TRANSACTIONS ON SCIENCE AND TECHNOLOGY

Growth and Biomass Production of Native Microalgae *Chlorella* sp., *Chlamydomonas* sp. and *Scenedesmus* sp. Cultivated in Palm Oil Mill Effluent (POME) at Different Cultivation Conditions

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ABSTRACT

The main objective of this research was to study native microalgae *Chlorella* sp., *Clamydomonas* sp. and *Scenedesmus* sp. growth and biomass productivity at different source of nutrient, inoculum concentration and aeration factor. Agricultural wastewater, palm oil mill effluent (POME) was used as a nutrient source which was compared with Bold's Basal Medium (BBM) by culturing the microalgae at different inoculum concentration in 1-Litre transparent vessel with 700ml of working volume. Fixed aeration rate, 1L/min was set for all with 14000 lux of light intensity at 25±2°C cultivation temperature. The results were also compared with the non-aerated cultures which only mixing was being provided for the microalgae growth. Based on the growth rate, 30% of inoculum shows the highest growth rate for all these three microalgae species *Chlorella* sp. (µmax =0.2712), *Chamydomonas* sp. (µmax =0.2547) and *Scenedesmus* sp. (µmax =0.1867) cultured in POME with air being supplied as the aeration factor. However, in BBM 30% of inoculum for *Chlamydomonas* sp. (µmax =0.5082) and *Scenedesmus* sp. (µmax =0.3402) manifest highest growth rate while *Chlorella* sp. does not exhibit significant effect at different inoculum concentration. The non-aerated culture condition shows 20% of inoculum for *Chlamydomonas* sp. (µmax =0.125) and *Chlorella* sp. (µmax =0.2052) cultures give the highest growth rate with POME. Similar results were obtained for *Chlamydomonas* sp. (µmax =0.1673) and *Chlorella* sp. (µmax =0.1855) cultured in BBM at non-aerated condition. *Scenedesmus* sp. shows the highest growth rate with 10% of inoculum at the non-aerated condition for both POME (µmax =0.1900) and BBM (µmax =0.1975). From the growth curves, the adaptation (lag phase) and exponential period of microalgae species were determined. Based on the results, all 3-species cultured in POME required 6 days for the lag phase and 14 days for the exponential phase which is doubled the period taken for BBM as the nutrient medium.

KEYWORDS: Microalgae, Wastewater, Palm Oil Mill Effluent (POME), Culture Condition, Nutrient Source

Data With Detail Description - *Biological* sciences
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INTRODUCTION

Microalgae capable of consuming nutrients for growth and absorb toxin in suspension. Apart from that, microalgae can be used in CO_2 fixation as it undergoes photosynthesis process where CO_2 will be consumed for growth and O_2 will be released as the respiration product. The simple cell structure of microalgae with a large surface area per unit volume of microalgae cell explained the rapid growth and cell division of microalgae. This special characteristic of microalgae allows them to consume nutrients at a high rate of consumption (Khan *et al.*, 2009). Microalgae cultivation under optimum growth conditions allows microalgae optimum cell division for every 3-4 hours. However, most species will take 1-2 days for the population to increase (Williams & Laurens, 2010). Microalgae metabolic mode can be divided into a few categories; heterotopic, autotrophic, mixotrophic, photoheterotrophic, and photoautotrophic (Brennan & Owende, 2010; Liang *et al.*, 2009). Some microalgae species can practise more than one metabolic mode at the same time. This phenomenon

happens to several microalgae species that has been studied such as *Chlorella* sp. and *Scenedesmus* sp. that are able to switch from autotrophic to heterotrophic mode depending on the source of nutrients available for utilization. Other studies reported that *Chlamydomonas* sp. also practises more than one metabolism mode when it is cultivated in POME and this multimode attribute is known as mixotrophic metabolism (Ding *et al.*, 2016).

Medium design and composition influence the growth and performance of microalgae. Insufficient nutrients provided during microalgae cultivation will slow down the growth rate and productivity of microalgae. There are growth and productivity requirements that need to be fulfilled in microalgae cultivation such as optimal supply of macronutrients (carbon, nitrogen, phosphorus), micronutrients (Ca, Mn, Fe, Zn,Cu), growth factors (vitamins, amino acids), functional nutrient for growth and product formation (non-growth associated and growth associated), physical factors (temperature, light, water activity, gas exchange) as well as the addition of additives (protectant, chelating and neutralizing agent). Commonly, commercial medium is used by most microalgae industries for cultivation. The cost for the medium sometimes exceed the gain from the biomass production. This has proven that commercialized medium is not feasible for the industries. Thus, the high cost of commercial medium for microalgae cultivation triggers the idea of using an available nutrient source from wastewater as an alternative way of microalgae cultivation. The high content of organic materials in wastewater such as POME provides low cost microalgae cultivation method which will benefit the industries for a longer-term period.

