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Original Research Article

Potential of fresh POME as a growth medium in mass production of *Arthrospira platensis*

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A B S T R A C T

Keywords

Arthrospira platensis; fresh POME; Growth; Pigments; Large-scale; Outdoor condition; Cost-effective medium

The prospects of utilizing fresh Palm Oil Mill Effluent (POME) as an alternative and inexpensive growth medium in *Arthrospira platensis* cultivation was evaluated in outdoor large scale cultivation system. The aim of this study is to find the optimum fresh POME concentration (T1; 0%, T2; 1%, T3; 2%, T4; 3% and T5; 4% v/v) for good growth and pigments production of *A. platensis* as in control (modified Kosaric medium). The relative performance of the different concentrations of fresh POME were investigated with respect to their productivity, specific growth rate and pigments production. *A. platensis* cultured in T2 (1% v/v fresh POME) had significantly higher ($p < 0.05$) productivity (0.211 ± 0.0034 g L^{-1} d^{-1}) and specific growth rate (0.250 ± 0.0026 μ d^{-1}) compared to control and other treatments. *A. platensis* in T2 also had shown high pigment contents in % dry weight: 1.045 ± 0.024 for chlorophyll, 0.573 ± 0.005 for carotenoid and 12.013 ± 0.110 for phycocyanin which were comparable with control and significantly higher ($p < 0.05$) compared to other treatments. Therefore, 1 % v/v fresh POME has been proven to be a suitable medium to culture *A. platensis* without adversely affecting its growth and pigments production.

Introduction

For the last ten years, Malaysia has been acknowledged as one of the world's leading producer and exporter of palm oil products. Based on the statistic obtained from Malaysian Palm Oil Board, Malaysia controls about 45% of total palm oil production in the world (Vairappan and Yen, 2008). But, this industry facing challenges in regulating disposal of the effluent resulting from extraction of oil from palm oil fruit bunches. Roughly, 5-5.7 tons of water needed in order to sterilize the palm fruit bunches and clarifying the extracted oil to produce 1 ton of crude palm oil while more than 50% of the water results as fresh palm oil effluent mill (POME) (Ahmad *et al*., 2003). Besides containing carbohydrates, protein and oil, fresh POME, a golden brownish liquid effluent, is high in organic compounds, biochemical oxygen demand and chemical oxygen demand which cause deterioration of water resources (Singh *et al*., 2010; Parkavi *et al*., 2011). But, in another perspective, these organic matters can be used as a vital nutrient source for aquatic plants especially for micro-algal growth (Phang and Ong, 1988; Servin-Reyssac, 1998; Hadiyanto *et al*., 2013).

Microalgae uses solar energy, while consuming organic compounds from nutrient rich wastewater to fix carbon substances, yielding biomass which has fine chemicals that are either unique to the algae or found at relatively high amount and have high market value (Kumar *et al*., 2011). At the same time, microalgae capable of utilizing organic pollutants and subsequently improves water quality (Markou *et al*., 2012). Besides bioremediation of waste water, microalgae are high in demand in many biotechnology sectors such as biofuels (Wang *et al*., 2008), cosmetics, pharmaceuticals (Raja *et al*., 2008), nutrition and food derivatives (Brown *et al*., 1997), aquaculture and pollution prevention (Lim *et al*., 2010) and animal feed (Chen and Lee, 2012) due to its commercially valuable biological byproducts. In this respect, *Arthrospira platensis* is one of the most promising microalga (Kumar *et al*., 2011).

A. platensis, which is alkalophilic in nature, fast growing and filamentous cyanobacterium, forms massive populations in tropical and subtropical bodies of water (Gami *et al*., 2011). *A. platensis* has been produced commercially due to high digestibility and protein content (50-70% of dry biomass), high essential amino acids, and then abundance of nutritional values (Morist *et al*., 2001). The United Nations (UN) world food conference acknowledged *A. platensis* as "the best food for tomorrow", and it is being focused as a food supplement (Kapoor and Mehta, 1993). Although, lipid content in *A. platensis* is not high, they could be a good substrate for biomethane and bioethanol production, due to the advantage of relative ease of harvesting (Pouliot *et al*., 1989) and fast growth rates (Markou *et al*., 2012).

