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Effect of microalgae-to-palm oil mill effluent (POME) ratio for rapid effective pollutants removal and biomass production

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ABSTRACT

In Malaysia, a conventional series of open ponding systems is mostly used to treat palm oil mills effluent (POME). However, the use of ponding systems is the current major challenge to the palm oil industry for POME treatment as it is not efficient and required long treatment process. The accidental discharge of POME with a high concentration of organic and inorganic pollutants will pollute the environment. Phycoremediation is the support solution due to its advantages such as environmentally friendly and cost-effective for pollutants removal processes. The aim of this study is to determine the efficiency of suspended free-cells *Chlorella vulgaris* in treating POME by investigating the effect of microalgae-to-10% palm oil effluent (POME) ratio on the removal of chemical oxygen demand (COD), biochemical oxygen demand (BOD_3), ammoniacal nitrogen (NH_3–N), and total phosphorus (TP) as well as biomass production from POME. At the end of 10 d treatment, the higher removal efficiency of COD, BOD_3, NH_3–N, and TP were obtained at the ratio of 1:10 with 79%, 90%, 100%, and 86%, respectively as compared to other ratios. The results also revealed that the ratio of 1:10 exhibited the best microalgae growth rate in terms of biomass concentration at 0.269 g/L. This treatment system could be a viable technology for sustainable and continuous POME treatment with simultaneous biomass production, considering the shorter treatment time required and its effectiveness in reducing the COD, BOD_3, NH_3–N, and TP from POME.

Keywords: *Chlorella vulgaris*; Microalgae cultivation; Biomass growth rate; Wastewater treatment; Nutrient removal efficiency; Sustainability

1. Introduction

The production of palm oil is ever-expanding, consequently, this directly causes an increase in the production of palm oil mills effluent (POME). The generation of POME is in a large quantity at a time and its conventional handling methods are not currently feasible, which leads to large quantities of untreated wastewater being produced. This wastewater commonly exceeds the industrial effluent standards set by the Ministry of Natural Resources and Environment and will end up being discharged into the aquatic environment [1,2]. Certainly, this causes damage to the aquatic environment and degenerates fragile aquatic ecosystems.

The conventional treatment technologies such as ponding systems (series of anaerobic, facultative, and aerobic/algae pond), hybrid ponding systems-open digesters, and hybrid ponding systems-hybrid open digester-extended aeration (installed at aerobic/algae ponds) [3] had been used to treat POME. Currently, pre-discharge POME treatment is scantily performed by palm oil industries, and this is mostly due to the inefficacy of conventional treatment technologies for complete removal of pollutants, predominantly time-consuming, environmentally unsustainable,

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and/or have high operational costs. In order to rectify these issues, a lot of polishing/tertiary technologies have been applied such as coagulation–flocculation [4], advanced oxidation [5], up-flow anaerobic sludge blanket [6], hybrid activated sludge-coagulation and flocculation–filtration [7], membrane filtration [8], plant [9], and microalgae [10,11]. Among these methods, the phycoremediation approach gains more attention because microalgae can use pollutants from wastewaters as a source of nutrients for growth resulting in simultaneous pollutants removal and biomass production within a short period of time [12,13]. Organic carbon and nutrients presence in wastewaters were assimilated and stored for growth and building up microalgae cells resulting in the reduction of pollutants level [11,12,14]. As a result, water quality can be improved. According to [15], the phycoremediation of wastewater coupled with biomass production were developed since 1950 and being practiced widely to date. Microalgae are microscopic photosynthetic organisms that can be obtained easily from different habitats [16]. POME with adequate nitrogen and phosphorus can be used as a growth media for microalgae cultivation [17]. Thus, the level of these nutrients can be reduced to the permissible discharge limits by the Ministry of Natural Resources and Environment.

