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The Prospects of the Cultivation of *Arthrospira platensis* under Outdoor Conditions in Malaysia

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Abstract

There is no virtual report on the commercial cultivation of *Arthrospira* in Malaysia beyond the laboratory scale probably because of the high costs of production and the lower yield which are highly interconnected with the algal cultivation techniques. One way to alleviate the production cost is through outdoor mass cultivation under natural conditions using all available resources. Therefore, the present study was conducted to investigate the prospects of the production of *Arthrospira platensis* under Malaysian tropical climate using enhanced cultivation techniques to reach a maximum yield. In this study, the growth and yield of *A. platensis* were investigated under three different cultivation conditions: laboratory (control), outdoor shaded (greenhouse, T1), and outdoor non-shaded (field, T2). The algal growth was measured through optical density, biomass dry weight, and chlorophyll *a* content. The algal yield was determined by calculating its productivity and specific growth rate. The *A. platensis* cultivation under outdoor non-shaded conditions achieved significantly higher growth ($p < 0.05$) with 1.62 ± 0.038 ABS of maximum optical density, 0.88 ± 0.020 g L^{-1} of maximum biomass dry weight, 8.77 ± 0.219 mg L^{-1} of maximum chlorophyll *a* content, 0.091 ± 0.0022 g L^{-1} d^{-1} of productivity and 0.220 ± 0.0017 µ d^{-1} of specific growth rate over a cultivation period of eight days. The present finding showed that the Malaysian climate is suitable for a satisfactory *A. platensis* productivity with proper cultivation techniques such as the pre-adaptation of the algal culture, inoculation in the late evening, continuous agitation and compensation of the evaporated culture medium.

Keywords: *Arthrospira platensis*; Biomass production; Outdoor cultivation; Cultivation techniques; Tropical climate

1. Introduction

Global warming and food insecurity associated with the inevitable increasing world population have been major global issues threatening humanity as a whole, specific societies, the economy, and nature over the past several decades. Human activities such as logging, deforestation, agricultural practices, waste disposal, and extensive usage of fossil fuels such as coal, oil and natural gas increased the emissions of CO_2, which is a major component of the greenhouse gasses responsible for global warming and climate change (Omer, 2008). Anthropogenic activities simultaneously with the escalating climate change resulted in water scarcity, depletion of cultivable land, and soil infertility, the three major bottlenecks hindering productive agricultural practices. Subsequently, failure in global food production to meet the rapidly-growing world population and the increasing demand for food ultimately resulted in global food crisis, starvation, malnutrition, morbidity and mortality. With the growing concerns about the impacts of global warming and food crisis, societies started to look for sustainable, economically-feasible, and environment-friendly technologies to mitigate the effects of global warming and sustain food production around the world. From this perspective, the photoautotrophic cultivation of microalgae gained plausible attention mainly because of its simultaneous CO_2 sequestration and the production of profitable bio-active compounds in a single process (Ravindran *et al*., 2016).

Considering the advantages of microalgal cultivation, research and developments began with commercial-scale production of *Chlorella*, *Scenedesmus*, *Arthrospira* and *Dunaliella* (Pulz and Gross, 2004; Borowitzka, 1999). Among them, *Arthrospira* (previously known as *Spirulina*) has attracted public and private interests due to its peculiar properties such as its large filamentous size (0.5 mm length); good for an easy harvesting, its effortlessly-digestible cell membrane and because it poses less risks regarding the external contamination due to its ability to

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grow in saline environments and alkaline conditions (up to pH 11) unlike other species (Pelizer *et al*., 2003). *Arthrospira* is a photosynthetic, blue-green, spiral-shaped and multicellular cyanobacterium, which has been consumed as food for thousands of years (Jensen *et al*., 2001). This microorganism is recognized as a potential dietary supplement due to its rich nutritional value such as the abundant protein content (50 – 70 % dry weight), low nucleic acid content, vitamins, pigments, minerals, polyunsaturated fatty acids (PUFAs) among others (Habib *et al*., 2008). Nowadays, the industrial production of *Arthrospira* has stepped into its fourth decade. In fact, this biological origin food, commonly known as Spirulina, is magnificently sustained in the market because of its well-established antioxidants, hypocholesterolemic, anti-inflammatory, antiviral, anticancer, as well as its hepatoprotection and immune-enhancing properties which have been confirmed through research findings (Makhlouf and Makhlouf, 2012; Wu and Ho, 2008).

Currently, the global commercial production of *Arthrospira* is estimated to be at about 8,000 metric tons per year (Vonshak *et al*., 2014), which is mainly contributed by China, followed by other producers in North America and the Asia-Pacific region (Belay, 2013). Concerning this low annual yield compared with the escalating global population, numerous studies are being conducted since the past two decades in order to maximize the worldwide *Arthrospira* production mainly through focusing on abiotic factors such as light intensity and wavelength, temperature, medium composition and concentration, pH and salinity in different cultivation systems including open ponds and photobioreactors under both indoor (laboratory) and outdoor conditions. Subsequently, the individual or the combined effects of these environmental factors on the productivity and biomass composition of *Arthrospira* under controlled laboratory conditions have been intensively investigated and well documented. These previous findings postulated that relatively high temperatures and a profuse supply of light are prerequisites for an optimal growth of this blue-green microalga (Belay, 2008).