A. platensis also has attained growing commercial interest and international demand due to its high value pigments besides high protein content, which have properties related to health benefits and wide pharmaceutical applications (Belay *et al*., 1993; Devanathan and Ramanathan, 2012; Mary Leema *et al*., 2010). There are three major light harvesting pigments present in *A. platensis* which are chlorophylls, phycobilliproteins (especially phycocyanin which is the most abundant) and carotenoids (Reis *et al*., 1998). These pigments aid in synthesizing many enzymes which are important for regulating our body's metabolism (Henrikson, 1989). Though early interest in these molecules focused mainly on their use as natural dyes, numerous studies had shown its applications in health foods, feed, therapeutics and diagnostics (Richmond, 1992; Becker, 1994; Walter *et al*., 2011).

To ensure economic viability of this venture, production cost must be lowered by reducing cost of the growth medium. In that manner, studies have been done in formulating cost-effective cultivation medium which is optimum for *A. platensis* growth and biochemical composition (Danesi *et al*., 2004; Bezerra *et al*., 2008;

Danesi *et al*., 2011). Accordingly, some researchers designed economical culture medium using a large portion of wastewater such as swine wastewater (Cheunbarn and Peerapornpisal, 2010), pig and cattle manure (Markou and Georgakakis, 2011), human urine (Feng and Wu, 2006), sago starch factory wastewater (Phang *et al*., 2000) and so on as nutrient supplement, at the same time used *A. platensis* to remediate the pollutants in that wastewater. However, microalgal cultivation in wastewater from human and animal for food and feed purpose may pose health risks.

Thus, in this study, fresh Palm Oil Mill Effluent (POME), non-toxic in nature (Lam and Lee, 2011) is suggested to be suitable supplementary nutrient in *A. platensis* cultivation proposed for beneficial application in pharmaceutical, cosmetics and food industries. Fertilizing properties of POME which contains appreciable amounts of macro- and micronutrients (Habib *et al*., 1998; Singh *et al*., 2010) is suitable to support good *A. platensis* growth. Besides that, fresh POME is readily available in Malaysia and it is also cost effective feed for *A. platensis* growth in commercial cultivation. At the same time, culturing *A. platensis* in POME offers inexpensive partial bioremediation of this agricultural waste (Parkavi *et al*., 2011) and utilizes CO_2 while producing O_2 which is good for the environment. Besides that, operation of open digestion ponds in POME treatment which release greenhouse gases such as methane and CO_2 to the environment (Yacob *et al*., 2006) and cause ground water and river pollution (Lam and Lee, 2011) can be reduced.

Previously, small scale studies have been done on Spirulina cultivation using digested or treated POME in laboratory condition (Parkavi *et al*., 2011; Fitria Yuli *et al*., 2012). The novelty of this study is to optimize productivity of *A. platensis* cultured in outdoor tanks (10 L) in the climate of Malaysia using fresh POME which were supplemented with commercial fertilizers as nitrogen, phosphorus source because fresh POME is lack of these macronutrients (Ma, 2000; Mohd Nasir *et al*., 2013) and sodium bicarbonate as carbon source and to maintain the alkalinity of culture medium. The aim of this study is to find the optimum fresh POME concentration (0%, 1%, 2% 3%, and 4% v/v) to culture *A. platensis* without affecting its productivity and pigments content as in control medium. Besides, to improve the growth measurement methods to highly represent the growth of *A. platensis* cultured in colored medium containing high suspended solids such as POME.

Materials and Methods

Arthrospira platensis culture

Pure culture of *A. platensis* was obtained from The Culture Collection of Algae at The University of Texas, Austin (UTEX) and pre-cultured in 20 L tanks containing 10 L of following culture medium: modified Kosaric medium (control), T1 (0% v/v POME), T2 (1% v/v POME), T3 (2% v/v POME), T4 (3% v/v POME), and T5 (4% v/v POME) for the pre-adaptation process of *A. platensis* in different treatments. Cultivation and adaptation process was done at Field 2, Agriculture Faculty, Universiti Putra Malaysia (UPM) under rain shelter.