Palm oil mill effluent (POME) is one of the wastes coming out from palm oil production industry. POME is an unpleasant, non-toxic suspension waste generated from palm oil milling process where a large amount of water and steam are being utilized and resulted in wastewater discharged. Native microalgae strains isolated from the local environment such as palm oil mill effluent and rubber waste water are used in current research to avoid biosafety issue and enhance adaptability of microalgae. This native microalgae strains will have high tolerance as it originates from the same environmental condition. The main objective of current research is to evaluate native microalgae growth and biomass productivity at different source of nutrient, inoculum concentration and aeration factor. Initial inoculum concentration is one of microalgae growth factors which need to be optimized. Higher inoculum concentration support growth and lessen the adaptation period of microalgae (Lau *et al.*, 1995). Study done by (Khalid *et al.*, 2016) shown the increment of inoculum concentration promoted better growth of the *Characium* sp. grown in POME. Other study has came out with the finding that higher inoculum concentration resulted in better biomass production (Ma *et al.*, 2012). However, too high inoculum concentration might cause internal shading phenomenon and stunted the growth (He et al., 2015). Thus optimization of initial microalgae seed concentration is important to counteract this issue. In this experiment, microalgae growth was totally depended on the atmospheric air. The ambient CO_2 percentage is 0.03% v/v which is below the microalgae growth requirement (Becker,1994). Mixing is important to provide sufficient turbulence to the medium and promote gas exchange between medium and the surrounding which will also overcome extreme pH condition (Gross, 2003). In addition, turbulence can enhance the microalgae productivity by ensuring the well distributed nutrient reaching microalgae cell for microalgae utilization and accumulation of organic matters can be avoided (Hariz *et al.*, 2017). Thick boundary layers between microalgae cell and the nutrient medium will be reduced and improve microalgae nutrient uptake rate (Mostert & Grobbelaar , 1987; Borowitzka ,1998). Sufficient turbulence for optimum mass transfer rate of nutrient and gas, promote light penetration by dispersing the accumulated particulate matters (Gross, 2003) at the same time to avoid energy wastage.

METHODOLOGY

Microorganism and Growth Medium

Native microalgae *Chlorella* sp. and *Chlamydomonas* sp. were isolated from palm oil wastewater while *Scenedesmus* sp. was isolated from rubber wastewater and the cultures were maintained in Bold's Basal Medium (BBM) at the temperature 25ºC. BBM is the commercial nutrient medium that consists of nutrient as stated in Table 1.

Table 1. Nutrient elements contain in BBM and Anaerobic POME

BBM Nutrient Element	Concentration (mol/L)	Anaerobic POME Nutrient Element	Concentration (mg/L)
NaNO3	2.94×10^{-3} M	**Ammoniacal-Nitrogen (NH_3-N)**	387
CaCl2	2H2O 1.70×10^{-4} M	**Ammonium (NH_4)**	498
MgSO4	7H2O 3.04×10^{-4} M	**Phosphorus (P)**	75
K2HPO4	3H2O 4.31×10^{-4} M	**Phosphate (PO_4^{-3})**	147
KH2PO4	1.29×10^{-3} M	**Chemical Oxygen Demand (COD)**	705
NaCl	4.28×10^{-4} M	**Biological Oxygen Demand (BOD)**	388
EDTA	1.71×10^{-4} M		
KOH	5.53×10^{-4} M		
FeSO4	7H2O 1.79×10^{-5} M		
H3BO3	1.85×10^{-4} M		
Trace elements	ZnSO4·7H2O 3.07×10^{-5} M, MnCl2·4H2O 7.28×10^{-6} M, MoO3 4.93×10^{-6} M, CuSO4·5H2O 6.29×10^{-6}M, Co(NO3)2·6H2O 1.68×10^{-6} M		

Other than BBM, anaerobic palm oil mill effluent (POME) was used as nutrient growth medium. Anaerobic POME was collected from the palm oil mill and stored at 4ºC. The POME sample was then centrifuged at 8000rpm for 10 minutes before being used for the experiment. This to ensure the suspended solid is removed from the POME sample before being introduced for microalgae cultivation. The characteristic of anaerobic POME used were showed in Table 1.

Cultivation Conditions and Analytical Determinations

The experiments were carried out in 1000 ml Erlenmeyer flasks containing growth medium, POME and BBM at different ratio of inoculated microalgae as stated in Table 2 and Table 3. The working volume for the experiment was 700ml for each flask and the remaining space was for the headspace to promote gas exchange. The runs were carried out in triplicates to ensure the consistency of the result. The same ratio of growth medium and inoculum concentration was used for all the three microalgae strains with different nutrient growth medium POME and BBM. For the non-aerated experiment set up, non-absorbance cotton wool was used as a stopper to allow gas exchange between the atmosphere air and the cultivated microalgae. The cultures were mixed at a fixed speed of 100 rpm by using the orbital shaker (SHO-1D Wise Shake). For the aerated experiment set up, fixed aeration rate at 1 Liter/min of air was supplied into the flasks by using the air pump (Big Boy BB-10200). The temperature for both experiments were maintained at 25±2°C with light exposure of 14000 lux of fluorescent lamp (Hitachi, Malaysia). The sample was taken daily, and the