The used of real wastewater POME as growth media can replace the synthetic nutrients media which is high in cost. At the end of the cultivation period, the biomass of microalgae with high protein, lipids, and starch can be converted for valuable bioproducts [18]. The following are some other benefits of phycoremediation: (i) increases dissolved oxygen (DO) levels via photosynthetic activity; (ii) fix CO_2 from the atmosphere as a source of carbon for growth thus reduce greenhouse gasses [12,15]. Inarguably, microalgae culture is an advantageous substitute for current conventional methods for pollutants removal of chemical oxygen demand (COD), biochemical oxygen demand (BOD_3), ammoniacal nitrogen (NH_3–N), and total phosphorus (TP) from POME and other wastewaters. Many studies had been reported the use of suspended free-cells to treat POME for simultaneous biomass production and pollutants removal [12,13,19]. The 10% POME was proven to be the best sample for pollutants removal and microalgae growth rate reported by the previous study [11]. However, the best ratio of *Chlorella vulgaris* to POME (v/v) for removal of COD, BOD_3, NH_3–N, and TP as well as biomass production have not been studied to date. Therefore, this study aims to determine the efficiency of *C. vulgaris* for POME treatment by investigating the effect of microalgae-to-10% palm oil effluent (POME) (v/v) ratio on pollutants removal and biomass production. In this study, the best ratio will be determined, considering the shorter retention time required to enable the 10% POME characteristics that meets the permissible discharge standards.

2. Materials and methods

2.1. Preparation of C. vulgaris cells culture

The species of microalgae used in this study was *C. vulgaris* which was supplied by the Commonwealth Scientific and Industrial Research Organization (CSIRO) Microalgae Research Centre (Tasmania, Australia). The microalgae cells was cultivated in MLA culture media at a volume ratio of 1:10 based on manual instruction by the CSIRO Microalgae Research Centre. The used MLA culture media for microalgae cells cultivation consists of $MgSO_4{\cdot}7H_2O$ (49.4 g/L), $NaNO_3$ (85.0 g/L), K_2HPO_4 (6.96 g/L), H_3BO_3 (2.47 g/L), H_2SeO_3 (1.29 mg/L), $NaHCO_3$ (16.9 g/L), $CaCl_2{\cdot}2H_2O$ (29.4 g/L), Na_2EDTA (4.36 g/L), $FeCl_3{\cdot}6H_2O$ (1.58 g/L), $NaHCO_3$ (0.60 g/L), $MnCl_2{\cdot}4H_2O$ (0.36 g/L), $CuSO_4{\cdot}5H_2O$ (1.0 g/L), $ZnSO_4{\cdot}7H_2O$ (2.2 g/L), $CoCl_2{\cdot}6H_2O$ (1.0 g/L), $Na_2MoO_4{\cdot}2H_2O$ (0.6 g/L). The culture condition was in accordance with the previous studies [10]. Duran bottles (Sigma-Aldrich's, Malaysia) opening was stuffed with cotton plugs, shaken at 150 rpm, illuminated under fluorescence light of 5,000 lux (12 h light:12 h dark conditions) at room temperature in the algal research laboratory (Department of Chemical and Environmental Engineering, Universiti Putra Malaysia). The axenic culture was maintained by two times routine subculturing to prolong their life and to expand the cells number as stock media. The microalgae cells were harvested prior to the phycoremediation study. All the chemicals used in this study were purchased from R&M Chemicals (Malaysia) suppliers.

2.2. Preparation of palm oil mill effluent

POME was collected from a wastewater treatment pond at Sime Darby Palm Oil Plantation Sdn. Bhd., Labu, Negeri Sembilan, Malaysia. The sample (POME) used for this work was collected from the final pond and stored in 20 L plastic containers. The sample was kept at 4°C to minimize biodegradation by bacterial activity. Before the treatment process, the POME sample was initially filtered to remove suspended sludge and microorganisms to reduce the high turbidity and dark brown of POME. The characteristics of the POME are shown in Table 1.

