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The effect of different nitrogen sources on continuous growth of *Arthrospira platensis* in simple floating photobiorector design in outdoor conditions

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Keywords: *Arthrospira platensis*, variable weather, floating photobioreactor, fertilizer, nitrogen source

Abstract

Malaysia rests in the tropic region where cloud covers and rains make it less favorable for outdoor *Arthrospira platensis (Spirulina)* cultivation. That is the most likely reason why there is no commercial *Spirulina* cultivation in Malaysia. Therefore the aim of this study is to find out whether *Spirulina* can be cultured in simple photobioreactor floating in water body. *A. platensis* was cultivated in simple water based floating photobioreactor and land based tank using different composition of nitrogen sources (Urea and Ammonium Nitrate). Experiment was conducted in outdoor conditions to assess the respond of different nitrogen sources on the cell density, dry biomass and total chlorophyll of *Spirulina* gained in simple photobioreactors in actual variable culture conditions. Results showed significantly higher biomass dry weight (g L^{-1}) with ammonium nitrate treated Spirulina under dry weather conditions for land based tank and water based photobioreactor at 3.026±0.058 and 4.687±0.154 respectively. Overall, productivity (g L^{-1} d^{-1}) and specific growth rate (μ d^{-1}) of Spirulina was highest with ammonium nitrate than urea for every cycles and photobioreactors under different weather patterns (wet, dry and mix). With current price of ammonium nitrate cheaper than urea suggesting that Spirulina can be cultured at lower cost in variable weather conditions such as Malaysia.

Introduction

Spirulina (*Arthrospira platensis*) has gained huge popularity for its benefits, having high protein contents, anti-oxidants, vitamins and other high nutritional values. With steady increase in human population, the demand for this unique cyanobacterium, Spirulina has been greater than before (Morist et al., 2001). However the price of Spirulina is still unaffordable for most people. In order to reduce the current market price of Spirulina, alternative nutrient sources were studied and investigated to replace expensive chemical fertilizers that have been used often in laboratory scale. Agricultural, industrial or domestic wastewaters have been proven beneficial for growing microalgae at commercial stage as these wastewaters have loads of essential nutrients that can be alternatively used as fertilizer sources. In addition to these implementations, removal or remediation of excess nutrient elements from wastewaters could also decrease impact to the environment (Metcalf, 2003).

Efforts to reduce cultivation cost using cheap fertilizer composition have been reported by substituting ammonium phosphate (De et al., 1999), urea (Danesi et al., 2002), ammonium salts (Carvalho et al., 2004) and ammonium chloride (Bezerra et al., 2007) as appropriate nitrogen sources which resulted better growth of *A. platensis.* In Malaysia with frequent rain and cloud cover formation, the suitable method of growing microalgae is through enclosed photobioreactor system (Naqqiuddin et al., 2014). Similar outdoor cultivation system was performed in two different countries: Oman and Malaysia, the growth of Spirulina was compared and results suggested weather patterns play significant role affecting yield of the microalgae (Al Mahrouqi et al., 2015a). Besides, the fatty acid profiles of grown Spirulina did not vary much with high salinity ranges in Oman though, sufficient acclimatization are necessary (Al Mahrouqi et al., 2015b). Enclosed photobioreactor isolate external factors such as rain and other contamination risks. Therefore the cultured microalgae and nutrient concentrations are protected from being diluted by rainwater and other contaminant such as other microalgal species and dust. It also make the culture more hygienic. Other advantages of floating enclosed photobioreactor system (water-based) was better temperature regulations as the maximum temperature reached was never too extreme compared to land based photobioreactor system (Naqqiuddin et al., 2014; Sukumaran et al., 2014).

From time to time there are countless photobioreactor systems were proposed from many countries (Ugwu et al., 2008). Since most of the studies are more focused of design and performance of photobioreactor, the information on the effect of nutrient formulation on growth and productivity in this system is very limited. Evaluation is necessary to decide on which fertilizer giving the high yield in term of biomass and productivity in a specific design of a photobioreactor system. A bench-scale airlift photobioreactor was tested for Spirulina cultivation to determine potential of the cultivation system in treating high strength wastewater. Using synthetic wastewater with varying ammonia/total nitrogen ratios, high biomass density and productivity at (3500-3800 mg L^{-1}) and (5.1 g m^{-2} d^{-1}) was achieved respectively (Yuan et al., 2011). However this is done in controlled environment.