microalgae biomass was determined by dry cell weight (DCW) analysis. The activity was done by using 0.45 μM micro fiberglass filter paper (GF/C) to filter the biomass and dry in 105°C drying oven for 24 hours. 10ml of samples were collected from the flasks by using measuring string and the samples were then centrifuged at 8000rpm for 10 minutes by using the centrifuge (Centrifuge 5804, Eppendorf) to separate the biomass from the supernatant. The supernatant was discarded, and distilled water was added to the remaining pellet of microalgae biomass and the liquid mixture was centrifuge for the second time with the same centrifugation speed. This to ensure the dissolved solid is being removed out of the sample before filtration process. The control experiment set up was prepared without microalgae being introduced into the growth medium for each experiment condition and it was ran together with the other samples. This is important for the analysis purpose as the filtered pellet from this control set up is used to determine the amount of suspended solid contained. For the biomass calculation, the following is the formula:

$$\text{DCW} = \frac{(\text{weight of filtered microalgae biomass} - \text{weight of blank filter paper})}{\text{Volume of sample collected}} \quad (1)$$

(a) (b)

Figure 1. Experiment set up for microalgae cultivation in BBM **a)** Cultivation of microalgae with air supply from the air pump; **b)** Cultivation of microalgae with mixing by using shaker

Table 2. Microalgae cultivation in BBM at different inoculum concentration

Inoculum Concentration	**10%**	**20%**	**30%**
Volume of Inoculum (ml)	70	140	210
Volume of BBM (ml)	630	560	490

(a) (b)

Figure 2. Experiment set up for microalgae cultivation in POME **a)** Cultivation of microalgae with air supply from the air pump; **b)** Cultivation of microalgae with mixing by using shaker

Table 3. Microalgae Cultivation in POME at different inoculum concentration

Inoculum Concentration	10%	20%	30%
Volume of Inoculum (ml)	70	140	210
Volume of POME(ml)	630	560	490

RESULT AND DISCUSSION

Based on the results from dry cell weight analysis, growth curve can be constructed from the logistic model by using MATLAB. The biomass productivity, ϱ and specific growth rate, μ can be obtained from this logistic model (Yang et al., 2011).

$$X = X_0 X_{max} e^{\mu_{max} t} / ((X_{max}-X_0) + X_0 e^{\mu_{max} t}) \quad (2)$$

X = microalgae concentration in the medium.

Table 4. Growth rate of microalgae cultivated with aerated growth condition

Nutrient Growth Medium	Bold Basal Medium (BBM)			Palm Oil Mill Effluent (POME)		
Inoculum Concentration (v/v)	10%	20%	30%	10%	20%	30%
Native Microalgae Strain	Growth Rate (μ_{max})					
Chlorella sp. UKM2	0.3231	**0.3636**	0.3537	0.2270	0.1517	**0.2712**
Chlamydomonas sp. UKM6	0.3076	0.2574	**0.5082**	0.0968	0.1260	**0.2547**
Scenedesmus sp. UKM9	0.2037	0.2369	**0.3402**	0.1557	0.1732	**0.1867**

Table 5. Growth rate of microalgae cultivated with non-aerated growth condition

Nutrient Growth Medium	**Bold Basal Medium (BBM)**			**Palm Oil Mill Effluent (POME)**		
Inoculum Concentration (v/v)	**10%**	**20%**	**30%**	**10%**	**20%**	**30%**
Native Microalgae Strain	**Growth Rate (μ_{max})**					
***Chlorella* sp. UKM2**	0.1635	**0.1855**	0.1445	0.1401	**0.2052**	0.0970
***Chlamydomonas* sp. UKM6**	0.1172	**0.1673**	0.1562	0.0981	**0.125**	0.0980
***Scenedesmus* sp. UKM9**	**0.1975**	0.0925	0.1352	**0.1900**	0.1629	0.0797

Cultivated microalgae strains with air supply as the aeration source showed higher growth rate compare to microalgae growth which mixing is provided as shown in Table 4 and Table 5. The reason this happened might be because of the turbulence factor in aerated condition enhance the growth of the microalgae strain in both BBM and POME. Turbulence as a result of air flux, producing different movement regimes of the cells within the media under an equal light intensity, could be an important factor. Adequate turbulence is important in this case of microalgae cultivation condition as it enhances mass transfer rate of nutrient to microalgae cell, promote gases exchange that can overcome photo-oxidative effect and thick boundary layer can be minimized (Mostert & Grobbelaar, 1987; Borowitzka, 1998; Gross, 2003; Hariz *et al.*, 2017). Other than that, efficiency of light utilization by microalgae can be improved by having optimum turbulence in cultivation condition. In addition, during photosynthesis process, microalgae consume carbon dioxide and gives out oxygen. Hence, the oxygen level in the cultivation system increases. However, an excessive amount of oxygen in the system can cause photo-oxidative and damages microalgae chlorophyll which results in the disruption of the photosynthesis process and reduces the productivity of microalgae. Aeration promotes mass transfer which allows the removal of excess oxygen released out from the medium during photosynthesis process by microalgae that can inhibit microalgae growth (Woertz *et al.*, 2009). Moreover, nutrient uptake rate can be enhanced by minimizing the boundary layer between microalgae cell and the surrounding nutrient concentration. It is proven that turbulence influences the microalgae productivity as an optimum turbulence enhances mass transfer rate and overcomes the thick boundary layer of unstirred suspension (Mostert & Grobbelaar, 1987; Borowitzka, 1999). Microalgae reproduction is also enhanced in a sufficient turbulence system compared to a still medium which can cause biomass accumulation.