Ten percent of pre-cultured *A. platensis* with 3.2 ± 0.13 g/L dry weight, was initially inoculated into each respective treatment. Each control and treatments was done in five replicates in a Completely Randomized Design. A negative control (without *A. platensis*) was done for control and each treatment. The *A. platensis* cultures and negative control were maintained until day 7 and agitated using aquarium aeration pump.

Preparation of control medium

Control medium was prepared based on Kosaric medium (Tompkins *et al*., 1995) with modification using commercial fertilizers as followed (g/L): 4.5 $NaHCO_3$, 0.25 NaCl, 0.1 $CaCl_2$, 0.2 $MgSO_4.7H_2O$, 0.221 Urea, 0.07 H_3PO_4, 0.242 KOH, 0.02 $FeSO_4.7H_2O$ and 0.5 mL/L of trace metals solution composed of following elements (g/L): 2.86 H_3BO_4, 1.81 $MnCl_2.4H_2O$, 0.22 $ZnSO_4.7H_2O$, 0.08 $CuSO_4.5H_2O$, 0.01 MoO_3, and 0.01 $COCl_2.6H_2O$. Urea was added to culture medium by fed-batch process (Danesi *et al*., 2002).

Preparation of POME based medium (Treatments)

Each POME based treatment was prepared using fresh palm oil mill effluent (POME) and supplemented with commercial fertilizers as followed (g/L): 4.5 $NaHCO_3$, 0.25 NaCl, 0.221 Urea, 0.07 H_3PO_4. Urea was added to culture medium by fed-batch process (Danesi *et al*., 2002). Fresh POME was obtained from Sri Ulu Langat Palm Oil Mill Sdn. Bhd. This wastewater was collected in high density polyethylene containers (20 L) from the primary discharge outlets one day before the experiment starts. Then, it was filtered thorough nylon cloth (30 µ mesh size) and stored in cold room at 4-8^oC prior to further usage. During the cultivation process, fresh POME was pulse-fed from day 0 until day 3 into each treatment as shown in table.1.

Growth Measurement

Three growth parameters were used throughout the study to precisely determine the growth pattern of *A. platensis*. Negative control was considered as blank for each growth parameters in order to increase reliability of the growth measurement. The optical density of *A. platensis* cultures was measured daily using spectrophotometer (Hitachi U-1900). First, the absorbance of the *A. platensis* cultural solution was manually scanned (wavelength range 550~700 nm), to test the cultures' optimum absorbed wavelength. Estimation of the *A. platensis* biomass was based on the tested absorbance values of microalgae under a spectrophotometer with 620 nm wavelength. Biomass dry weight was determined every alternate days followed the method by Sorokin (1973). Chlorophyll *a* was extracted with 95 % ethanol and evaluated spectrophotometrically (Hitachi U-1900) at 664 and 649 nm according to Lichtenthaler (1987). It was done every alternate days.

Productivity

Productivity was calculated using the following equation according to Danesi *et al* (2011):

$$P_x = (X_m - X_i)(T_c)^{-1}$$

where: P_x = productivity (g L^{-1} day^{-1})
X_i = initial biomass concentration (g L^{-1})
X_m = maximum biomass concentration (g L^{-1})
T_c = cultivation time related to the maximum biomass concentration (days)

Specific growth rate

Specific growth rate (μ) was calculated by the following formula according to Markou *et al* (2012):

$$\mu = (\ln X_m - \ln X_i)(T_c)^{-1}$$

where: X_i = initial biomass concentration ($g\ L^{-1}$)
X_m = maximum biomass concentration ($g\ L^{-1}$)
T_c = cultivation time related to the maximum biomass concentration (days)

Pigments analysis

Upon reaching stationary phase on day 7, *A.platensis* was harvested using nylon cloth (25 μ pore size). Harvested samples were freeze-dried at -40°C for 72 hours and kept in freezer at -80°C before being analyzed. Chlorophyll and carotenoid was extracted with 95 % ethanol in a water bath at 70°C for 5 minutes and evaluated spectrophotometrically (Hitachi U-1900) at 664, 649, 470 nm followed the method by Lichtenthaler (1987). Phycocyanin was extracted using 0.1M phosphate buffer (pH 7) (Walter *et al*., 2011) and evaluated spectrophotometrically (Hitachi U-1900) at 615 and 652 nm according to Bennett and Bogorad (1973).