Being a tropical country, the Malaysian humid climate with its favourable temperatures ranging from 25 °C to 35 °C as well as the high intensity and duration of sunlight could be very advantageous in the cultivation of *Arthrospira* throughout the year given that there is neither winter nor cold seasons. There were few pilot scale studies conducted in both indoor and outdoor conditions of the local climate (Fagiri *et al*., 2013; Phang *et al*., 2000) showing the prospective of the *Arthrospira* cultivation in Malaysia. However, the commercial production of this microalga is not extensively applied in Malaysia up to now due to the lack of know-how and awareness of its potential profitability, the high cost of production and the low biomass yield. Hence, cultivation techniques should be enhanced in order to optimize *Arthrospira* productivity through economical, feasible and applicable approaches. One way to achieve this goal is by growing this blue-green alga under outdoor conditions using natural solar radiation at ambient temperatures (Vonshak, 1997). Outdoor microalgal production is a complex system synergistically affected by a large number of variables including the quantity of solar radiation, photoperiod, fluctuating temperatures, humidity changes, salinity and pH (Borowitzka, 1999). Those who efficiently simulated the natural ecosystems for the *Arthrospira* production by surmounting the problems raised proved successful and sustained in this industry.

Many suggestions, improvements and constraints associated with the outdoor mass production of *Arthrospira* have been discussed before but, these were mostly related to the seasonal regions (Vonshak and Richmond, 1988). Based on the previous investigations, diurnal fluctuations that impose photo-inhibition, light limitation (Lu and Vonshak, 1999), and hot temperatures above 40 °C (Chanawongse *et al*., 1994) during a significant part of the day are considered as major setbacks for outdoor cultivation as they restrain the culture from achieving its maximum productivity by decreasing the photosynthesis activity, which sometimes causes cell death (Chaiklahan *et al*., 2007). In some former studies, outdoor *Arthrospira* cultivation showed a marked decrease in cell density while better growth was observed under laboratory conditions with continuous light (Vonshak and Richmond, 1985) and outdoor shaded conditions (Vonshak and Guy, 1992). There is lack of information on the cultivation of *Arthrospira* in non-shaded outdoor conditions in the Malaysian tropical climate with 12:12 photoperiod where the intensity of sunlight used to be above 1,000 µmol m^{-2} s^{-1}, and the ambient temperature exceeding 40 °C during the midday. Henceforth, the present study was conducted under three different cultivation conditions in laboratory, outdoor shaded and outdoor non-shaded sites to investigate the prospects of the *Arthrospira* cultivation under Malaysian tropical climate using proper techniques to reach the maximum algal yield.

2. Materials and Methods

2.1. Arthrospira platensis Culture

An axenic culture of *A. platensis* was obtained from The Culture Collection of Algae at The University of Texas, Austin (UTEX). This microalgal species was cultured and pre-adapted in indoor and outdoor conditions using standard Kosaric medium through batch cultivation for two months to facilitate the *A. platensis* adaptation to the new environmental conditions.

2.2. Experimental Cultivation Conditions for Optimum A. platensis Growth

The indoor cultivation of *A. platensis* was experimented under controlled conditions in a pure culture room of Plant Physiology Laboratory, Biology Department at the Faculty of Science, Universiti Putra Malaysia (UPM) at 27.5 ± 1 °C with a continuous illumination (24 h photoperiod) of 27 ± 2 µmol m^{-2} s^{-1} using Philips TLD fluorescent light. Concurrently, the outdoor growth study was conducted in shaded and non-shaded cultivation sites under fluctuating environmental conditions with 12:12 photoperiod. Accordingly, the growth of *A. platensis* under shaded outdoor conditions was investigated in a greenhouse located in the Biology Department. Meanwhile, non-shaded outdoor cultivation of this cyanobacterium was experimented in Field 2, Faculty of Agriculture, UPM.

2.3. Preparation of Experimental Growth Medium

Standard Kosaric medium (SKM) was prepared in half concentration as described by Sukumaran *et al*. (2018) as follows (g L^{-1}): 4.500 $NaHCO_3$, 0.250 NaCl, 0.010 $CaCl_2$, 0.050 $MgSO_4 \cdot 7H_2O$, 0.625 $NaNO_3$, 0.125 K_2HPO_4, 0.250 K_2SO_4, 0.025 $FeSO_4 \cdot 7H_2O$ and 0.5 mL L^{-1} of trace metals solution composed of the following elements (g L^{-1}): 2.86 H_3BO_3, 1.81 $MnCl_2 \cdot 4H_2O$, 0.22 $ZnSO_4 \cdot 7H_2O$, 0.08 $CuSO_4 \cdot 5H_2O$, 0.01 MoO_3, and 0.01 $COCl_2 \cdot 6H_2O$.