2.3. Experimental set up for phycoremediation studies

Based on the previous study [11], the 10% POME exhibited the highest pollutants removal and biomass concentration as compared to 25%, 50%, 75%, and 100% POME at a fixed ratio of microalgae cells culture to POME (1:5, v/v). In this study, the concentration of POME as culture media was fixed at 10%. Three different ratios of microalgae to POME (1:1, 1:5, and 1:10) were prepared in 500 mL bottles as shown in Table 2. All samples were aerated via orbital shaker (Brand Smith) at 150 rpm to incorporate oxygen and distribute nutrients efficiently throughout the culture media to ensure successful *C. vulgaris* cultivation [24]. The bottles were cotton plugged to filter the transport of air into and out of the bottles to prevent fungal spores and bacterial contaminations. The phycoremediation studies were performed in a batch culture at room temperature, shaken at 150 rpm, and illuminated under fluorescence light of 5,000 lux (12 h light:12 h dark conditions). Each sample was collected every 3 d for growth rate, COD, BOD, NH_3–N, and TP analysis. The pollutants removal efficiency was calculated using Eq. (1):

$$\text{Pollutant removal}\ (\%) = \left[\frac{(C_0 - C_i)}{C_0}\right] \times 100\% \quad (1)$$

Table 1
Characteristics of raw POME from the final pond

Parameter	POME	Strength classification of untreated wastewater [20–22]	Department of Environment discharge limitation [23]
COD, mg/L	3,665 ± 91.92	Strong	1,000
BOD_3, mg/L	1,015 ± 120.21	Strong	50
NH_3–N, mg/L	7.47 ± 0.66	Weak	100
TP, mg/L	9.5 ± 0.71	Medium	–
pH	8.27 ± 0.02	–	5

Table 2
Composition of POME concentration and microalgae as suspended free-cells

Ratio of microalgae to POME (v/v)	Dilution factor (concentration)	Labeling	Volume of distilled water (mL)	Volume of raw POME (mL)	Total volume of POME (mL)	Volume of microalgae (mL)
1:1	10% POME	*A*1, *A*2	180	20	200	200
1:5	10% POME	*B*1, *B*2	180	20	200	40
1:10	10% POME	*C*1, *C*2	180	20	200	20

where C_0 (mg/L) and C_i (mg/L) are the mean values of pollutant concentrations at the initial time t_0 (day) and time t_i (day), respectively.

2.4. Analytical method

2.4.1. Optical density measurements and biomass concentration

The OD of microalgae cells was measured using UV-Vis spectrophotometer (Brand GENESYS™ 10S, USA) at an optimum wavelength of 680 nm (OD_{680}). Approximately 1 mL of the sample was put into a clean UV-cuvette to measure the absorbance value of the microalgae cells growth rate. POME without microalgae was used as a blank sample. A calibration curve was prepared by plotting OD vs. biomass concentration (in dry weight, g/L). The OD of microalgae cells was recorded and biomass concentration was obtained from the calibration curve.

2.4.2. Chemical oxygen demand

The COD procedure is based on the chemical decomposition of organic and inorganic contaminants, dissolved, or suspended in the sample. The test aims to study the efficiency of *C. vulgaris* in the removal of COD from POME. The HACH DR/4000 spectrophotometer is a powerful scanning spectrophotometer that is ideally suited for applications in diverse fields especially in water and wastewater treatment (HACH Company, 2017). This test was carried out for all the samples using DR/4000 Spectrophotometer (Brand HACH, USA) by following HACH DR/4000 Method 8000 (Reactor Digestion Method). Prior to COD analysis, 2 mL of samples were added to each vial, shaken, and the samples were digested in HACH COD reactor for 120 min. After digestion, the samples were allowed to cool down to room temperature. The samples were then tested for COD in HACH DR/4000 spectrophotometer with HACH Program 2720 for high range (HR). The readings obtained were in mg/L COD which are defined as the mg of O_2 consumed per liter of sample.