While in microenvironment condition (sterile aerated photobioreactor), a comparative study has suggested potential of utilizing alternative nitrogen sources such as urea and ammonium to reduce production costs of commercial algae farm as it has shown positive results in response to the growth of *A. platensis* following after the standard nitrogen sources in Kosaric medium, sodium nitrate (Costa et al., 2001). In this study, we studied the effects of different nitrogen sources of Urea, $(NH_2)_2CO$ and Ammonium Nitrate, NH_4NO_3 to determine best grown *A. platensis* in our simple designed photobioreactors that were located in outdoor condition with different weather pattern.

Materials and methods

Cultivation location

Arthrospira platensis (Spirulina) was supplied by Laboratory of Plant Physiology, Faculty of Science, Universiti Putra Malaysia and was cultivated in Tilapia Fish Hatcheries Lake, University Agricultural Park (UPM).

Experimental Design

Preparation of Land and Water Based photobioreactor (PBR) designs

Vertically cylindrical simple land based photobioreactors (working volume of 20L) were constructed at dimension: π $(20.5cm)^2$ (50cm) with High-Density Polyethylene (HDPE) flexible plastic bag. Floating water based photobioreactors (PBR); angular floating photobioreactors were constructed by joining several pieces (5L) plastic bottles (PET) with dimensions: 16cm x16cm x110cm (Naqqiuddin et al., 2014). All photobioreactors was aerated with standard diaphragm aquarium air pump (SONIC P-125, 85L/min, 0.04MPa) with standardized aeration rate at 0.7 L L^{-1} min^{-1}. The photobioreactors were placed in tilapia fish pond located in outdoor conditions under direct sunlight penetration.

Acclimatization

Three cycles of experiments were carried out (10 cultivation days for each cycle) of *A. platensis* cultivation. Each cycle of cultivation experiencing different weather conditions which can be classified into 3 different categories: Wet weather conditions (L1/W1) was the first cycle experiencing more rain, cloudy and less sunny conditions during the culture period; Dry weather conditions in second cycle (L2/W2) which was mostly sunny and clear sky in most of the culture period; Mix weather conditions (L3/W3) experiencing about equal rainy and sunny conditions during the culture period was the third cultivation cycle. Sufficient adaptation phase was arranged for *A. platensis* to be grown in outdoor conditions before used for actual experiment.

Medium preparation

Kosaric medium was prepared following Tompkins et al., (1995) with minor modification using commercial fertilizer for both nitrogen sources (g L^{-1}): 0.221 Urea, $(NH_2)_2CO$ and 0.214 Ammonium Nitrate, NH_4NO_3; other nutrients (g L^{-1}): 5.0 $NaHCO_3$, 0.25 NaCl, 0.1 $CaCl_2$, 0.2 $MgSO_4.7H_2O$, 0.07 H_3PO_4, 0.242 KOH, 0.02 $FeSO_4.7H_2O$ and 0.5 mL L^{-1} of trace metals solution composed of following elements (g L^{-1}): 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 $COCl_2.6H_2O$ (Sukumaran et al., 2014). Urea and ammonium nitrate was added to culture medium by using fed-batch method (pulse-fertilization) (Danesi et al., 2002).

Measurement of growth, productivity and specific growth rate

Light intensity in ($\mu mol.m^{-2}.s^{-1}$): (Licor Li-250), culture temperature (°C) (Fisher Scientific) and pH of the culture medium: (Mettler Toledo Model 330) were recorded daily. Growth performance parameter determination based on the following methods: Optical density (absorbance: at spectral range of 620nm) and Chlorophyll a (665 645 and 630 nm) (Sukumaran et al., 2014): (Hitachi U-1900). Biomass dry weight ($g\ L^{-1}$) following method in Borowitzka (1991) was analyzed daily. Productivity ($g\ L^{-1}d^{-1}$) and specific growth rate ($\mu\ d^{-1}$) calculated following formulas as mentioned below. Data analysis using SPSS software (Version 21) through One-way ANOVA, Tukey HSD and bivariate correlation for comparison between growth parameters readings.

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 ($\mu\ d^{-1}$) 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)

Results

Growth of A. platensis in simple photobioreactors (PBR) with different nitrogen sources

Different nitrogen treatments of urea or ammonium nitrate (AmmN) for *A. platensis* cultivated in land-based PBR (L), and water-based PBR (W) were measured through absorbance, ABS (620nm). For land-based PBR (L) (**Fig. 1**), the ABS result for the first cultivation cycle (wet weather conditions): Urea (L1), AmmN (L1) was not significantly different ($p > 0.05$) from the third cycle: Urea (L3), AmmN (L3). Cultivation in dry weather conditions showed significantly higher optical density ($p < 0.05$) compared to wet weather conditions and mix weather conditions. During the 10 days cultivation period, highest average mean ± SE of the optical density was achieved with culture treated in the second cultivation cycle, AmmN (L2): 1.783±0.021. For water-based PBR (W) (**Fig. 2**), *A. platensis* treated with AmmN (W2) in dry weather conditions showed significantly higher absorbance reading (ABS) ($p < 0.05$) compared to other cycles. The absorbance (ABS) optical density readings of Spirulina were achieved significantly lowest ($p < 0.05$) with urea as nitrogen source under wet weather condition (W1).