Environmental Growth Factors

Light intensity (Licor Li-250), air and culture temperature (Fisher Scientific) and air humidity (Fisher Scientific) was recorded daily from 7 am to 7 pm with 2 hours gap. pH of the culture medium was recorded on daily basis using pH meter (Mettler Toledo).

Data Analysis

Productivity, specific growth rate and pigments production of *A. platensis* cultured in different POME concentrations were analysed using SPSS software version 21 through one-way independent analysis of variance (ANOVA) and followed by Tukey HSD (Honestly Significant Difference) multiple comparison test. Correlation between each growth parameter readings was determined using SPSS software version 21 through bivariate correlation.

Results and Discussion

Growth of *A.platensis* in different concentrations of fresh POME

Growth of *A. platensis* cultured in control and POME based medium which were expressed in terms of optical density, biomass dry weight and chlorophyll *a* are shown in Figure 1, 2 and 3 respectively. Based on these three growth measurements, there was no presence of lag phase in each treatment due to the preadaptation process. Next, *A. platensis* cultured in T2 (1 % v/v fresh POME) reached maximum cell concentration compared to control and all other treatments. For example, maximum biomass (dry weight) achieved by *A. platensis* grown in T2 was 1.79 ± 0.028 g/L and it was followed by control (1.59 ± 0.023 g/L). Moreover, maximum cell concentration of *A. platensis* was increased in medium with decreasing POME concentration from T5 (4 % v/v fresh POME) to T2 (1 % v/v fresh POME). Besides that, T1 (0 % v/v fresh POME) showed the lowest growth and maximum cell concentration among the treatments

Based on Table 2, the three growth measurements (optical density, biomass dry weight and chlorophyll *a*) were significantly ($p < 0.01$) correlated. Thus, using negative control (medium without *A.platensis*) as blank for each measurement is proven and reliable method to highly represent growth of *A. platensis* cultured in colored and turbid medium such as POME.

Based on Table 3, productivity and specific growth rate of *A. platensis* cultured in T2 (1% v/v fresh POME) were significantly higher ($p < 0.05$) compared to control and other treatments. Next, Table 4 shows pigments content (% dry weight) in *A. platensis* cultured in control and POME based medium. Based on this table, *A. platensis* grown in control and T2 had significantly higher ($p < 0.05$) chlorophyll content. Furthermore, carotenoid content was significantly higher ($p < 0.05$) in T2 while phycocyanin content was significantly ($p < 0.05$) higher in control. Besides that, productivity, specific growth rate and pigments content decreased in *A. platensis* cultured in medium with increasing concentration of POME. Moreover, *A. platensis* grown in T1 (0 % v/v fresh POME) had significantly lower productivity, specific growth rate and pigments content compared to control and other treatments

Environmental growth factors measurement throughout the study

Average light intensity, relative humidity, air temperature and culture temperature for every day throughout the cultivation period are shown in Figure 4 and 5. Light intensity and temperature, which are the important factors in *A. platensis* growth, were relatively higher during daytime from 11 am to 5pm while relative humidity was lower during these hours. Figure 6 shows pH of the culture medium. The pH decreased with increasing concentration of fresh POME from T2 to T5 due to acidic nature of fresh POME. It was observed that pH of POME based medium (T2 to T5) started to increase after day 4. This was due to the pulse-feed of fresh POME with increasing amount from day 0 until day 3.