2.4. Experimental Design

A. platensis cultured under laboratory conditions was considered as control to study the growth and productivity of *A. platensis* in shaded outdoor (greenhouse; T1) and non-shaded outdoor (field; T2) conditions. Control, T1 and T2 were cultured in five replicates in 5 L sterilized polyethylene bottles containing 4 L working volume. Consequently, 10% (v/v), which is 400 mL of the pre-adapted algal culture was transferred into 3.6 L cultivation medium of the respective control and treatments. Two holes were designed on the cap of the bottle to hold tubing for aeration and gas exchange respectively. The algal culture was aerated using an aquarium air pump (80W, 220V - 240V, Hailea®) through standard 3/16-inch diameter airline tubing with an air stone suspended in the middle of the bottle to provide continuous mixing and agitation. The cultivation period was fixed at eight days based on preliminary observation where the algal culture under non-shaded outdoor conditions started to experience a stationary phase on day nine and onwards. The cultivation process was conducted during mostly sunny and clear skies with minimum rainfall weather conditions.

2.5. Measurement of Environmental Factors during A. platensis Cultivation

Throughout the *A. platensis* cultivation period, the environmental parameters including light intensity (µmol m^{-2} s^{-1}) and air temperature (°C) were recorded daily from seven am to seven pm with a two-hour interval period using a light meter (Li-250, LI-COR® Biosciences) and thermometer (Traceable® Big-Digit Four-Alert Alarm, Fisher Scientific) respectively.

2.6. Measurement of A. platensis Growth

The growth of *A. platensis* cultured in control, T1 and T2 was measured using three growth parameters, namely: optical density, biomass dry weight, and chlorophyll *a* content to precisely determine the growth pattern of this microalga. The algal sample was homogenized each time before being analysed to avoid the sedimentation of cells, which could adversely affect the precision of measurements. The optical density of *A. platensis* cultures was measured daily using spectrophotometer (Hitachi U-1900) at 620 nm (Sukumaran *et al*., 2014).

The biomass dry weight of *A. platensis* was determined gravimetrically every alternate day following Lee and Shen (2004). After being filtered with pre-washed, dried and pre-weighed glass microfiber filter papers (GF/C, 47 mm Ø, 1.2 µm pore sizes, Whatman®) under vacuum, the cells were rinsed with distilled water in order to eliminate leftover mineral salts and other possible extracellular material. After removing the excess moisture through a two-stage vacuum pump, the filtered cells were dried in an oven (Memmert) at 60 – 70 °C for twenty-four hours and were then weighed to determine the dry biomass weight.

Chlorophyll *a* was determined spectrophotometrically on day eight after extraction with 95 % ethanol for five minutes in a water bath at 70 °C (Lichtenthaler, 1987) and subsequent refrigeration at 4 °C for twenty-four hours under dark conditions for a maximum chlorophyll extraction. The absorbance was measured at 664 and 649 nm through spectrophotometer (U-1900, Hitachi) against prepared blank and the chlorophyll *a* concentration was computed following Lichtenthaler and Buschmann (2001).

2.7. Productivity of A. platensis

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

$$P_X = (X_m - X_i)(T_c)^{-1} \quad (1)$$

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}), and

T_c = cultivation duration related to X_m (days).

2.8. Specific Growth Rate of A. platensis

Specific growth rate of *A. platensis* was calculated according to Madkour *et al*. (2012) using the following equation:

$$\mu_m = (\ln X_m - \ln X_i)(T_c)^{-1} \quad (2)$$

where,

μ_m = maximum specific growth rate (µ d^{-1})

X_i = initial biomass concentration (g L^{-1}),

X_m = maximum biomass concentration (g L^{-1}), and

T_c = cultivation duration related to X_m (days).

2.9. Data analysis

The maximum biomass dry weight, chlorophyll *a* content, productivity and specific growth rate of *A. platensis* cultured in control and the two treatments (T1 and T2) were analyzed using SPSS software (version 21) by the one-way independent analysis of variance (ANOVA) followed by Tukey HSD (Honestly Significant Difference) multiple comparison test.

3. Results

The average light intensity and air temperature throughout the cultivation period are shown in Figure 1. Light intensity and atmospheric temperature were relatively higher in non-shaded outdoor (field) conditions compared to shaded outdoor (greenhouse) and indoor (laboratory) conditions during daytime. Under the controlled laboratory conditions, light intensity and air temperature were stable at 24.66 ± 0.308 µmol m^{-2} s^{-1} and 27.5 ± 0.44 °C respectively throughout the day. Besides, the solar radiation under shaded outdoor conditions varied from 1.2 µmol m^{-2} s^{-1} to 367.0 µmol m^{-2} s^{-1} throughout the day. The average atmospheric temperature in this conditions varied from 24.7 °C - 28.7 °C in the morning, 31.5 °C - 34.6 °C during midday and 31.3 °C - 32.1 °C in the late afternoon. Meanwhile, the average light intensity in non-shaded outdoor conditions fluctuated between 4.2 µmol m^{-2} s^{-1} - 413.8 µmol m^{-2} s^{-1} in the morning, 1,007.0 µmol m^{-2} s^{-1} - 1,311.7 µmol m^{-2} s^{-1} during midday, and

27.2 µmol m^{-2} s^{-1} - 500.3 µmol m^{-2} s^{-1} in the late afternoon. The average ambient temperature in this conditions fluctuated between 25.5 °C - 29.9 °C in the morning, 35.8 °C - 39.9 °C during midday, and 32.8 °C - 35.6 °C in the late afternoon.