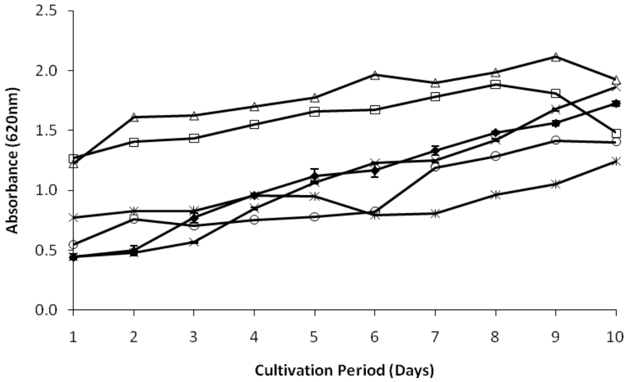

Fig. 1 Absorbance of *A. platensis* grown with urea and ammonium nitrate (AmmN) supplied in land-based simple photobioreactors (PBR) (L: Land-based photobioreactor) for 10 days of cultivation. Urea (L1): (✚); AmmN (L1): (×); Urea (L2): (□); AmmN (L2): (△); Urea (L3): (✳); AmmN (L3): (○). Cultivation cycle: First (1), wet weather conditions; Second (2), dry weather conditions; Third (3), mix weather conditions. Values are presented as Mean ± SE (n = 3).

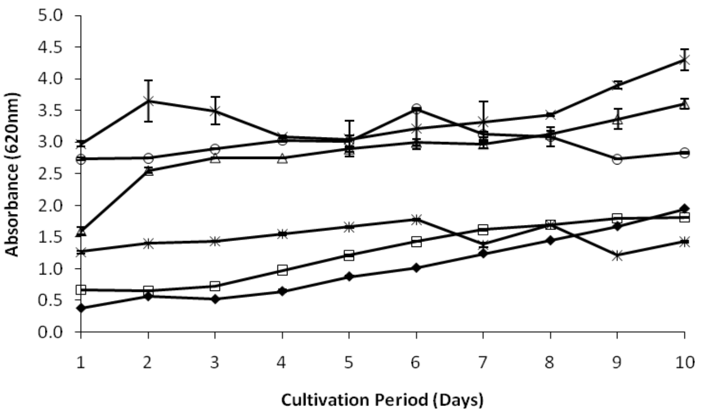

Fig. 2 Absorbance of *A. platensis* grown with urea and ammonium nitrate (AmmN) supplied in water-based simple photobioreactors (PBR) (W: Water-based photobioreactor) for 10 cultivation days. Urea (W1): (◆); AmmN (W1): (□); Urea (W2): (△); AmmN (W2): (×); Urea (W3): (✻); AmmN (W3): (○). Cultivation cycle: First (1), wet weather conditions; Second (2), dry weather conditions; Third (3), mix weather conditions. Values are presented as Mean ± SE (n = 3).

Although optical density was convenient, biomass dry weight (g L^{-1}) is important to determine best yield based on filtered dried powder of Spirulina *(A. platensis).* For land-based PBR (**Fig. 3**), treatment with ammonium nitrate (AmmN) during dry weather condition (L2) had average dry weight (g L^{-1}), 3.026±0.058 was significantly higher ($p < 0.05$) than treatment with Urea. Furthermore, second cycle (dry weather condition) treated *A. platensis* had significantly higher ($p < 0.05$) dry weight (g L^{-1}) collected compared to other cycles of different weather conditions. Water-based PBR (**Fig. 4**) results showed both AmmN and Urea treatments yielded highest significantly ($p < 0.05$) during second cycle of cultivation (dry weather condition) with average dry weight (g L^{-1}), 4.687±0.154 and 4.083±0.128 respectively. Land-based PBR had highest total chlorophyll (**Fig. 5**) during third cultivation cycle with AmmN treatment, which was not significantly different ($p > 0.05$) from Urea (L3) and AmmN (L2) though significantly different ($p < 0.05$) than other cycles. Highest total chlorophyll was achieved with AmmN (W3) are not significantly different ($p > 0.05$) from AmmN (W2). However, these treatments were significantly different ($p < 0.05$) compared to other cycle treatments (**Fig. 6**).