This study showed, 1 % v/v fresh POME had enhanced growth and productivity of *A. platensis* without adversely affecting its pigments content as in control medium (Table 4). Even though, fed-batch addition of urea was applied in control medium which is a evidently feasible method (Danesi *et al*., 2011; Morocho Jacome *et al*., 2012), loss of urea as ammonia from this open cultivation system was out of control due to relatively high temperature in Malaysia, especially during day time (Figure 5) and also alkaline condition of the culture medium (Figure 6) (Kasim *et al*., 2009; Mohd Yusuff *et al*., 2009). On the other hand, presence of appropriate amount of humic and fulvic acids in 1 % v/v fresh POME (Rosliza *et al*., 2009; Yaser *et al*., 2013) effectively promoted retention of ammonium in culture medium which improved growth and development of *A. platensis* compared to control medium. Properties of these acidic materials such as low pH and high cation exchange capacity (CEC) reduced ammonia volatilization from the medium by inhibiting urease activity and delaying hydrolysis of urea (Ameera *et al*., 2009; Latifah *et al*., 2011; Ahmed *et al*., 2012) in POME based medium.

Besides that, growth and high value pigments of *A. platensis* cultured in medium with increasing POME concentration from T2 (1 % v/v fresh POME) to T5 (4 % v/v fresh POME) decreased. This phenomenon can be evidently related with the presence of

Table.1 Pulse-feeding information of fresh POME into 10 L of culture from day 0 until day 3

Treatments	POME concentrations (% v/v)	Total POME (mL) in 10 L of culture.	Fed-batch (mL)			
			Day 0	Day 1	Day 2	Day 3
T1	0	No addition of POME	0	0	0	0
T2	1	100	10	20	30	40
T3	2	200	20	40	60	80
T4	3	300	30	60	90	120
T5	4	400	40	80	120	160

Figure.1 Optical density of *A. platensis* grown in different POME concentrations. Control; (◆), Treatment 1; (■), Treatment 2; (▲); Treatment 3; (✕), Treatment 4; (+), Treatment 5; (●). Values are presented as mean ± SE (n = 5).

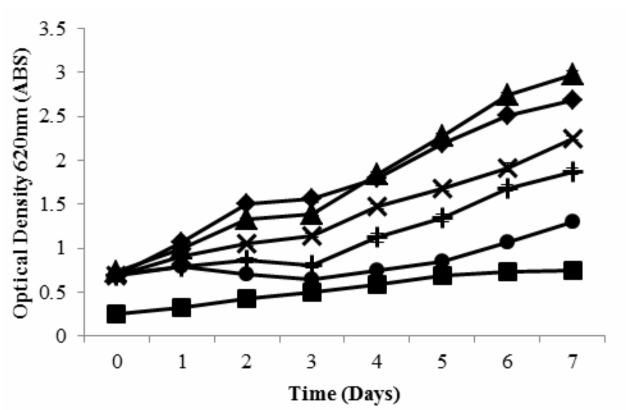

Figure.2 Biomass (g L^{-1}) dry weight of *A. platensis* grown in different POME concentrations. Control; (◆), Treatment 1; (■), Treatment 2; (▲); Treatment 3; (✕), Treatment 4; (+), Treatment 5; (●). Values are presented as mean ± SE (n = 5).

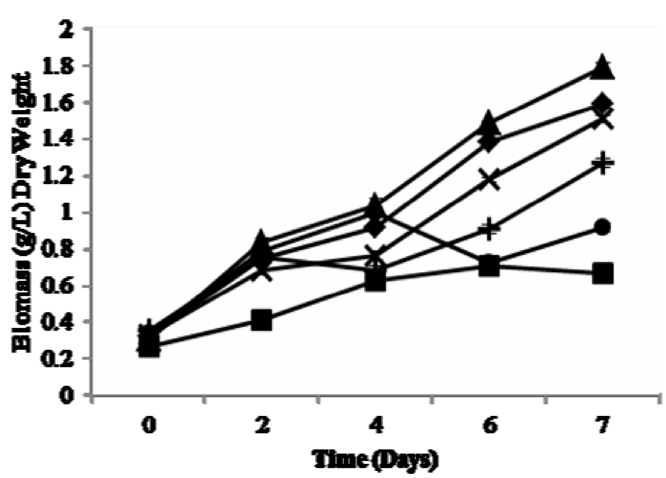

Figure.3 Chlorophyll *a* (mg L^{-1}) of *A. platensis* grown in different POME concentrations. Control; (◆), Treatment 1; (■), Treatment 2; (▲); Treatment 3; (✕), Treatment 4; (+), Treatment 5; (●). Values are presented as mean ± SE (n = 5).