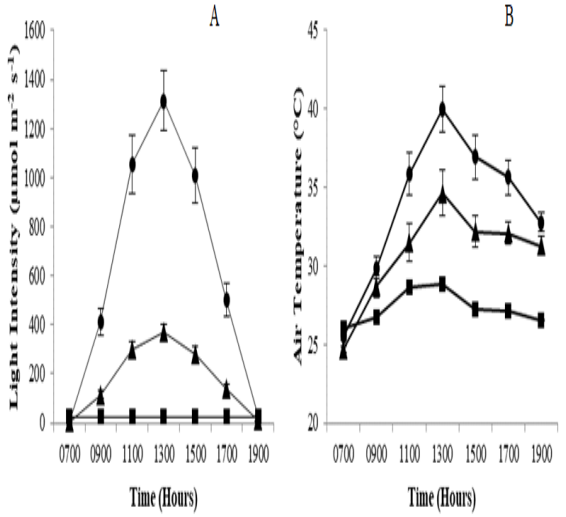

Figure 1. Average light intensity (A) and air temperature (B) throughout the cultivation duration in the laboratory; (■), shaded outdoor in greenhouse; (▲) and non-shaded outdoor in field; (●). Values are presented as mean ± SE (n = 8)

The result of growth curves based on optical density and biomass dry weight of the *A. platensis* culture under three culture conditions were illustrated in Figure 2. Based on the plotted growth curves, there was no presence of lag phase due to the preadaptation process. The growth curves also show incredible growth of *A. platensis* under non-shaded outdoor conditions compared to shaded outdoor and indoor controlled conditions.

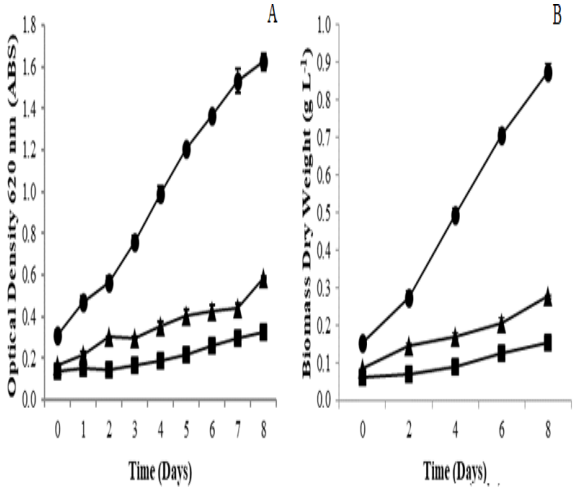

Figure 2. Optical density (A) and biomass dry weight (B) of *A. platensis* grown in different cultivation conditions. Control in laboratory; (■), T1 in shaded outdoor in greenhouse; (▲), T2 in non-shaded outdoor in field; (●). Values are presented as mean ± SE (n = 5)

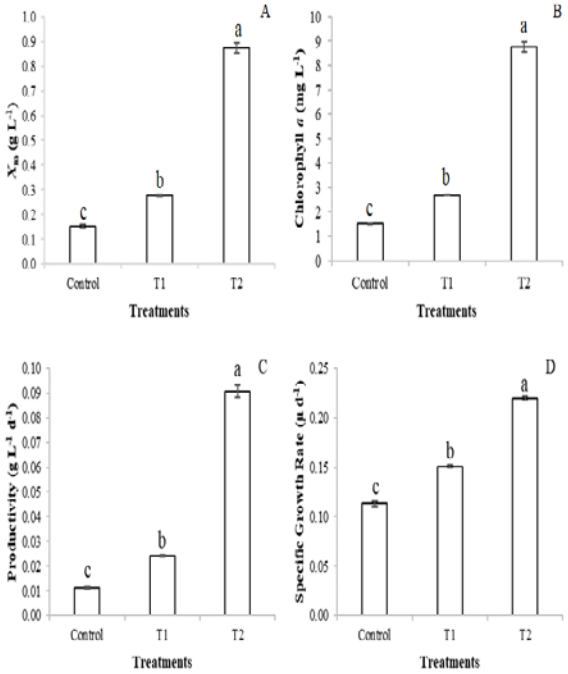

Figure 3. Maximum biomass dry weight, X_m (A), chlorophyll *a* (B), productivity (C) and specific growth rate (D) of *A. platensis* grown in different cultivation conditions. Control; (laboratory), T1; (shaded outdoor in greenhouse), T2; (non-shaded outdoor in field). Values are presented as mean ± SE (n = 5). Means with different letters (a-c) differ significantly ($p < 0.05$)

Figure 3 shows the maximum yield of *A. platensis* on day eight in terms of: biomass dry weight, chlorophyll *a* content, productivity and specific growth rate. This blue-green alga produced significantly higher ($p < 0.05$) biomass dry weight, chlorophyll *a* content, productivity and specific growth rate under non-shaded outdoor conditions (T2) with 0.88 ± 0.020 g L^{-1}, 8.77 ± 0.219 mg L^{-1}, 0.091 ± 0.0022 g L^{-1} d^{-1} and 0.220 ± 0.0017 µ d^{-1} respectively, followed by shaded outdoor conditions (T1) with 0.28 ± 0.005 g L^{-1}, 2.68 ± 0.022 mg L^{-1}, 0.024 ± 0.0004 g L^{-1} d^{-1} and 0.151 ± 0.0014 µ d^{-1} respectively. Meanwhile, microalga cultured in control under laboratory conditions achieved significantly lower ($p < 0.05$) biomass dry weight (0.15 ± 0.007 g L^{-1}), chlorophyll *a* (1.52 ± 0.024 mg L^{-1}), productivity (0.011 ± 0.0005 g L^{-1} d^{-1}) and specific growth rate (0.113 ± 0.0027 µ d^{-1}).

