dosage of PAM increases the percentage of turbidity removal up to a certain point, after which the addition of more flocculants will lower the turbidity removal percentage.

The flocculation process alone could not significantly reduce the concentration of organic matter and nutrient from the POME as shown in Fig. 2. Nevertheless, there was slight reduction of the observed parameters before and after pre-treatment using PAM.

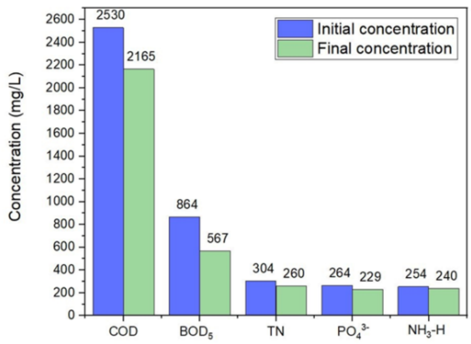

Fig. 2. Nutrient removal percentages for POME after using PAM as flocculant.

High levels of organic matter were found in POME with concentration of 2530 mg/L and 864 mg/L for COD and BOD respectively. The COD value of POME was reduced by 14.4% to 2165 mg/L and BOD value dropped to 567 mg/L equivalent to 34.3% lower from initial concentration. Other parameters such as TN and PO_4^{3-} were also reduced by 14.4% and 13.2% respectively. The lowest removal was NH_3-N with just 5% decrement from 254 mg/L to 240 mg/L. After the pre-treatment, the pollutants concentration was still considerably high for environmental discharge. Therefore, the POME was subjected to further remediation process using microalgae.

B. Microalgae Growth and Nutrient Removal Using Pre-treated POME

Table IV show the specific growth rate of microalgae in POME pre-treated using PAM. Results show that all the microalgae able to sustain growth with highest growth rate was achieved by UKM3. The growth rate achieved in present study was higher than previous study using the same species by as reported by Syafaini *et al.* [20]. In their study, the growth rate of UKM3, UKM6 and UKM9 in POME without PAM pretreatment were 0.17 day^{-1}, 0.15 day^{-1} and 0.14 day^{-1} respectively. In another study by Farhan *et al.* [10], the growth rate of 0.155 day^{-1} was reported for UKM9 in anaerobic POME. Therefore it can be concluded that the application of PAM in POME was able to stimulate better growth of the microalgae. The result also higher than the reported 0.17 day^{-1} in previous study by Kamarudin *et al.* [21] using *Chlorella vulgaris* in 100% POME concentration.

TABLE IV: SPECIFIC GROWTH RATE OF MICROALGAE IN PRE-TREATED POME

Microalgae	Specific Growth Rate, day^{-1}
UKM3	0.2864
UKM6	0.2484
UKM9	0.2310

The BOD and COD removal rates for different microalgae are shown in Fig. 3a and Fig. 3b. It was observed that the residual BOD in the POME decreased rapidly with time during the first 8 days especially for UKM3, before slowed down thereafter.

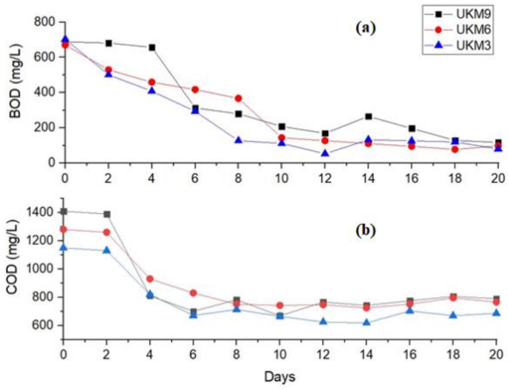

Fig. 3. a) BOD removal and b) COD removal for three species of microalgae.

The same trend can be seen for COD where the concentration reduces markedly at the start of the cultivation until the 6th day of the cultivation. This rapid assimilation of BOD and COD from the early stage of the cultivation could be attributed to the high adaptability of the microalgae in the POME. This would suggest better light availability for the microalgae resulted from lower turbidity in pretreated POME using PAM. Thus microalgae are able to grow instantly using light as the energy source as well as organic carbon assimilation for carbon source [22]. This is supported by Tangahu *et al.* [23] which in their study, statistically higher ($p<0.05$) removal rates and removal efficiencies were determined with higher light supplies.

The PO_4^{3-} and NH_3-N concentrations in POME also decreased over the cultivation period. The PO_4^{3-} removal generally showed comparable pattern with BOD and COD as illustrated in Fig. 4a.

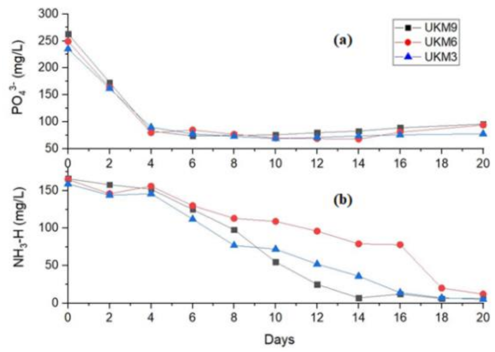

Fig. 4. a) PO_4^{3-} and b) NH_3-N removal profiles in POME using microalgae.