Table 4 shows the summary of the specific growth rate of the three microalgae species UKM2, UKM6 and UKM9 in aerated cultivation condition in comparison to their differences of nutrient source from different medium used. As the result obtained, growth of these three strains showed higher biomass productivity in BBM compare to POME. This can be explained based on the nutrient contain in BBM is complete for the growth requirement of microalgae in comparison to POME. Nutrient is one of the most critical factors for microalgae productivity (Stephens et al., 2010). The limitation of nutrient components in the effluent can cause a low growth rate of microalgae. In POME the level of nutrient content may fluctuated from time to time depending of palm oil mill activities. Sometimes, the level of organic compound is too high that might cause inhibition towards microalgae and affect the nutrient uptake performance. The tolerance towards the organic compound is totally depending on microalgae species as different species shows different tolerance towards highly concentrated medium such as POME. A strong relationship is shown between microalgae growth and the availability of nutrients for microalgae consumption (Khalid et al., 2016). This happened as microalgae assimilate the nutrient components from wastewater, the biomass increases and resulted in positive growth of microalgae at the same time reduce nutrient or known as pollutant in the wastewater. The chemical composition of microalgae biomass, $C_{106}H_{263}O_{110}N_{16}$ represents the fraction of elements contained in the biomass cell and it proves that the elements in the biomass are the keys of microalgae requirement and elements involved (Stumm & Morgan, 1993). A research has proven that highly concentrated POME results in low growth rate and biomass yield of *Chlamydomonas* sp. which is grown in 100% v/v and 50% v/v of POME dilution (Ding *et al.*, 2016).

In terms of microalgae strains sensitivity, BBM shows the least growth limitation compared to other medium (Milling et al., 1988). The extremely high concentration of nutrient such as nitrogen and phosphorus can inhibit microalgae growth. The agricultural wastewater is an example of wastewater with high nitrogen and phosphorus concentration (Wilkie & Mulbry, 2002) which sometimes might not be suitable for microalgae growth. POME is loaded with abundance of nutrient that might not be suitable to certain microalgae species especially the low resistance towards concentrated wastewater. Microalgae might take a long time to adapt to the environment with a high nutrient load and low light penetration condition that limits its growth and performance in nutrient removal (Ding *et al.*, 2016). However, different microalgae species show differences in their tolerance towards high nutrient concentration.

Meanwhile, light penetration is another factor that is important and causing low growth and productivity when light is limited. In POME microalgae cultivation, light penetration is poor due to the high turbidity of POME which is about 460 NTU and it appears in extremely dark brown in colour which is limiting the light entering the cultivation system. The high amount of suspended solid that cause the internal shading and prevent certain amount of light from penetrating through the medium for microalgae utilization. The light penetrated the cultivation column might be unevenly distributed and limits the growth. It is important to reduce the suspended solid in POME by having the pre-treatment process such as flocculation to have effective microalgae cultivation environment. Optimum mixing speed or aeration rate can also overcome this internal shading problem as it prevents the accumulation of particulate matters that block light penetration.

Aeration source and nutrient growth medium are not the only parameters being considered in this experiment, the microalgae inoculum concentration is the other factor being tested as it is important and might influence the growth pattern of all these three strains. Based on the result in Table 4, 30% of inoculum concentration of UKM6 and UKM9 shows the highest growth rate in BBM compare to the 20% and 10% of the inoculum strain. While UKM2 does not give any significance difference at 10%, 20% and 30% initial inoculum concentration. However, in POME all the three strains have highest growth rate at 30% inoculum concentration with air supply as the aeration source. The data proved that high inoculum concentration required to cultivate microalgae in POME as it is important for the adaptation of microalgae to the environment as higher initial inoculum concentration will enhance the microalgae adaptability to the growth environment. The result of specific growth rate from Table 5 showed that 20% of inoculum concentration resulted in highest growth for UKM2 and UKM6 in both BBM and POME in non-aerated condition while 10% of inoculum concentration gave the highest growth for UKM9 for both BBM and POME. In BBM, microalgae do not show significance effect on adaptation period compare to the adaptation period in POME as it might take longer time to adapt due to the nutrient availability factor and other particulate matters present in POME. The inoculum concentration also influences the exponential period of microalgae growth as higher inoculum concentration shows exponential phase that start at early stage of growth curve compare to lower inoculum concentration. This is important to optimize the initial inoculum concentration as it able to reduce the lag phase and enhance the adaptability of microalgae in the system especially for wastewater treatment where treatment period will be faster.