4. Discussion

The growth and productivity of *A. platensis* were significantly higher ($p < 0.05$) under outdoor non-shaded conditions compared to laboratory and shaded outdoor conditions probably due to one or to a combination of factors. Meteorological factors particularly irradiance flux and temperature have highly influenced the growth of microalga under outdoor conditions as these two factors largely vary throughout the day as the consequences of diurnal effect, cloud cover, rainfall and sometimes haze. In the present investigation, the average light intensity in outdoor non-shaded conditions was about 3.6-fold higher than shaded outdoor conditions (between 0700 and 1900); about forty times higher than laboratory conditions (between 1100 and 1700). On average, the recorded light intensity in shaded outdoor conditions was about 11-fold

higher than the laboratory conditions (between 1100 and 1700). Meanwhile, on average, the temperature in shaded (greenhouse) and non-shaded outdoor (field) conditions was higher about 5 °C and 10 °C respectively than in laboratory conditions (between 1100 and 1700). Moreover, the average temperature was increased about 10 °C and 15 °C in shaded and non-shaded outdoor conditions respectively from 0700 to 1300.

Besides, the light intensity and temperature reached the maximum during midday, which exceeded 1,300 µmol m^{-2} s^{-1} and 40 °C respectively under outdoor non-shaded conditions. Previous studies conducted under outdoor conditions showed reduced growth rate, photosynthetic efficiency, and chlorophyll content during such peak hours mainly due to photoinhibition (Vonshak *et al*., 2014; Torzillo *et al*., 1998; Chanawongse *et al*., 1994). Photoinhibition is light-induced photo-oxidative stress occurring at light intensities above the saturation of the photosynthetic rate, which causes severe photo-damage to photosynthetic pigments and, in extreme cases, a total loss of the algal culture (Soletto *et al*., 2008; Jensen and Knutsen, 1993). Moreover, the culture temperature between 35 °C and 37 °C was found to be optimum for microalgal biomass productivity (Richmond, 1988) while, a further increase in temperature was observed to hinder the growth rate due to inactivation of PSII activity (Chaiklahan *et al*., 2007). Previous investigations conducted on outdoor cultivation with 12:12 photoperiod also emphasized the night biomass loss due to respiration during the dark cycle (Edmundson and Huesemann, 2015; Phang *et al*., 2000), which influences the net *Arthrospira* productivity.

Despite the former reports on the possible limitations and inhibitions associated with microalgal farming in the natural ecosystem, *A. platensis* cultivation under outdoor non-shaded conditions in the present investigation, produced satisfactory growth within a cultivation period of eight days. The maximum algal growth in outdoor non-shaded conditions was 2.8, 3.2, 3.3, 3.8 and 1.5-fold higher than in shaded outdoor conditions and 5.0, 5.7, 5.8, 8.0 and 1.9-fold higher than in laboratory conditions in terms of optical density, biomass dry weight, chlorophyll *a* content, productivity and specific growth rate respectively. The present result seems better than a recent study conducted by Fagiri *et al*. (2013) who cultured *A. platensis* strain UTEXLB2340 under outdoor Malaysian conditions using Zarrouk's medium with a maximum optical density of 1.04 ABS (at 560 nm) over a cultivation period of twenty days. The growth of this blue-green alga cultured in reduced amounts of nutrients (half concentration Kosaric medium) within eight days in the present study is considered slightly better compared to the previous investigations conducted under indoor and outdoor conditions in various regions with longer cultivation periods as shown in Table 1.

A number of cultivation techniques were implemented in the present study aiming to maximize the productivity of this cyanobacterium under outdoor cultivation by reducing the inhibitory effects of the fluctuating extreme environmental conditions. Initially, the *A. platensis* culture was pre-adapted to the outdoor conditions through repeated batch cultivation processes for about two months. Previous studies demonstrated that algal cells acclimatized to the changes in growth conditions were less susceptible to the stress factors associated with the new environment compared to the non-acclimatized culture (Grobbelaar, 2007; Vonshak *et al*., 1996). Such acclimation involves an increase in the respiratory activity and a partial retrieval of photosynthetic activity in the *Arthrospira* cells after the initial drop due to exposure to stress conditions (Vonshak *et al*., 1988). The absence of lag phase in the growth curves (Figure 2) of *A. platensis* cultured in different conditions showed the adapted stage of this microalga to the new environment (outdoor conditions). Next, inoculation of alga for the new cycle was done during sunset to encourage the initial culture growth rate throughout the night under cool and dark conditions as the new culture is less tolerant to high light and temperature occurring during midday under outdoor conditions due to low cell density (Vonshak *et al*., 2014).