2.4.3. Biochemical oxygen demand

The test aims to study the efficiency of *C. vulgaris* in the removal of BOD_3 from POME. This test was carried out for all the samples using Method 5210B coupled with DO meter. Prior to BOD_3 analysis, dilution water was prepared. The dilution water was prepared by adding 10 mL of phosphate buffer solution (pH 7), 10 mL of magnesium sulfate solution ($MgSO_4{\cdot}7H_2O$), 10 mL of calcium chloride solution ($CaCl_2{\cdot}2H_2O$), and ferric chloride solution ($FeCl_3{\cdot}6H_2O$) to 10 L of sterile distilled water and left for aeration for at least 8 h. Then, 299 mL of the aerated dilution water was added into BOD bottles (labeled as day 0 and 3) containing 1 mL of the sample. The samples in the BOD bottles labeled as day 0 were measured directly using DO meter (Brand Shen Zhen Yieryi Technology, China). Meanwhile, the BOD bottles labeled as day 3 were wrapped with aluminum foil and further stored in the incubator at 20°C for 3 d. The reading of D_0 was again taken on day 3. The BOD_3 of each sample from day 0 to 10 was measured using the following equation:

$$\mathrm{BOD}_3, \left(\mathrm{mg/L}\right) = \frac{\left(D_0 - D_3\right)}{P} \tag{2}$$

where D_0 (mg/L), D_3 (mg/L), and P are the dissolved oxygen (DO) of the diluted sample immediately after preparation, DO of the diluted sample after 3 d incubation at 20°C (mg/L), and the decimal volumetric fraction of the sample used, respectively.

2.4.4. Ammoniacal nitrogen

The NH_3–N content in the samples was determined using the HACH DR/4000 spectrophotometer (HACH

Company, 2017). The test aims to study the efficiency of *C. vulgaris* in the removal of NH_3–N from POME. This test was carried out for all the samples using HACH DR/4000 spectrophotometer by following HACH DR/4000 Method 10031 (Salicylate Method). Prior to AN analysis, 1 packet of ammonia salicylate reagent powder pillow, 1 packet of ammonia salicylate reagent powder pillow, and 0.1 mL of sample were added into each vial. The vials were shaken and left for a reaction for about 20 min. Then, the samples were tested for NH_3-N content using HACH DR/4000 spectrophotometer with HACH Program 2465 for HR.

2.4.5. Total phosphorus

The phosphorus content in the samples was determined using the HACH DR/4000 spectrophotometer by following HACH DR/4000 method 10127 (molybdovanadate method with acid persulfate digestion). The test aims to study the efficiency of *C. vulgaris* in the removal of phosphorus from POME. Prior to the TP analysis, 5 mL of sample, and 1 packet of potassium persulfate powder pillow were added to each vial and the samples were digested in COD Reactor for 30 min. After digestion, the samples were allowed to cool down to room temperature and each vial was then added with 2 mL of 1.54 N sodium hydroxide and 0.5 mL of molybdovanadate reagent. Then, the samples were left for a reaction for about 7 min and further measured for TP in HACH DR/4000 spectrophotometer with HACH Program 3040 for HR.

2.4.5. Hydrogen ion concentration

The pH of the sample was determined based on Model 3505 user guide using pH meter (Brand Jenway, UK).

2.5. Statistical analysis

All the experiments were carried out in duplicate and data points were analyzed in duplicate. The analysis of variance (ANOVA) SPSS version 20 was used to determine the statistical significance among treatments at $p < 0.05$.