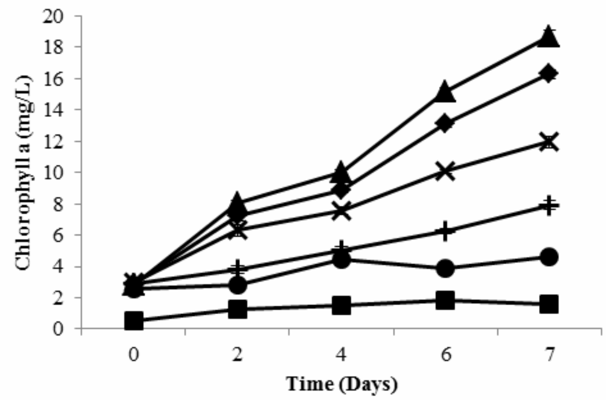

Table.2 Results of correlation between the three growth parameters

Treatments	Biomass & OD (pearson correlation, r)	OD & Chlorophyll a (pearson correlation, r)	Biomass & Chlorophyll a (pearson correlation, r)
Control	0.998	0.960	0.967
T1	0.964	0.775	0.794
T2	0.978	0.985	0.964
T3	0.936	0.978	0.981
T4	0.958	0.935	0.945
T5	0.921	0.892	0.962

*correlation is significant at the 0.01 level (2-tailed).

Table.3 Results of productivity (g L^{-1} d^{-1}) and specific growth rate (µ) of *A. platensis* grown in different POME concentrations

Treatments	Productivity (g L^{-1} d^{-1})	Specific growth rate (µ d^{-1})
Control	0.181 ± 0.0034^{b}	0.227 ± 0.0031^{b}
T1	0.057 ± 0.0019^{e}	0.130 ± 0.0037^{d}
T2	0.211 ± 0.0034^{a}	0.250 ± 0.0026^{a}
T3	0.168 ± 0.0034^{b}	0.218 ± 0.0066^{b}
T4	0.130 ± 0.0031^{c}	0.181 ± 0.0042^{c}
T5	0.082 ± 0.0022^{d}	0.142 ± 0.0016^{d}

Each value is presented as mean ± SE (n = 5). Means within each column with different letters (a-e) differ significantly ($p < 0.05$).

Table.4 Pigments content (% dry weight) in *A. platensis* cultured in control and treatments with different POME concentrations

Treatments	Chlorophyll (% Dry weight)	Carotenoid (% Dry weight)	Phycocyanin (% Dry weight)
Control	1.026 ± 0.023[a]	0.533 ± 0.004[b]	13.585 ± 0.192[a]
T1	0.235 ± 0.014[e]	0.175 ± 0.004[f]	4.636 ± 0.229[e]
T2	1.045 ± 0.024[a]	0.573 ± 0.005[a]	12.013 ± 0.110[b]
T3	0.793 ± 0.016[b]	0.484 ± 0.008[c]	9.770 ± 0.104[c]
T4	0.623 ± 0.022[c]	0.384 ± 0.011[d]	6.108 ± 0.098[d]
T5	0.509 ± 0.011[d]	0.330 ± 0.005[e]	5.011 ± 0.103[e]

Each value is presented as mean ± SE (n = 5). Means within each column with different letters (a-f) differ significantly ($p < 0.05$).

Figure.4 Average light intensity (μmol/m²s) (□) and relative humidity (%) (◆) throughout the cultivation duration. Values are presented as mean ± SE (n = 8)

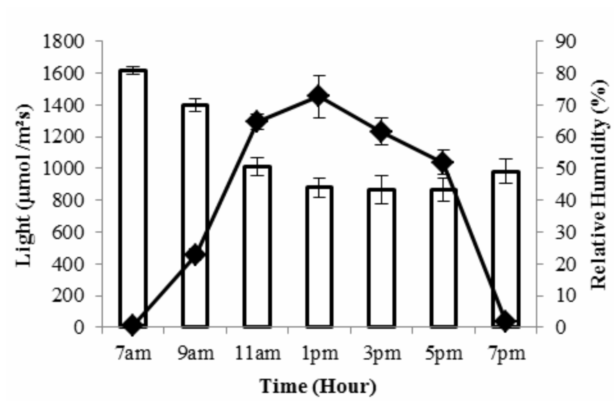

Figure.5 Average air temperature (°C) (◆) and culture temperature (°C) (■) throughout the cultivation duration. Values are presented as mean ± SE (n = 8)