Next, the culture was continuously aerated throughout the cultivation period. Agitation is an important operational factor to reduce the prolonged exposure of algal cells to photo-inhibition and photo-limitation (Richmond, 2004) while allowing for a better assimilation of nutrients by *A. platensis* and enhancing gas exchange (Ogbonda *et al*., 2007). Accordingly, Vonshak *et al.* (2014) noted significantly smaller reduction in the maximal photochemical efficiency of PSII at the higher turbulence compared to the cultures with the lower turbulent flow during midday. A similar result was observed in other former studies (Grobbelaar, 1994; Richmond, 1992). Besides, the evaporated culture medium in the present investigation due to the high temperature during midday was compensated by tap water in order to maintain the salinity and pH of the growth conditions. This was done to avoid the sudden salinity and alkalinity shock during the cultivation period, which could hinder the algal productivity (Zeng and Vonshak, 1998). On the whole, the aforementioned cultivation techniques can be applied in such a way to achieve superior growth and productivity of alga compared to those occurring under indoor controlled conditions and shaded outdoor conditions.

Table 1. Growth of *Arthrospira* in present study compared to previous investigations

Medium	X_m (g L^{-1})	P_X (g L^{-1} d^{-1})	μ_m (d^{-1})	Chl a_{max} (mg L^{-1})	Duration	Cultivation conditions	References
Zarrouk's	1.18	0.075	0.109	8.81	15 d	Laboratory (30 °C in 50 µmol photons m^{-2} s^{-1} with 14:10 h light:dark PP)	Kumari *et al*. (2015)
Zarrouk's	0.58	0.031	0.175	23.22	18 d	Laboratory (30±1 °C in 50 µmol photons m^{-2} s^{-1} with 12:12 h light:dark PP)	Raoof *et al*. (2006)
Zarrouk's	0.72	0.030	0.074	-	450 h	Greenhouse (30 °C with 12:12 h light:dark PP)	Colla *et al*. (2007)
50% Zarrouk's	0.99	0.044	0.106	5.71	20 d	Greenhouse (Transparent jars)	Goksan *et al*. (2007)
20% Zarrouk's	0.90	0.059	-	-	18 d	Greenhouse with forced aeration (25.3°C under 12:12 h light:dark PP)	Walter *et al*. (2011)
20% Zarrouk's	1.33	0.054	0.160	-	≈ 25 d	Greenhouse (open bioreactors)	Andrade and Costa (2008)
SOT + underground water	0.80	0.048	0.187	5.12	16 d	Greenhouse (semi-outdoor)	Kim *et al*. (2007)
SOT	1.05	0.042	-	-	20 d	Outdoor	Toyoshima *et al*. (2015)
50% Zarrouk's	1.20	0.079	0.177	-	15 d	Outdoor (Transparent jars)	Singhal and Kumar (2017)
50% SKM	0.88	0.091	0.220	8.77	8 d	Outdoor	Present study

X_m: Maximum biomass dry weight; P_X: Productivity; μ_m: Maximum specific growth rate; Chl a_{max}: Maximum chlorophyll *a*; d: Days; h: Hours; PP: Photoperiod

5. Conclusion

A. platensis cultivation under outdoor non-shaded conditions (field) gave superior results compared to indoor laboratory and outdoor shaded conditions (greenhouse). Previously, it was assumed that Malaysia has unfavorable cultivation conditions for the microalgal growth due to the frequent cloud covering and precipitation. However, in the present investigation, Malaysian climatic conditions were proven to support a maximum yield of *A. platensis* with appropriate cultivation methods such as pre-adaptation of the algal culture, inoculation at late evening, continuous agitation and compensation of the evaporated culture medium. Unlike the controlled laboratory conditions, microalgal production in the real environment using the available resources potentially reduced the production cost as it requires less energy and supervision. Integration of the algal production system into natural conditions, as per being practiced in the agricultural sectors for the crop farming, paves the way towards the practical application of algal farming in Malaysia for commercial purposes. On the other hand, this study also highlighted the significance of the acclimatization process in the optimization of algal growth and productivity, especially when introducing the microalgal cultures to the new cultivation conditions through avoiding the presence of the lag phase and a shorter culture period. Besides, the utilization of half concentration growth medium (SKM) with satisfactory algal growth also reduced beneficially the cost of algal production by discounting the nutrient cost. On the whole, the present study proved that the cultivation of *A. platensis* in outdoor conditions under Malaysian tropical climate through proper cultivation methods is technically and economically viable.

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References

Andrade MR and Costa JAV. 2008. Outdoor and indoor cultivation of *Spirulina platensis* in the extreme south of Brazil. *Z Naturforsch C.*, **63(1-2)**: 85-90.

Belay A. 2008. Spirulina (*Arthrospira*): Production and quality assurance. *In*: Gershwin ME and Belay A (Eds.), **Spirulina in Human Nutrition and Health.** CRC Press, Taylor and Francis Group, Boca Raton, FL, pp. 1-25.

Belay A. 2013. Biology and industrial production of *Arthrospira* (*Spirulina*). *In*: Richmond A and Hu Q (Eds.), **Handbook of Microalgal Culture: Applied phycology and biotechnology.** Blackwell Publishing Ltd., Oxford, London, pp. 339-358.

Borowitzka MA. 1999. Commercial production of microlalgae: Ponds, tanks, tubes and fermentors. *J Biotechnol.*, **70(1-3)**: 313-321.