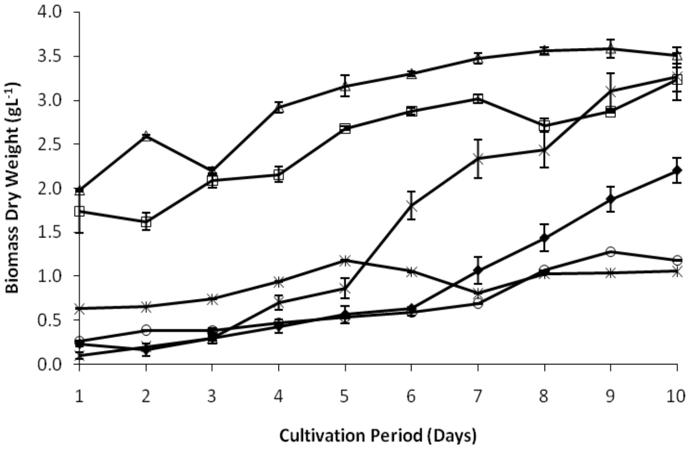

Fig. 3 Biomass dry weight (g L^{-1}) of *A. platensis* grown with urea and ammonium nitrate (AmmN) supplied in land-based simple photobioreactors (PBR) (L: Land-based PBR) for 10 days of cultivation. Urea (L1): (◆); AmmN (L1): (×); Urea (L2): (□); AmmN (L2): (△); Urea (L3): (✻); AmmN (L3): (○). Cultivation cycle: First (1), wet weather conditions; Second (2), dry weather conditions; Third (3), mix weather conditions. Values are presented as Mean ± SE (n = 3).

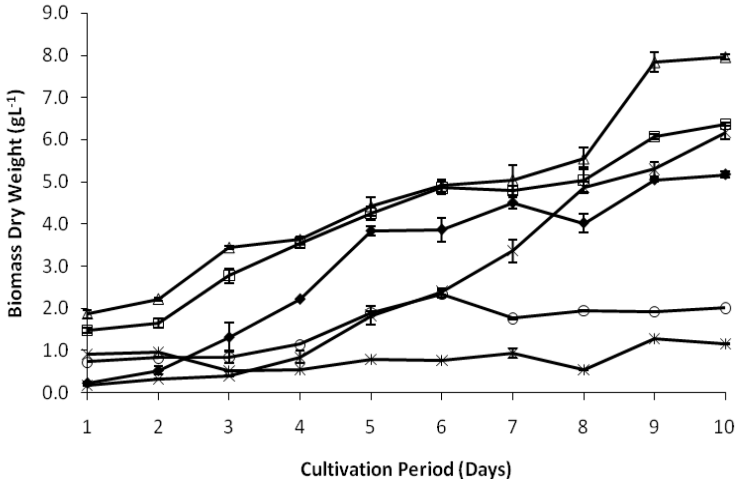

Fig. 4 Biomass dry weight (g L^{-1}) of *A. platensis* grown with urea and ammonium nitrate (AmmN) supplied in water-based simple photobioreactors (PBR) (W: Water-based PBR) for 10 days of cultivation. Urea (W1): (◆); AmmN (W1): (×); Urea (W2): (□); AmmN (W2): (△); Urea (W3): (✳); AmmN (W3): (○). Cultivation cycle: First (1), wet weather conditions; Second (2), dry weather conditions; Third (3), mix weather conditions. Values are presented as Mean ± SE (n = 3).