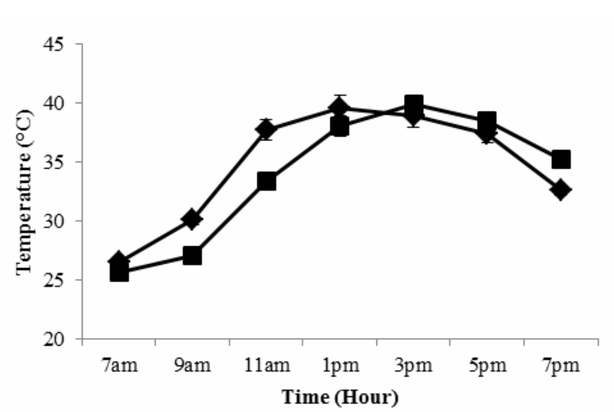

Figure.6 pH of *A. platensis* grown in different POME concentrations. Control; (◆), Treatment 1; (■), Treatment 2; (▲); Treatment 3; (✕), Treatment 4; (+), Treatment 5; (●). Values are presented as mean ± SE (n = 5).

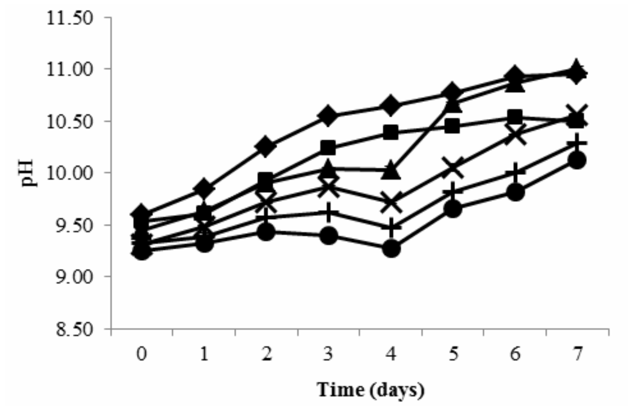

Table.5 Comparison of productivity and specific growth of Spirulina from previous studies with present study

Medium	Reactor	Condition	Productivity (g L^{-1} d^{-1})	Specific growth rate (µ d^{-1})	Duration	References
Zarrouk's	Photobioreactor (20L)	Greenhouse	0.030	0.074	450 h	Colla *et al* (2007)
Digested sago effluent	High rate algal pond (60 L)	Outdoor	0.088	0.570	16 d	Phang *et al* (2000)
Digested POME	Erlenmeyer flask (1L)	Indoor	0.029	0.128	7 d	Fitria Yuli *et al* (2012)
Olive-oil mill effluent	Photobioreactor (0.5 L)	Indoor	0.110	0.173	14 d	Markou *et al* (2012)
T2	Tank (10 L)	Outdoor	0.211	0.250	7 d	Present study

microalgal growth inhibitory factors in fresh POME such as chelating substances especially tannins (Habib *et al*., 2003; Yaser *et al*., 2013) and humic acid (Mohd Yusuff *et al*., 2009), high suspended solids (18000 ppm) (Ahmad *et al*., 2003) and dark coloration which inhibit light penetration and also low pH (4.7) (Ahmad *et al*., 2003). Intensity of these inhibitory factors increased with increasing POME concentration and concurrently affects growth of *A. platensis*. Therefore, in this study, fresh POME was diluted to the lower concentrations (1 to 4 % v/v) and pulse-fed into the culture medium in order to reduce those negative effects.