According to Figure 3-6, the comparison of microalgae growth pattern can be made at different nutrient medium used. Microalgae growth in POME showed longer lag phase of growth about 6 days which is doubled the lag phase of microalgae in the commercial medium, BBM about 3 days at average. The same pattern showed for the exponential phase where microalgae took up to 14 days in POME compare to BBM which is at average of 7 days. The microalgae growth pattern study is important as it is related to the hydraulic retention time (HRT) for microalgae based treatment process. It is important to have optimum HRT for the system as too short HRT will resulted in insufficient time for the microalgae to adapt and efficiently utilize the nutrient in the effluent. If the HRT is too long, the thick boundary of the suspension liquid will start to form and cause limitation of light to penetrate through for microalgae cultivation system. It is proven that with an optimum HRT, the competition between species that might lead to nutrient and CO_2 limitations can be avoided.

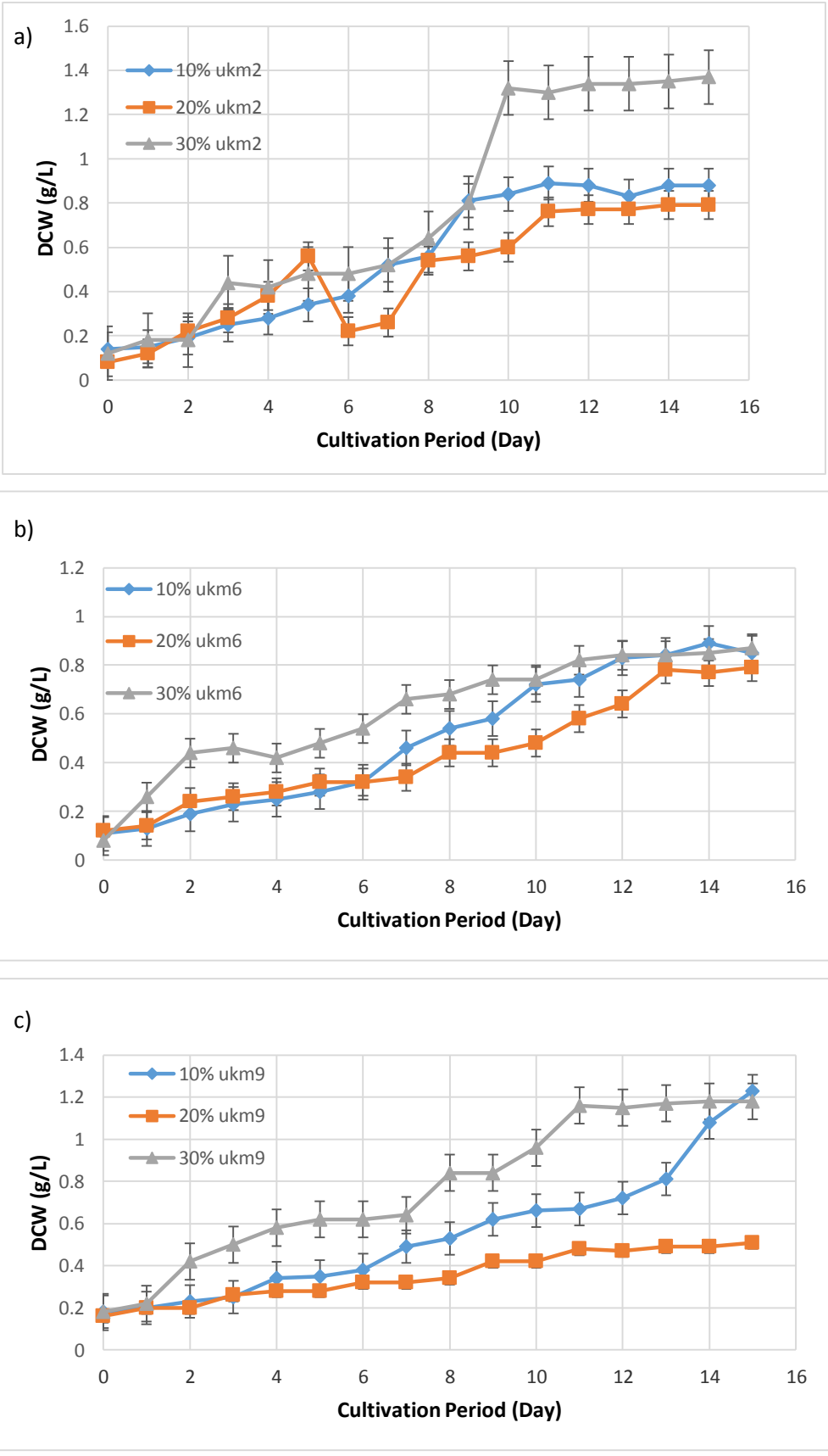


Figure 3. Dry cell weight (DCW) growth curve in Bold's Basal Medium (BBM) with air supply as the aeration source **a)** Growth curve of *Chlorella* sp. (UKM2), **b)** Growth curve of *Chlamydomonas* sp. (UKM6), **c)** Growth curve of *Scenedesmus* sp. (UKM9)