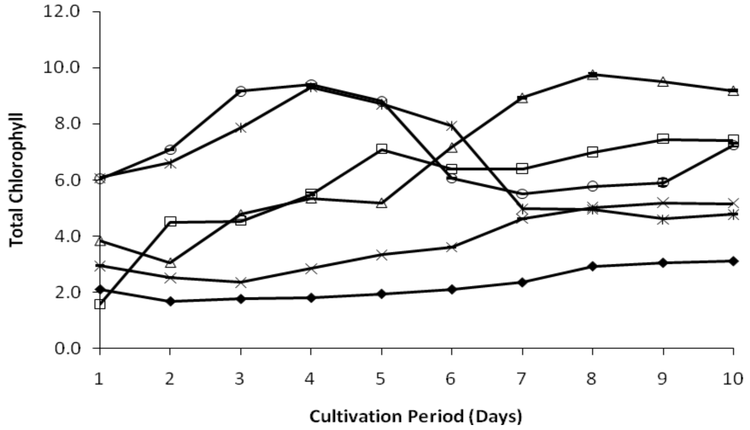

Fig. 5 Total Chlorophyll readings of *A. platensis* grown with urea and ammonium nitrate (AmmN) supplied in land-based simple photobioreactors (PBR) (L: Land-based PBR) for 10 days of cultivation. Urea (L1): (◆); AmmN (L1): (×); Urea (L2): (□); AmmN (L2): (△); Urea (L3): (✳); AmmN (L3): (○). Cultivation cycle: First (1), wet weather conditions; Second (2), dry weather conditions; Third (3), mix weather conditions. Values are presented as Mean ± SE (n = 3).

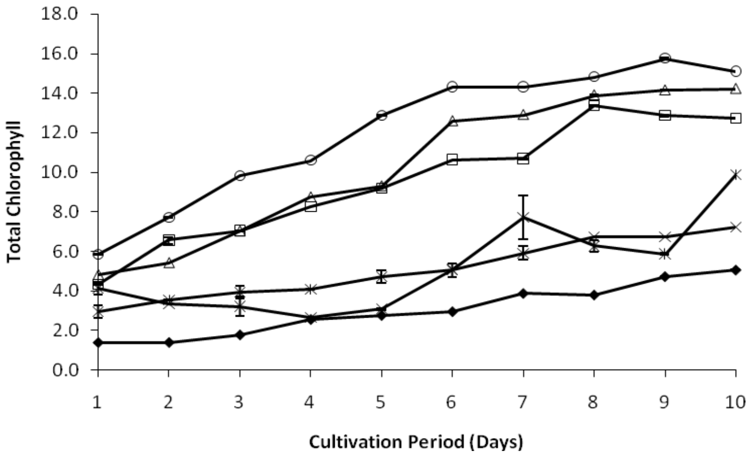

Fig. 6 Total Chlorophyll readings of *A. platensis* grown with urea and ammonium nitrate (AmmN) supplied in water-based simple photobioreactors (PBR) (W: Water-based PBR) for 10 days of cultivation. Urea (W1): (◆); AmmN (W1): (×); Urea (W2): (□); AmmN (W2): (△); Urea (W3): (✳); AmmN (W3): (○). Cultivation cycle: First (1), wet weather conditions; Second (2), dry weather conditions; Third (3), mix weather conditions. Values are presented as Mean ± SE (n = 3).

Results collected from this study with different nitrogen sources could contribute to a better understanding of the microalgae, *Arthrospira platensis* grown in simple photobioreactors under different cycles and weather conditions. As shown in **Table 1**, the differences on the productivity of Spirulina cultured in the simple land photobioreactors between cycles and types of fertilizer used are significant; F = 22.22, ($p < 0.05$). The differences are significant for the specific growth rate as well; F = 54.70, ($p < 0.05$). Whereas, the differences in the simple water based photobioreactors for productivity and specific growth rate of *A. platensis* are widely significant; F = 947.73, ($p < 0.05$) and F = 709.74, ($p < 0.05$) respectively. The performance of Spirulina can be analysed based on the correlation results as shown in **Table 2** and **3** to determine which nutrient supplementation could fit better either using urea or ammonium nitrate under different weather conditions. The gap of Spirulina biomass dry weight and optical density achieved were close to 1 except for Spirulina treated urea for land and water based during dry and mix weather conditions.

Table 1 **Results of productivity (g L^{-1} d^{-1}) and specific growth rate (μ d^{-1}) of *A. platensis* grown with urea and ammonium nitrate (AmmN) supplied in land (L) and water-based (W) photobioreactors (PBR) for 10 days of cultivation. Cultivation cycle: First (1), wet weather conditions; Second (2), dry weather conditions; Third (3), mix weather conditions.**

Cycle	Productivity for land-based (L) PBR (g L^{-1} d^{-1})	Specific growth rate for land-based (L) PBR (μ d^{-1})	Productivity for water-based (W) PBR (g L^{-1} d^{-1})	Specific growth rate for water-based (W) PBR (μ d^{-1})
Urea (1)	0.197 ± 0.011^{b}	0.226 ± 0.010^{b}	0.493 ± 0.009^{b}	0.309 ± 0.034^{b}
AmmN (1)	0.317 ± 0.028^{a}	0.358 ± 0.034^{a}	0.598 ± 0.015^{a}	0.350 ± 0.009^{a}
Urea (2)	0.150 ± 0.037^{bc}	0.064 ± 0.019^{c}	0.489 ± 0.009^{b}	0.146 ± 0.005^{c}
AmmN (2)	0.154 ± 0.010^{bc}	0.057 ± 0.003^{c}	0.610 ± 0.004^{a}	0.145 ± 0.005^{c}
Urea (3)	0.043 ± 0.001^{d}	0.051 ± 0.001^{c}	0.025 ± 0.001^{d}	0.024 ± 0.001^{e}
AmmN (3)	0.092 ± 0.000^{cd}	0.151 ± 0.001^{b}	0.127 ± 0.001^{c}	0.101 ± 0.001^{d}

