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Impact of physico-chemical parameters on the physiological growth of *Arthrospira* (*Spirulina platensis*) exogenous strain UTEXLB2340

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Spirulina platensis **is an attractive human food supplement, animals feed and of numerous medicinal uses. The effect of physico-chemical factors on the physiological growth rates as optical densities (O.D.) of *S. platensis* strain (UTEXLB2340) were investigated. Experiments were conducted at different pH levels, light intensities, and temperature regimes, and monitored for 20 days. The half concentration of Zarrouk media was found to be suitable for cultivation of this strain under indoor (1.14 O.D.) and outdoor conditions (0.99 O.D.). The nitrogen source sodium nitrate (2.5 gl^{-1}) can be replaced by inexpensive and low concentration of urea fertilizer (0.12 gl^{-1}). The growth rates of this strain were optimum at light intensities of 1500 to 2500 lux of continuous light (0.99 to 1.71 O.D.) or 2500 lux of intermittent light (1.32 O.D.). The optimal growth was recorded at lower pH 7 to 8 (1.19 to 1.76 O.D.), and temperature of 25 to 35°C (1.13 to 1.71 O.D.). Therefore, this microalga can be cultivated for commercial uses in the available natural ponds where the average temperature reached 30°C and light intensities fluctuated between 1000 to 4000 lux in the morning, 12,000 to 22,000 lux midday, and 7000 to 10,000 lux in the noon.**

Key words: *Arthrospira* (*Spirulina*) *platensis*, indoor and outdoor cultures, light intensity, pH, sodium nitrate, temperature, urea.

INTRODUCTION

Arthrospira (*Spirulina*) *platensis* is a planktonic, filamentous cyanobacterium that grows in tropical, subtropical and temperate water habitats (Vonshak, 1997; Colla et al., 2007; Rodrigues et al., 2011; Madkour et al., 2012). This microalga deserves special attention due to its nutritional properties as high single cell protein for human food supplement, animal and fish feed. It produces numerous bioactive compounds such as vitamins, essential amino acids, minerals, polysaturated fatty acids, biopigments, and antioxidents (Kamat, 1995; Abu et al., 2007; Pandey and Tiwari, 2010; Pandey et al., 2010; Madkour et al., 2012; Thirumala, 2012). It has been used as an alternative healthy food for malnutrition, growth stimulation, anticancer, and enhancing milk secretion (Richmond, 1986a; Ogbonda et al., 2007). The microalgae industries have been receiving increasing interest and utilized in both traditional and biotechnological processes during the last decades (Celekli et al., 2009; Thirumala, 2012). Increase in human population stimulating the search for alternative food and feed sources through exploiting biotechnological techniques for cultivation of useful microalgae (Venkataraman et al., 1981; Celekli et al., 2009; Jitendra

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et al., 2012). Many algal genera such as *Spirulina*, *Chlorella*, *Dunaliella*, and *Scenedesmus* were used to fulfill these purposes (Pulz and Gross, 2004). The physiological growth and large-scale production of *Spirulina* depends on many physical, environmental and nutritional factors. The most important are nutrients availability and composition, pH, light and temperature under both indoor and outdoor conditions (Abu et al., 2007; Colla et al., 2007; Madkour et al., 2012). The challenging cultivation of *S. platensis* depends on nutrients availability (Paoletti et al., 1975; Richmond, 1986; Rodrigues et al., 2010; 2011). Although nitrates are commonly used as growth media, many studies supported the benefit of using alternative cheaper nutritional sources such as ammonia and urea (Bezerra et al., 2008; Rodrigues et al., 2010). This will help to avoid difficulties encountered in cultivation with only one cultivation source (Piorreck et al., 1983; Stanca and Popovici, 1996; Danesi et al., 2002, 2011; Volkman et al., 2008; Rodrigues et al., 2010). The inhibition of the growth was less marked with urea comparable to ammonia (Converti et al., 2006; Celekli and Dönmez, 2006; 2009). Different low cost media were used for cultivation of *Spirulina* such as Zarrouk media (Cola et al., 2007; Jitendra et al., 2012; Madkour et al., 2012; Thirumala, 2012).

The pH plays apparent role in the metabolic activities of microalgae (Richmond, 1986b; Rafiqual et al., 2005; Ogbonda et al., 2007). It strongly affects biomass production (Celekli and Dönmez, 2006; 2009), chemicals dissociation and cell physiology (Kim et al., 2007; Ogbonda et al., 2007; Celekli et al., 2009). Therefore, the effect of different pH levels on the growth of microalgae was continuously evaluated under different environmental conditions (Kim et al., 2007; Ogbonda et al., 2007; Celekli et al., 2009). On the other hand, light is the most important factor representing the main source of energy for *S. platensis* (Soletto et al., 2008). The effect of light and temperature under both indoor and outdoor conditions on the algal growth was under continuous investigation (Richmond, 1986; Richmond and Grobbelaar, 1986; Torzillo et al., 1986, 1991; Torzillo and Vonshak, 1994; Boussiba et al, 1988; Pandey et al., 2010). At lower temperature, the photo inhibition effect results in both low final cell concentration and productivity of *S. platensis* (Richmond and Grobbelaar, 1986; Boussiba et al, 1988; Pandey et al., 2010). Photoinhibition is a reduction of the photosynthetic activities with high light intensity (Soletto et al., 2008). This is associated with climatic variances and the photoperiods (Tamiya et al., 1953; Samuelsson et al., 1985; Jensen and Knutsen, 1993; Danesi et al., 2011).