However, the reduction of NH_3-N occurred more gradually in contrast to PO_4^{3-} as observed from Fig. 4b. For instance, there was only slight decrease of NH_3-N by UKM9 until the day 4, before notable depletion of the concentration between days 4 to day 14. The lower assimilation of NH_3-N at the beginning of the cultivation was probably caused by the presence of other nitrogen species namely NO_3, NO_2 and organic-N, such as urea and amino acids. Microalgae are often able to utilize these different nitrogen sources based on its availability in the environment [24]. Thus it may directly interfere with the NH_3-N uptake from the medium.

The average nutrient removal rates were not markedly different among the three microalgae, as shown Table V.

TABLE V: NUTRIENT REMOVAL EFFICIENCY USING MICROALGAE FOR PRETREATED POME USING PAM

Microalgae	Removal percentage (%)				
	COD	BOD	TN	NH_3-N	PO_4^{3-}
UKM3	48.2	78.2	59.1	96.2	60.7
UKM6	47.5	82.3	56.9	96.0	70.0
UKM9	56.7	78.0	47.2	95.2	60.0

However, there was distinct differences were found between COD and NH_3-N removal by each microalgae. For example, the highest COD removal achieved by UKM9 was 56.7% compared to 95.2% removal of NH_3-N by the same species. This possible reason was due to the higher initial concentration of COD in the medium. The result for UKM3 and UKM6 were comparable, where both species removed about 96% of NH_3-N, almost 50% of COD and average 80% of BOD from the medium. In term of PO_4^{3-}, UKM6 achieves the highest efficiency among the three tested species with 70.0% removal.

Generally, the efficiency of nutrient removal corresponds to the growth rate of the microalgae species. Highest removal of TN and NH_3-N was obtained by UKM3 which shows the highest growth rate of 0.286 day^{-1}. Although UKM6 achieved higher BOD removal than UKM3, it was not statistically different ($p>0.05$). On the other hand, UKM9 which produced lowest growth rate at 0.231 day^{-1} recorded lowest removal efficiency for all tested parameters except for COD. This discrepancy might be associated with the specific microalgae-bacteria interaction in the POME. As reported by Farhan *et al.* [25], the interaction between UKM9 and bacteria in POME produced the highest COD removal percentages among the tested species.

The COD removal in the present study range from 48% to 57% is much higher than 31% obtained by Farhan *et al.* [10] using UKM9 in undiluted anaerobic POME. Another study performed by Kamyab *et al.* [26], 34% of COD removal was attained using diluted POME from facultative pond. As for BOD removal, Emparan and Harun, [27] reported better removal efficiency of 81%–90%, however it was achieved using much diluted sample, of which only 10% POME concentration used in the experiment. This study also produced higher TN and PO_4^{3-} removal than previously determined by Bajunaid *et al.* [12] which reported removal of 37% of TN and 52% of PO_4^{3-} obtained after 25 days of POME treatment using UKM9. In term of NH_3-N removal, the average rate of 95.8% obtained in this study was in accordance with the results previously reported by Emparan and Harun [27] and Tan *et al.* [28], wherein a maximum of

96.0% and 91.3% removal were achieved respectively. Therefore, the present work successfully demonstrated that microalgae bioremediation can be practically improved by using pre-treated POME using PAM for further enhancement of the treatment process.

In the midst of controversial sentiment given by the European countries on palm oil industry, Malaysia is totally committed to implement a cleaner production initiative. The government has introduced the Malaysian Sustainable Palm Oil (MPSO) Certification Scheme to ensure responsible and sustainable palm oil production in Malaysia. The application of microalgae for POME treatment has tremendous potential to assist palm oil industry achieving key sustainability issues embedded in MSPO's principles. The microalgae based POME treatment is highly suitable to attain the fifth principle; which required a plan to reduce significant pollution and emission. As shown in this study, the application of two-stage PAM-microalgae for POME treatment has enhanced the efficiency of microalgae in assimilating nutrient from POME. This will contribute to preserving the local biodiversity from wastewater pollution. Nevertheless, scaling up microalgae cultures for industrial production is a complex task. There are some concerns and obstacles that need to be tackled to turn the theoretical promise into practical and economic success.

IV. Conclusion

The application of PAM as flocculants in pre-treatment prior to microalgae bioremediation process has successfully enhanced the wastewater treatment. A remarkable 46.5% reduction of POME turbidity was achieved under optimum dosage. The increase of microalgae growth rate as compare to previous studies without the pre-treatment, has verified the high potential of this proposed two-stage treatment process. All three microalgae species successfully removed more than 95% NH_3-N. The highest removal of BOD and PO_4^{3-} of 82% and 70% respectively was achieved by UKM6. Although many microalgae based system have been developed for removing pollutants from wastewater, there is still a need to improve their performance. The present work provides a better insight into integrating PAM-microalgae system to further enhance nutrient removal process in POME.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

M. Z. Rusli– conducted the research, analyzed the data and wrote the paper; A. A. H. Khalid– supervised the experiment, write the paper; G. T. Ding– interpretation of the results; M. S. Takriff– Critical reviewing and final approval of the last version to be submitted. All authors had approved the final version. All authors had approved the final version.

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