3. Results and discussion

3.1. Biomass concentration of suspended free-cells

The growth rate of microalgae cells in POME are graphically represented in Fig. 1. The lag-phase/adaption phase period for adaptation to the POME media was found to be short about 2 d for sample ratio 1:1. There is no apparent lag phase/adaption phase for both sample ratio 1:5 and 1:10. During the exponential phase/log phase/growth phase, the biomass concentration progressively increased from sample ratio 1:10 (day 0–10), sample ratio 1:5 (day 0–8), and sample 1:1 (day 0–8) reflects the active photosynthetic activity of microalgae cells. The stationary phase and/or death phase lasted from day 8 to the end of treatment for sample 1:5 and 1:1, respectively. There is a statistically significant difference ($p < 0.05$) on the biomass concentration between different samples. The biomass concentration increases from an initial concentration of about 0.041, 0.045, and 0.041 g/L to 0.099, 0.174, and 0.269 g/L for sample 1:1, sample 1:5, and sample 1:10, respectively. The photosynthetic cells consumed pollutants such as organic and inorganic compounds as a source of nutrients for growth. Sample ratio 1:10 exhibited the best biomass concentration at the end of treatment, as compared to other samples where it achieved 85% of the increase in biomass concentration. As the ratio of microalgae to POME (v/v) increases (1:10 > 1:5 > 1:1), the biomass concentration decreases. These findings suggest that the ratio of microalgae to POME (v/v) is an indicator of the active photosynthetic activity and growth rate of microalgae cells. Too high-density culture resulting in the self-shading, caused the active photosynthesis activity decreased caused by poor utilization of light, as also previously reported [9,25,26]. This phenomenon inhibits the growth rate of microalgae cells that reduced the assimilation rate of pollutants within cells. The biomass concentration achieved in this study was higher, as compared to [27] and [28]; however lower than that obtained by Selmani et al. [29]. This indicated that high biomass production depends on the types of microalgae cells, nature, and severity of wastewaters to be used in the treatment system.

3.2. COD removal efficiency

The COD concentration in 10% POME throughout the treatment period using suspended free-cells *C. vulgaris* are graphically represented in Fig. 2. There is a statistically significant difference ($p < 0.05$) on the COD removal efficiency

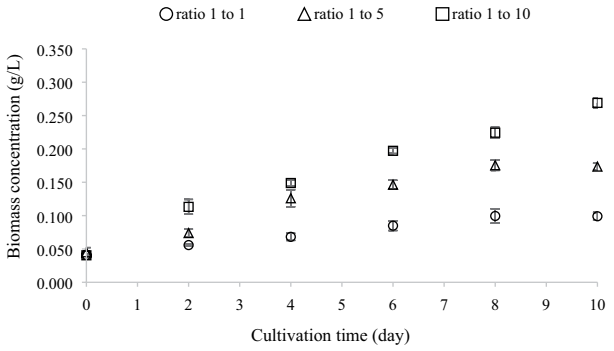

Fig. 1. Biomass concentration vs. time with different ratio of microalgae to 10% POME.

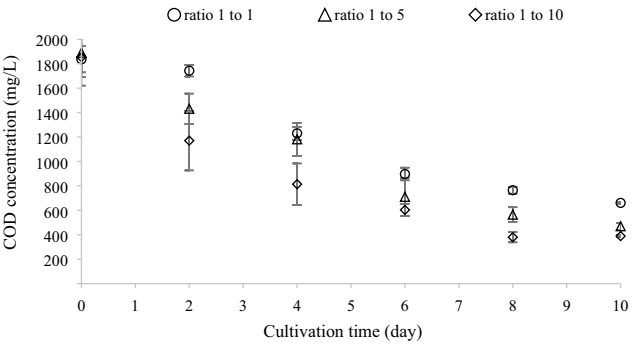

Fig. 2. COD vs. time with different ratio of microalgae to 10% POME.

between different samples. Sample ratio 1:1, 1:5, and 1:10 removed 64% (661 ± 86.27 mg/L), 75% (470 ± 42.43 mg/L), and 79% (389 ± 33.94 mg/L) of the initial COD concentration (1,860 ± 27.22 mg/L) for 10 d of treatment, respectively. The gradual COD reduction toward the end of treatment was due to the consumption of organic and inorganic carbon as a substrate by microalgae cells through mixotrophic metabolism [10]. During the treatment process, the active reaction of photosynthesis and cellular respiration generates energy which was further used for bio-synthesis in dark conditions [30]. Organic material from wastewater can be also be removed by the natural bacteria through the decomposition process [31]. However, a slight increment of COD concentration may have occurred throughout the treatment due to the excretion of allogenic organic matter [12] and glycolic acid [32] by photosynthetic microalgae cells. Lower COD removal of 48% [12], 29% [33], and 63% [30] had been previously reported using *Chlorella* sp. for POME treatment as compared to this study. This indicated that this treatment system was more suitable for a rapid and effective treatment for the removal of COD from POME. The successful use of microalgae cells to reduce COD from sewage wastewater has also been reported by some other researchers [34].