Chaiklahan R, Khonsarn N, Chirasuwan N, Ruengjitchatchawalya M, Bunnag B and Tanticharoen M. 2007. Response of *Spirulina platensis* C1 to high temperature and high light intensity. *Kasetsart J - Nat Sci.*, **41(1)**: 123-129.

Chanawongse L, Lee YK, Bunnag B and Tanticharoen M. 1994. Productivity of the cyanobacterium *Spirulina platensis* in cultures using sunlight. *Bioresour Technol.*, **48(2)**: 143-148.

Colla LM, Reinehr CO, Reichert C and Costa JAV. 2007. Production of biomass and nutraceutical compounds by *Spirulina*

platensis under different temperature and nitrogen regimes. *Bioresour Technol.,* **98(7)**: 1489-1493.

Danesi EDG, Rangel-Yagui CO, Sato S and de Carvalho JCM. 2011. Growth and content of *Spirulina platensis* biomass chlorophyll cultivated at different values of light intensity and temperature using different nitrogen sources. *Braz J Microbiol.,* **42(1)**: 362-373.

Edmundson SJ and Huesemann MH. 2015. The dark side of algae cultivation: Characterizing night biomass loss in three photosynthetic algae, *Chlorella sorokiniana*, *Nannochloropsis salina* and *Picochlorum* sp. *Algal Res.,* **12**: 470-476.

Fagiri YMA, Salleh A and El-Nagerabi SAF. 2013. Impact of physico-chemical parameters on the physiological growth of *Arthrospira* (*Spirulina platensis*) exogenous strain UTEXLB2340. *Afr J Biotechnol.,* **12(35)**: 5458-5465.

Goksan T, Zekeriyaoglu A and Ak I. 2007. The growth of *Spirulina platensis* in different culture systems under greenhouse condition. *Turk J Biol.,* **31(1)**: 47-52.

Grobbelaar JU. 1994. Turbulence in mass algal cultures and the role of light/dark fluctuations. *J Appl Phycol.,* **6(3)**: 331-335.

Grobbelaar JU. 2007. Photosynthetic characteristics of *Spirulina platensis* grown in commercial-scale open outdoor raceway ponds: What do the organisms tell us? *J Appl Phycol.,* **19(5)**: 591-598.

Habib MAB, Parvin M, Huntington TC and Hasan MR. 2008. **A review on culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish.** Food and Agriculture Organization of the United Nations, Rome, Italy.

Jensen GS, Ginsberg DI and Drapeau MS. 2001. Blue-green algae as an immuno-enhancer and biomodulator. *J Am Nutraceutical Assoc.,* **3(4)**: 24-30.

Jensen S and Knutsen G. 1993. Influence of light and temperature on photoinhibition of photosynthesis in *Spirulina platensis*. *J Appl Phycol.,* **5(5)**: 495-504.

Kim CJ, Jung YH, Ko SR, Kim HI, Park YH and Oh HM. 2007. Raceway cultivation of *Spirulina platensis* using underground water. *J Microbiol Biotechnol.,* **17(5)**: 853-857.

Kumari A, Pathak AK and Guria C. 2015. Cost-effective cultivation of *Spirulina platensis* using NPK fertilizer. *Agric Res.,* **4(3)**: 261-271.

Lee YK and Shen H. 2004. Basic culturing techniques. *In*: Richmond A (Ed.), **Handbook of Microalgal Culture: Biotechnology and applied phycology.** Blackwell Publishing Ltd., Oxford, London, pp. 40-56.

Lichtenthaler HK. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.,* **148**: 350-382.

Lichtenthaler HK and Buschmann C. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *In*: Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DS, Schwartz SJ *et al*. (Eds.), **Current Protocols in Food Analytical Chemistry.** John Wiley and Sons, Inc., New York, NY, pp. F4.3.1-F4.3.8.

Lu C and Vonshak A. 1999. Photoinhibition in outdoor *Arthrospira platensis* cultures assessed by polyphasic chlorophyll fluorescence transients. *J Appl Phycol.,* **11(4)**: 355-359.

Madkour FF, Kamil AE and Nasr HS. 2012. Production and nutritive value of *Spirulina platensis* in reduced cost media. *Egypt J Aquat Res.,* **38(1)**: 51-57.

Makhlouf R and Makhlouf I. 2012. Evaluation of the effect of Spirulina against Gamma irradiation induced oxidative stress and tissue injury in rats. *Int J Appl Sci Eng Res.,* **1(2)**: 152-164.

Ogbonda KH, Aminigo RE and Abu GO. 2007. Influence of aeration and lighting on biomass production and protein biosynthesis in a *Spirulina* sp. isolated from an oil-polluted brackish water marsh in the Niger Delta, Nigeria. *Afr J Biotechnol.,* **6(22)**: 2596-2600.

Omer AM. 2008. Energy, environment and sustainable development. *Renew Sustain Energy Rev.,* **12(9)**: 2265-2300.

Pelizer LH, Danesi EDG, Rangel CO, Sassano CEN, Carvalho JCM, Sato S and Moraes IO. 2003. Influence of inoculum age and concentration in *Spirulina platensis* cultivation. *J Food Eng.,* **56(4)**: 371-375.