* Each value is presented as Mean ± SE (n = 3). Means within each column with different letter (a-e) differs significantly ($p < 0.05$).

Table 2 Results of correlation between growth parameters (ABS – absorbance; Biomass – dry weight; TC – total chlorophyll) for land based (L) photobioreactor (PBR) in 3 different cultivation cycle: first (1), wet weather conditions; second (2), dry weather conditions; third (3), mix weather conditions.

Cycle	Biomass & ABS of land-based PBR (pearson correlation, r)	Biomass & TC of land-based PBR (pearson correlation, r)	ABS & TC of land-based PBR (pearson correlation, r)
Urea (1)	0.890	0.936	0.836
AmmN (1)	0.954	0.949	0.929
Urea (2)	0.661	0.815	0.775
AmmN (2)	0.893	0.857	0.824
Urea (3)	0.584	0.066	-0.320
AmmN (3)	0.954	-0.400	-0.453

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

Table 3 Results of correlation between growth parameters (ABS – absorbance; Biomass – dry weight; TC – total chlorophyll) for water based (W) photobioreactor (PBR) in 3 different cultivation cycle: first (1), wet weather conditions; second (2), dry weather conditions; third (3), mix weather conditions.

Cycle	Biomass & ABS of water-based PBR (pearson correlation, r)	Biomass & TC of water-based PBR (pearson correlation, r)	ABS & TC of water-based PBR (pearson correlation, r)
Urea (1)	0.881	0.792	0.540
AmmN (1)	0.936	0.782	0.842
Urea (2)	0.736	0.843	0.747
AmmN (2)	0.980	0.344	0.318
Urea (3)	0.729	-0.205	-0.405
AmmN (3)	0.958	-0.404	-0.431

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

The gap of differences between the biomass of land based photobioreactor (L) and water based photobioreactor (W) through the correlation analyses were not too extensive except under dry and mix weather condition as shown in **Table 4**. Nonetheless, the amount of total chlorophyll gained was persistently corresponding to the amount of light intensity recorded during each cycles. On daily average for 3 cycles with different weather patterns, the amount of light intensity and the culture temperature were as shown in **Fig. 9**. Highest culture temperature was achieved with land based photobioreactor reached up to 33.4 °C. While, highest Spirulina culture temperature reached 32.2 °C. PH was not much affected by the presence of different types of nitrogen sources as long as the growth of *A. platensis* is escalating, number of cells would increasing and so the pH would rise with mounting oxygen gaseous produced as shown in **Fig. 7** & **8**.

Table 4 Results of correlation between growth parameters (ABS – absorbance; Biomass – dry weight; TC – total chlorophyll) of both land and water-based (L&W) photobioreactor (PBR) in 3 different cultivation cycle: first (1), wet weather conditions; second (2), dry weather conditions; third (3), mix weather conditions.

Cycle	Biomass & ABS of (L&W) PBR (pearson correlation, r)	Biomass & TC of (L&W) PBR (pearson correlation, r)	ABS & TC of (L&W) PBR (pearson correlation, r)
Urea (1)	0.881	0.792	0.540
AmmN (1)	0.936	0.782	0.842
Urea (2)	0.736	0.843	0.747
AmmN (2)	0.980	0.344	0.318
Urea (3)	0.729	-0.205	-0.405
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* Correlation is significant at the 0.01 level (2-tailed).