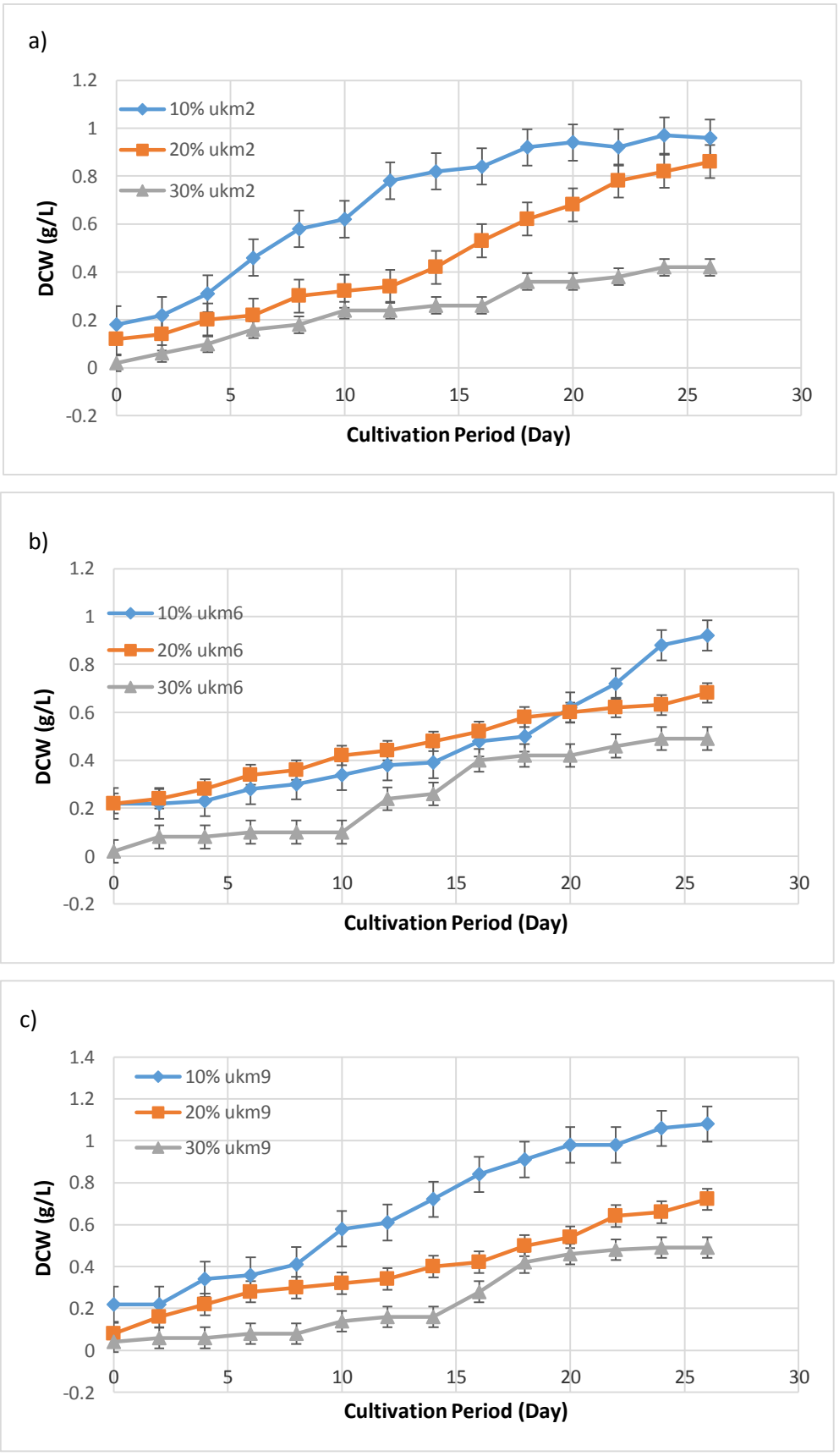
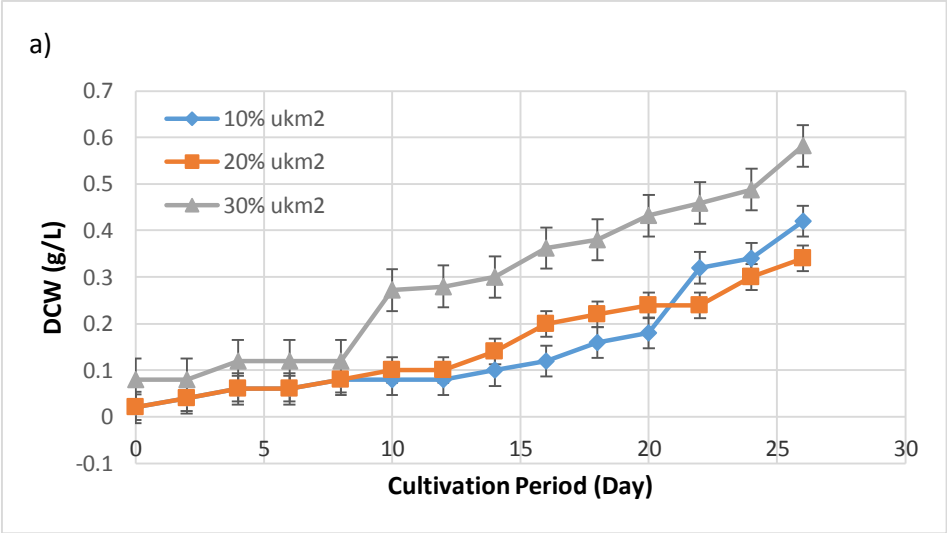
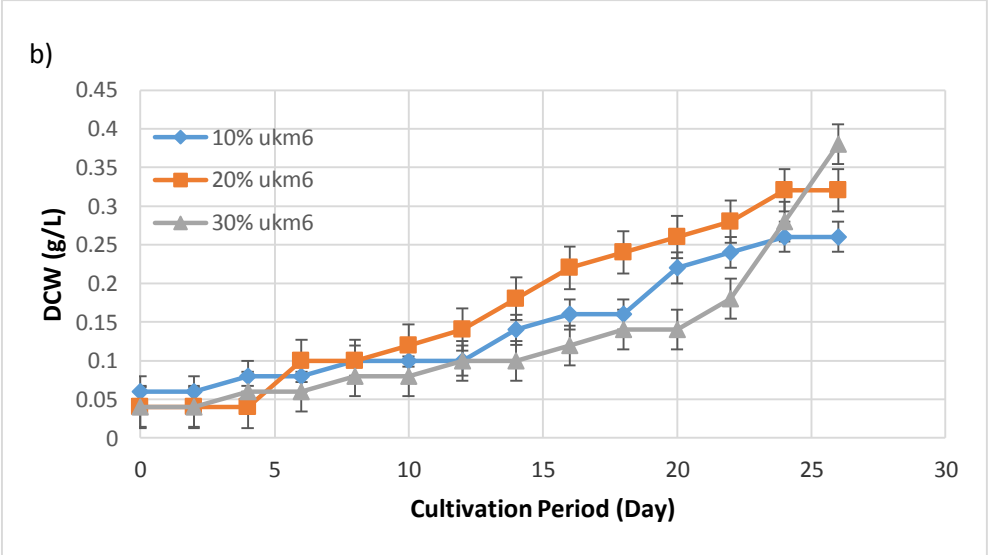