Phang SM, Miah MS, Yeoh BG and Hashim MA. 2000. *Spirulina* cultivation in digested sago starch factory wastewater. *J Appl Phycol.,* **12(3-5)**: 395-400.

Pulz O and Gross W. 2004. Valuable products from biotechnology of microalgae. *Appl Microbiol Biotechnol.,* **65(6)**: 635-648.

Raoof B, Kaushik BD and Prasanna R. 2006. Formulation of a low-cost medium for mass production of *Spirulina*. *Biomass Bioenergy,* **30(6)**: 537-542.

Ravindran B, Gupta SK, Cho WM, Kim JK, Lee SR, Jeong KH *et al*. 2016. Microalgae potential and multiple roles - current progress and future prospects: An overview. *Sustainability,* **8(12)**. Online publication.

Richmond A. 1988. *Spirulina*. *In*: Borowitzka MA and Borowitzka LJ (Eds.), **Microalgal Biotechnology**. Cambridge University Press, Cambridge, London, pp. 85-121.

Richmond A. 1992. Open systems for the mass production of photoautotrophic microalgae outdoor: Physiological principles. *J Appl Phycol.,* **4(3)**: 281-286.

Richmond A. 2004. Biological principles of mass cultivation. *In*: Richmond A (Ed.), **Handbook of Microalgal Culture: Biotechnology and applied phycology.** Blackwell Publishing Ltd., Oxford, London, pp. 125-177.

Singhal G and Kumar S. 2017. Growth of *Spirulina maxima* in different physical conditions. *IOSR J Pharm Biol Sci.,* **12(3)**: 40-44.

Soletto D, Binaghi L, Ferrari L, Lodi A, Carvalho JCM, Zilli M and Converti A. 2008. Effects of carbon dioxide feeding rate and light intensity on the fed-batch pulse-feeding cultivation of *Spirulina platensis* in helical photobioreactor. *Biochem Eng J.,* **39(2)**: 369-375.

Sukumaran P, Nulit R, Halimoon N, Simoh S, Omar H and Ismail A. 2018. Formulation of cost-effective medium using urea as a nitrogen source for *Arthrospira platensis* cultivation under real environment. *Annu Res Rev Biol.,* **22(2)**: 1-12.

Sukumaran P, Nulit R, Zulkifly S, Halimoon N, Omar H and Ismail A. 2014. Potential of fresh POME as a growth medium in mass production of *Arthrospira platensis*. *Int J Curr Microbiol Appl Sci.,* **3(4)**: 235-250.

Torzillo G, Bernardini P and Masojidek J. 1998. On-line monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by high oxygen concentration and low temperature and its effect on the productivity of outdoor cultures of *Spirulina platensis* (cyanobacteria). *J Phycol.,* **34(3)**: 504-510.

Toyoshima M, Aikawa S, Yamagishi T, Kondo A and Kawai H. 2015. A pilot-scale floating closed culture system for the multicellular cyanobacterium *Arthrospira platensis* NIES-39. *J Appl Phycol.,* **27(6)**: 2191-2202.

Vonshak A and Guy R. 1992. Photoadaptation, photoinhibition and productivity in the blue-green alga, *Spirulina platensis* grown outdoors. *Plant Cell Environ.,* **15(5)**: 613-616.

Vonshak A and Richmond A. 1985. Problems in developing the biotechnology of algal biomass production. *Plant Soil*, **89**: 129-135.

Vonshak A and Richmond A. 1988. Mass production of the blue-green alga *Spirulina*: An overview. *Biomass*, **15(4)**: 233-247.

Vonshak A, Guy R and Guy M. 1988. The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. *Arch Microbiol.*, **150(5)**: 417-420.

Vonshak A, Kancharaksa N, Bunnag B and Tanticharoen M. 1996. Role of light and photosynthesis on the acclimation process of the cyanobacterium *Spirulina platensis* to salinity stress. *J Appl Phycol.*, **8(2)**: 119-124.

Vonshak A, Laorawat S, Binnag B and Tanticharoen M. 2014. The effect of light availability on the photosynthetic activity and productivity of outdoor cultures of *Arthrospira platensis* (*Spirulina*). *J Appl Phycol.*, **26(3)**: 1309-1315.

Vonshak A. 1997. Outdoor mass production of *Spirulina*: The basic concept. *In*: Vonshak A (Ed.), ***Spirulina platensis (Arthrospira)*: Physiology, cell-biology and biotechnology.** Taylor and Francis Ltd., Gunpowder Square, London, pp. 79-99.

Walter A, de Carvalho JC, Soccol VT, de Faria ABB, Ghiggi V and Soccol CR. 2011. Study of phycocyanin production from *Spirulina platensis* under different light spectra. *Braz Arch Biol Technol.*, **54(4)**: 675-682.

Wu L and Ho JA. 2008. Antioxidative and hepatoprotective effects of *Spirulina*. *In*: Gershwin ME and Belay A (Eds.), **Spirulina in Human Nutrition and Health.** CRC Press, Taylor and Francis Group, Boca Raton, FL, pp. 119-151.

Zeng MT and Vonshak A. 1998. Adaptation of *Spirulina platensis* to salinity-stress. *Comp Biochem Physiol A Mol Integr Physiol.*, **120(1)**: 113-118.