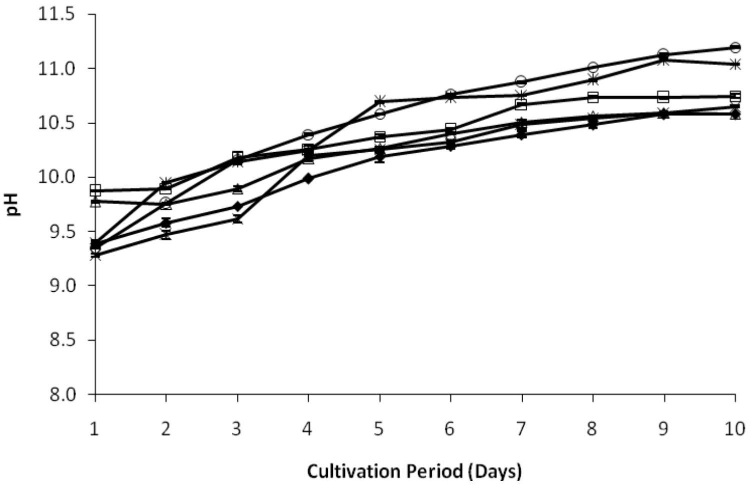

Fig. 7 PH readings of *A. platensis* grown with urea and ammonium nitrate (AmmN) supplied in land-based simple photobioreactors (PBR) (L: Land-based photobioreactor) for 10 days of cultivation. Urea (L1): (◆); AmmN (L1): (×); Urea (L2): (□); AmmN (L2): (△); Urea (L3): (✳); AmmN (L3): (○).Cultivation cycle: First (1), wet weather conditions; Second (2), dry weather conditions; Third (3), mix weather conditions. Values are presented as Mean ± SE (n = 3).

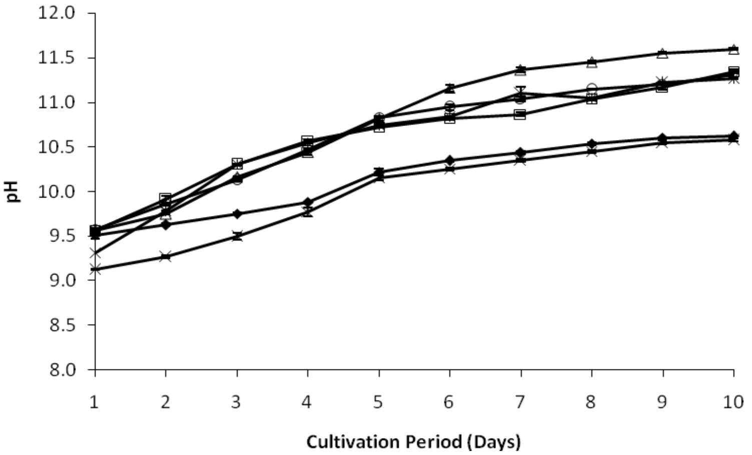

Fig. 8 PH readings of *A. platensis* grown in water-based simple photobioreactors (PBR) (W: Water-based PBR) for 10 days of cultivation. Urea (W1): (◆); AmmN (W1): (×); Urea (W2): (□); AmmN (W2): (△); Urea (W3): (✳); AmmN (W3): (○).Cultivation cycle: First (1), wet weather conditions; Second (2), dry weather conditions; Third (3), mix weather conditions. Values are presented as Mean ± SE (n = 3).

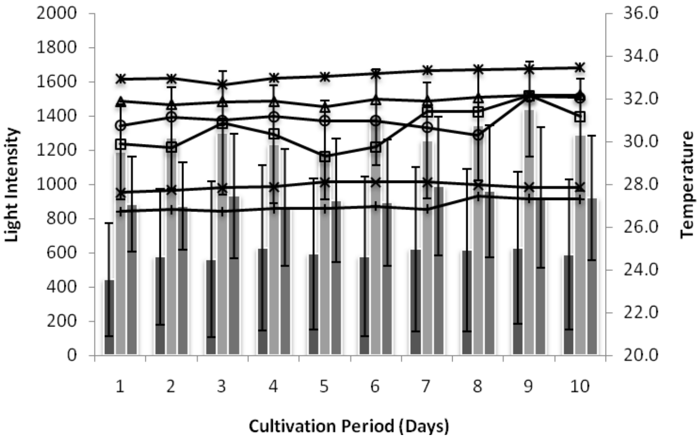

Fig. 9 Temperature (°C) and light intensity (µmol.m^{-2}.s^{-1}) readings during 10 days of *A. platensis* cultivation. Photobioreactors: Land-based (L): L1, (×); L2, (✱); L3, (○); Water-based (W): W1, (+); W2, (△); W3, (□). Cultivation cycle: First, wet weather conditions- ▮; Second, dry weather conditions- ▮; Third, mix weather conditions- ▮. Values are presented as Mean ± SE (n = 3).