Figure 4. Dry cell weight (DCW) growth curve in Palm Oil Mill Effluent (POME) with air supply as the aeration source **a)** Growth curve of *Chlorella* sp. (UKM2), **b)** Growth curve of *Chlamydomonas* sp. (UKM6), **c)** Growth curve of *Scenedesmus* sp. (UKM9)





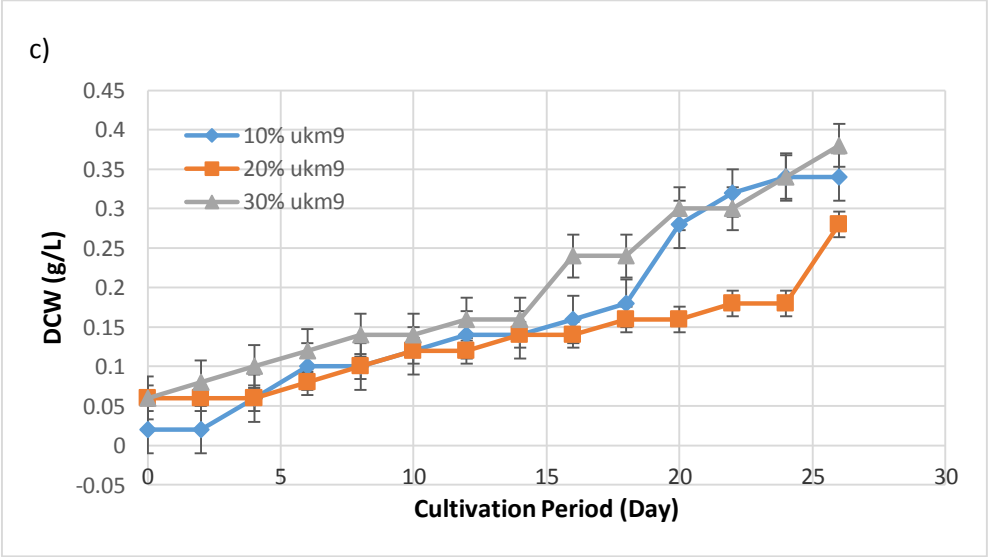


Figure 5. Dry cell weight (DCW) growth curve in Bold's Basal medium (BBM) with agitation by mixing **a)** Growth curve of *Chlorella* sp. (UKM2), **b)** Growth curve of *Chlamydomonas* sp. (UKM6), **c)** Growth curve of *Scenedesmus* sp. (UKM9)