Chelating agents mainly tannins are also known as anti-nutrients because they often lower absorption of some minerals especially iron into the living cells (Ashok and Upadhyaya, 2012). These compounds form insoluble complex with divalent ions particularly iron, making them less available for absorption (Brune *et al*., 1989; Hassan *et al*., 2003; Afsana *et al*., 2004; Ashok and Upadhyaya, 2012). When POME concentration increasing, formation of tannin-iron complexes also increased while availability of iron for *A.platensis* consumption been reduced. Concomitantly, pigments content in *A.platensis* cultured in medium with higher POME concentration decreased due to the reduction in the number of iron-containing protein within the photosynthetic apparatus and also structural alteration of thylakoid membranes and phycobilisomes (Kudo *et al*., 2000). Xing *et al* (2007) also proved that iron deficiency causes pigment reduction in phytoplankton. Besides that, decreasing photosynthetic efficiency in medium with increasing POME concentration also accompanied with reduction in carbon fixation which leads to decrease in cell volume (van Leeuwe and Stefels, 1998).

Next, as concentration of fresh POME increased, increasing turbidity and darker color of growth medium due to the presence of tannic, humic and fulvic acids (Yaser *et al*., 2013), limit light penetration and photosynthesis reaction through shading effect in the cultivation system (Habib *et al*., 2003). This phenomenon changed the POME based treatments (T2 to T5) to mixotrophic and heterotrophic state. When photosynthetic green algae grow in mixotrophic condition, light and organic carbon consumed as carbon source while, only organic carbon is the key carbon source in heterotrophic condition (Abreu *et al*., 2012). Consequently, rate of photosynthesis metabolism decreased while rate of oxidative glucose metabolism increased in the culture medium with increasing POME concentration. As a result, photosynthetic pigments reduced in darker medium (Madhyastha and Vatsala, 2007). Hence, microalgae have greater growth rate under mixotrophic condition than heterotrophic and autotrophic condition as proved by Bhatnagar *et al* (2011) and Kong *et al* (2011). Similar result was obtained in this study where productivity and specific growth rate of *A. platensis* cultured in T2 (1 % v/v fresh POME) (mixotrophic condition) were significantly higher ($p < 0.05$) compared to control medium (photoautotrophic condition) and other POME based treatments (heterotrophic condition).

Although growth inhibitory factors exist in POME, productivity, specific growth rate and pigments production of *A. platensis* cultured in POME based medium (T2 to T5) were significantly higher ($p < 0.05$) than T1, treatment without POME addition (0% v/v fresh POME). This suggests *A. platensis* benefited the organic nutrients available in fresh POME for growth and productivity. Thus, these types of investigations are presently applicable since low cost biomass production derived from the use of an inexpensive and easily available medium such as fresh POME. Besides that, this study also added value to this wastewater as a potential fertilizer for Spirulina growth which could be economically viable and environmental friendly.

Furthermore, this investigation also produced more prominent and comparable findings with previous literatures. This is proved in Table 5 where the productivity and specific growth rate of *A. platensis* cultured in T2 (1 % v/v fresh POME) within 7 days were comparatively higher compared to the previous studies.

This study also could be a key factor for developing a competitive process for the production of high value products such as pigments. Spirulina in nature contains appreciable amount of pigments, expressed in % dry weight: 0.37 for carotenoid, 1.00 for chlorophyll, and 14.00 for phycocyanin (Henrikson, 1989). In present study, *A. platensis* cultured in T2 contained higher amounts of carotenoid (0.573 ± 0.005 % dry weight), and similar amount of chlorophyll content (1.045 ± 0.024 % dry weight) compared to previous study done by Henrikson (1989). However, lower phycocyanin content was (12.013 ± 0.110 % dry weight) probably due to the different cultivation condition such as temperature (Figure 5), light intensity (Figure 4), photoperiod (12:12) and so on.

1 % v/v fresh POME is optimum for *A. platensis* growth and pigments production in the climate of Malaysia due to the presence of appropriate amount of humic and fulvic acids which reduce loss of ammonia from urea. Besides, fresh POME contains appreciable amount of vital nutrients for algal growth thereby, addition of macro and micro minerals except nitrogen and phosphorus can be omitted which could reduce the cost of production. This study also gives a good view on fresh POME as a cheaper and easily available organic fertilizer source in Malaysia to culture *A. platensis* and also to increase the efficiency of urea in the growth medium. Thus, further investigations should be done in commercial cultivation of *A. platensis* using fresh POME for food and feed purposes.

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