3.3. BOD_3 removal efficiency

BOD used the capability of microorganisms using the oxidizing agent of molecular oxygen to oxidize organic material to CO_2 and water [34]. The BOD level was increased with a higher concentration of organic matter present in the water body. The complete decomposition of organic matter by microorganisms required more dissolved oxygen (DO) resulted in the reduction of DO level and also increment of BOD level. Therefore, a lower DO level indicates poor water quality which dangerous to aquatic life. The removal of BOD_3 from POME among different samples is graphically represented in Fig. 3. There is a statistically significant difference ($p < 0.05$) on the BOD_3 removal efficiency between different samples. The BOD_3 from each sample was gradually decreased starting from day 0 to 10. The total BOD_3 removal from initial concentration towards final concentration for sample ratio 1:1 (70%), sample ratio 1:5 (81%), and sample ratio 1:10 (90%). During the treatment period, the BOD_3 reduction from all samples reflects the removal of dissolved organic compounds and derivates [35]. The reduction of BOD_3 was higher than COD from all samples. This is greatly attributed to the degradation process that occurred through biological activity rather than the chemical agent [36]. These results are comparable with other studies, that shows *Spirulina plantesis* (78% removal) [37] and *C. vulgaris* (62% removal) [28] have a potential as an alternative agent for removal of BOD from POME.

3.4. NH_3–N removal efficiency

An excessive concentration nitrogen level in the wastewater contributed to the eutrophication problem. According to [34], nitrogen is an essential element for microalgae growth. Therefore, the cultivation of microalgae cells in nitrogen-rich wastewater can improve its water quality together with the production of valuable biomass. The gradual reduction of NH_3–N by *C. vulgaris* via assimilation was obtained from day 0 to 10 as represented graphically in Fig. 4. There is a statistically significant difference ($p < 0.05$) on the NH_3–N between different samples. The reduction of NH_3–N and TN corresponded to the uptake of nitrogen by microalgae cells and the volatilization process since the treatment systems were shaken [38]. Results indicated that the maximum removal of NH_3–N was observed to be 82%, 96%, and 100% for sample ratio 1:1, 1:5, and 1:10, respectively. However, a slight increment of NH_3–N concentration may have occurred throughout the treatment due to the decomposition of dead microalgae cells resulting in the release of consumed NH_3–N back into the wastewater media [27]. Cultivation of suspended free-cells of *C. vulgaris* in the POME also resulted in the noticeable reduction of total nitrogen (TN) up to 82% [19] and NH_3–N up to 61% [37] removal efficiency, respectively.

3.5. TP removal efficiency

Phosphorus is also an essential nutrient for microalgae growth, cellular process, mechanisms of energy transfer, and biosynthesis of DNAs [21,39,40]. The orthophosphates, H_2PO4^- and HPO_4^{2-} are preferred form of phosphorus to be assimilated by plants including microalgae [21]. Phosphorus synthesized protein in the plants, which are responsible for the continuous production of new tissue

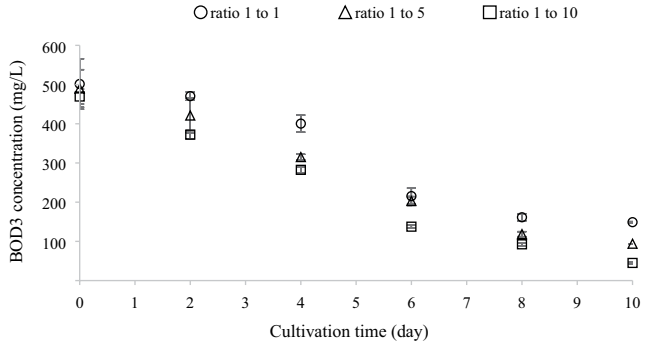

Fig. 3. BOD_3 vs. time with different ratio of microalgae to 10% POME.