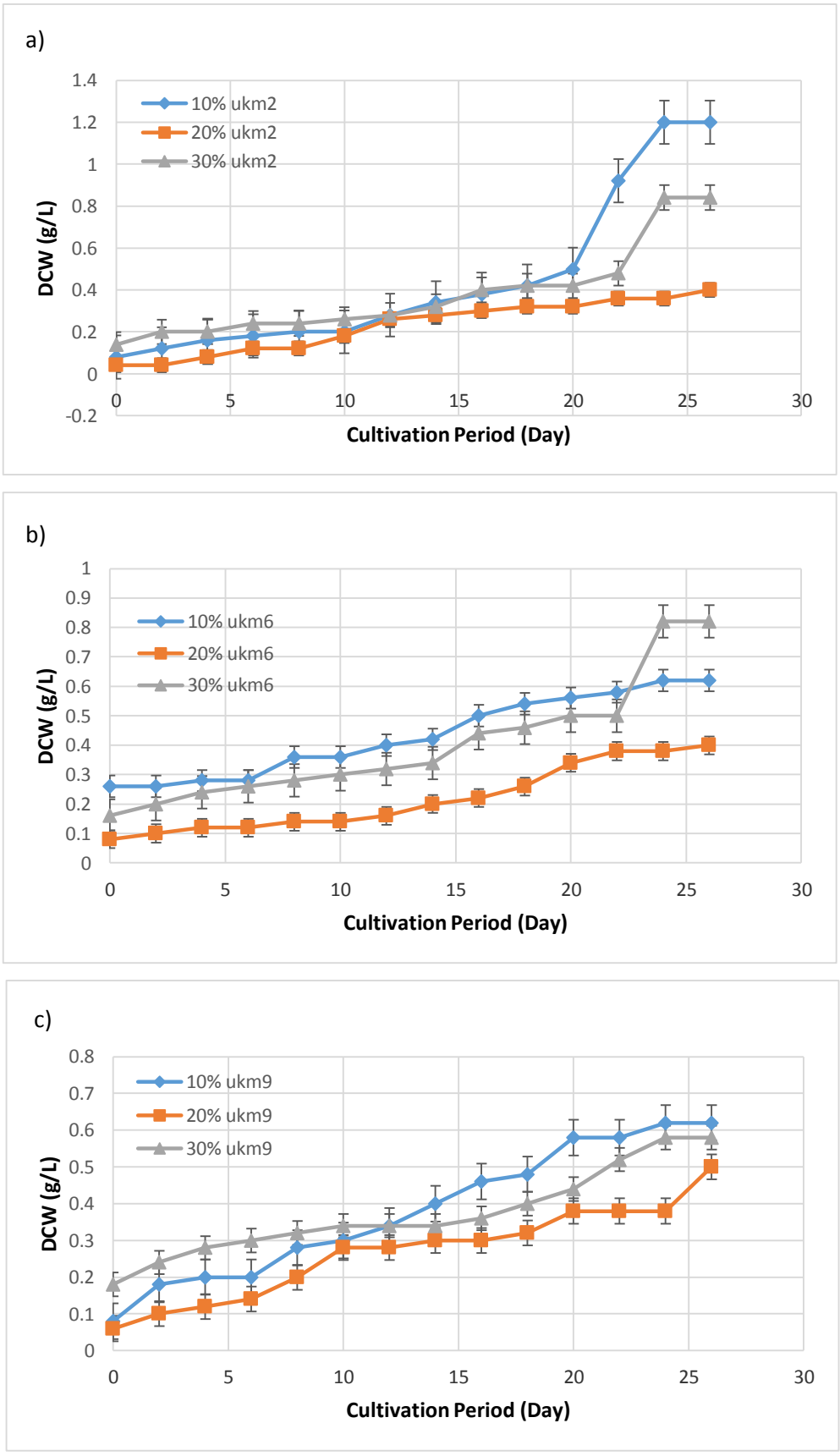


Figure 6. Dry cell weight (DCW) growth curve in Palm Oil Mill Effluent (POME) with agitation by mixing **a)** Growth curve of *Chlorella* sp. (UKM2), **b)** Growth curve of *Chlamydomonas* sp. (UKM6), **c)** Growth curve of *Scenedesmus* sp. (UKM9)

CONCLUSION

The main objective of this research was to study native microalgae *Chlorella* sp., *Clamydomonas* sp., and *Scenedesmus* sp. growth and biomass productivity at different source of nutrient, inoculum concentration and aeration factor. The findings proved that turbulence, nutrient availability, initial inoculum concentration influence the microalgae growth and productivity. The results show that POME can be the alternative of the commercial microalgae nutrient medium and promotes the cost-effective strategies of microalgae cultivation system towards a better environment.

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