Discussion

The proportion and types of nutrient used play significant role influencing growth rate Spirulina. However in this study, the design of simple constructed photobioreactors itself would hypothetically gives either benefits of boosting growth of Spirulina based on nutrients fed under different weather conditions or despondently degrade the cultured Spirulina. Under dry weather conditions, a cultivation system requires efficient temperature regulating system to balance immediate fluctuated of culture temperature which could also affect the solubility rates or dispersion efficiency of nutrient (Lomas & Glibert, 1999). Without sufficient acclimatization, Spirulina cultures might collapse under extreme weather conditions (Al Mahrouqi et al., 2015a; 2015b). Some of reported studies on utilizing different nitrogen sources for Spirulina cultivations were reported by Cohen et al., (1987), Richmond (1990), Manabe et al., (1992), Gibbs (1995), Chen & Zhang (1997), Costa et al. (2001), Matsudo et al., (2009) and Ak (2012). However most of reported finding conducted in different scale of cultivation and studied in indoor conditions unlike in this study where the cultivation was set up under outdoor conditions. Most growth parameters would be affected under the outdoor conditions especially the factor of light; photolimitation limits growth and chlorophyll contents (Danesi et al., 2004) while, photoinhibition from extreme light intensity would cause damages to the algae cells and dropped the total chlorophyll gained (Chojnacka and Noworyta, 2004). Factor of temperature was experimentally proven affecting protein synthesis during nitrogen hydrolyzation process occurrence in alkaline condition. This indirectly accumulated fatty acid composition also when the temperature increased (Ogbonda et al., 2007). High evaporation rate during high temperature also increased the rate of ammonia loss which later limits nitrogen conversion factors and the algae cell growth (Danesi et al., 2002). Hence, pulse feeding was introduced to counter huge losses of ammonia within short period under high temperature and evaporation rates condition.

In lab-scale condition, 0.01 M urea was observed promoting biomass production better compared to acid ammonium phosphate and ammonium nitrate with the same amount (Costa et al., 2001). Batch feeding was suggested for Spirulina cultivation to avoid any inhibitory of ammonia concentration in the medium (Matsudo et al., 2009). Ammonium nitrate provides preferred type of nitrogen sources (ammonia) and reserved (nitrate) for *A. platensis* (Boussiba, 1989). In outdoor conditions, excessive evaporation might occur due to increasing culture temperature during dry and semi dry weather condition. The constant presence of nitrate from ammonium nitrate supply adequate nitrogen amount with ammonia and less nitrate is converted into ammonia by nitrate reductase (Hatori & Myers, 1996). Even though nitrogen source is a limiting factor for the growth of Spirulina, too high concentration of ammonia would easily damage Spirulina cells. Hence, Ferreira et al., (2010) recommended ammonium nitrate as nitrogen sources for tubular photobioreactor system to prevent nitrogen loss by off-gassing and water evaporation as nitrogen sources from urea tend to diminish faster compared to ammonium nitrate after being dissolved in culture medium (Shimamatsu, 2004; Cruz-Martinez et al., 2015).

Previous study has shown that the cell productivity, cell composition and the yield of specific products are affected by the culture medium (Imamoglu *et al*., 2007). In a large scale production of Spirulina, suitable culture medium should be the important factors in order to optimize the microalgae biomass (Sankar and Ramasubramaniam, 2012). High concentration of salts in the culture medium is needed by these photosynthetic microalgae that will possess higher biomass and productivity (Satyanarayana et al., 2011). One of the significant effects that play an important role in altering growth rate and primary productivity is nutrient concentration in the medium which has been critically reviewed by Healey (1973). There are major difference for the growth of Spirulina and their biomass production between open and closed cultivation system which can be associated with the light contact of the culture and the atmosphere (Grobbelaar, 2009). According to Borowitzka and Moheimani (2013), there are two large scale systems for algal cultivation; outdoor and indoor system. In the outdoor system, the microalgae are exposed to the environment directly, whereas in indoor cultivation is when microalgae are cultured within a wholly enclosed, tubular vessel. They also stated that, outdoor cultivation may be used for waste water treatment and it is the main system to produce high production of microalgae commercially (Borowitzka and Moheimani, 2013). All in all, outdoor productions offered amazing opportunities for experimental and research of mass microalgae work (Grobbelaar, 2009).

This study was carried out to provide an inexpensive growth medium for Spirulina that can be used in a simple developed photobioreactor system. Medium used was based on original Kosaric's medium; however the performance of dry biomass and productivity rate of Spirulina depends more on the techniques used to distribute the fertilizers in culture medium, the sources type of nutrients and the proportion fixed for the growth medium. It has been proven through this experiment that ammonium nitrate projected better performance as a nitrogen source at lower cost.

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