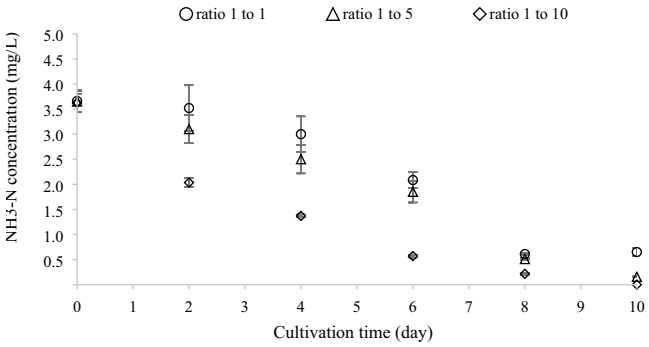

Fig. 4. NH_3–N vs. time with different ratio of microalgae to 10% POME.

through cells division and also associated with complex energy transformations [35]. TP removal from %10 POME in the presence of *C. vulgaris* from all samples are graphically represented in Fig. 5. There is a statistically significant difference ($p < 0.05$) on the TP between different samples. The reduction of phosphorus concentration in all samples shows that phosphorus was taken up from 10% POME by the *C. vulgaris*. According to [41], the mechanism of phosphorus removal occurs through assimilation into biomass and dynamics of intracellular polyphosphate compounds, as well as precipitation at high pH. There are some other studies that reported a fluctuated trend of TP concentration over the treatment period. The photosynthetic microalgae cells produces DO which further consumed by bacterial for a breakdown of organic matter resulting in the increment of TP concentration [27,42]. Overall, the highest percentage of TP removal was 87% which achieved by sample ratio 1:10 on day 8. Meanwhile, about 61% and 67% of TP removal were achieved by sample ratio 1:1 and 1:5 on day 10, respectively. The high removal efficiency of phosphorus from the POME reached over 95% obtained by [25] and [34].

4. Conclusions

The ratio of microalgae to POME (v/v) such as 1:1, 1:5, and 1:10 were found to have significant effects on the COD, BOD_3, NH_3–N, and TP removal efficiency together with microalgae cells growth rate (biomass concentration). The *C. vulgaris* can effectively reduce the aforementioned pollutants from 10% POME within short treatment time, which meets the permissible discharge standard set by the Department of Environment Malaysia. The ratio of microalgae to POME at 1:10 was shown to have the highest impact on the pollutants removal efficiency and biomass concentration. The maximum removal efficiency of COD (79%), BOD_3 (90%), NH_3–N (100%), and TP (86%) were obtained from this ratio. This noteworthy phycoremediation approach with shorter retention time for POME treatment and biomass production can be a potential viable alternative technology system for a conventional series of open ponding systems that consumes several months of retention time. Phycoremediation of POME has been proven to be an eco-friendly technology at minimal maintenance cost. This sustainable treatment system can easily be integrated into existing process flowsheets of palm oil mills. Based on the above noteworthy accomplishment, thus it is recommended that continues POME treatment through phycoremediation can be used as a viable alternative solution to wastewaters including POME in the future.

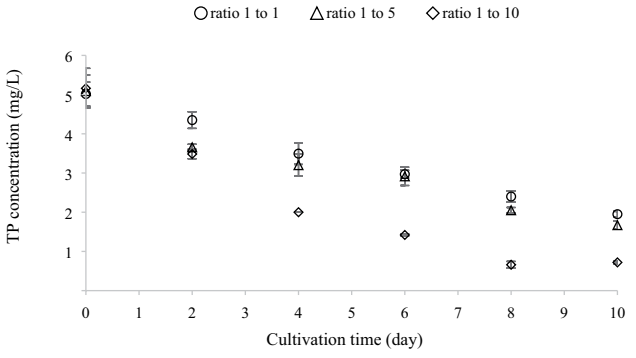

Fig. 5. TP vs. time with different ratio of microalgae to 10% POME.

Author contributions

R.H. conceived the ideas of the paper and supervised the study. Q.E. and J.A.K. designed and conducted the analyses and led the writing of the manuscript. Q.E. and J.A.K. collected the data in the field and contributed to the writing. All authors contributed to writing the present paper and gave their final approval for submission.

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