The studies of the major limitations on the growth of *S. platensis* were carried in open-race way (Richmond et al., 1990). Economically, the open-air system is useful in the large-scale production compared to expensive closed systems (Borowitzka, 1999). Indoor controlled cultivation of *Spirulina* sp. facilitates checking of the simultaneous effect of light and temperature (Danesi et al., 2011). Therefore, the present study was undertaken to evaluate the physiological growth as optical density of exogenous strain of *S. platensis* (UTEXLB2340) under indoor and outdoor conditions of Malaysia. Different concentrations of Zarrouk media, urea fertilizer, sodium nitrate, pH levels, light intensities, and temperature regimes were investigated. Hopefully these findings will help in introducing microalgae industry in Malaysia and exploiting several hundred natural ponds available.

MATERIALS AND METHODS

Microorganism strain and cultivation media

In the present study, *S. platensis* strain namely UTEXLB2340 was obtained from the culture collection centre of the University of Texas, Austin, USA. The strain was inoculated in 2 l Erlenmeyer flask with 1 l of sterilized Zarrouk media containing (gl^{-1}): $NaHCO_3$, 8.0; $NaNO_3$, 2.5; NaCl, 1.6; KCl, 1.0;K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; $(NH_2)_2CO$, 0.1; EDTA-Na_2, 0.08; $CaCl_2.2H_2O$, 0.04 and $FeSO_4.7H_2O$, 0.01. The media was sterilized at 12°C under pressure of 15 Lb/in^2 for 20 min. The inoculated flasks were incubated at 30°C with cool white florescent lamps of continuous light intensity of 1500 lux and continuous aerator (10 vvm), and kept as stock cultures in the algal culture room of the Institute of Biological Sciences, University of Malaya.

Inoculum preparation

For inoculum preparation, an aliquot from the stock culture was added to the sterilized conical flask (500 ml) and diluted with Zarrouk media until the algal biomass of optical density 0.2 and 0.5 O.D. were maintained using spectrophotometer at 560 nm. The algal biomass of 0.5 O.D. was used as initial inoculum to study the effect of different concentration of Zarrouk media, light intensity and urea on the physiological growth of this strain under both outdoor and indoor conditions, whereas the concentration of 0.2 O.D. was used as inoculum for testing the indoor impacts of pH and temperature on the growth of the alga (Canizares et al., 1995).

Effect of outdoor, indoor conditions and media on *Spirulina* growth

The outdoor experiment was carried out in 45 cm Plexiglass tanks of 30 cm width and depth. The tanks were located in area of 40 m^2 in the Institute of Biological Sciences, University of Malaya. The top of the shelter was sealed by cement roof with the western side covered by perforated folding cover to maintain light intensity of 100,000 lux in a sunny day which is normally reduced by cloud. The light intensity in the outdoor area was fluctuating between 1000-4000 lux in the morning, 12,000-22,000 lux midday, and 7000-10,000 lux in the noon, with the average temperature of between 24-35°C. The tanks were filled with 15 L of Zarrouk media of full, half, and quarter concentrations of the ingredients. The media were inoculated with 1.5 L of algal inoculum of 0.05 optical densities with continuous aeration (10 vvm) throughout the duration of the experiment.

For the indoor culture experiment, the algal culture room of the Institute of Biological Sciences, University of Malaya was used. The room was supplied with continuous light illumination from cool white florescent lamps of 1500 lux and average temperature of 30°C. For inoculation, 150 ml of sterilized Zarrouk media of full, half, and quarter concentrations of the ingredients in 250 ml conical flasks was adjusted to pH 9 using sodium hydroxide (40% w/v). Four replicates from each concentration were inoculated with 15 ml of the inoculum (0.05 O.D.), stopped by cotton wool, covered by aluminum foil, sealed with sealing film and were then shaken on rotatory shaker of 140 rpm.

Effect of urea and pH on *Spirulina* growth

For testing the efficiency of urea fertilizer as feasible and cheaper nitrogen source, 0.12 gl^{-1} urea were used as alternative to 2.5 gl^{-1} sodium nitrate ($NaNO_3$) in Zarrouk media. The other culture conditions were similar to that of indoor experiment by inoculating 150 ml of Zarrouk media with 15 ml algal inoculum of 0.05 O.D. in 250 ml conical flasks. The algal growth was measured as optical density and compared with the growth in Zarrouk media containing 2.5 gl^{-1} sodium nitrate. To determine the effect of pH on the growth and biomass production of this strain, 150 ml Zarrouk media in 250 Erlenmeyer conical flasks with different pH (7, 8, 9, 10, and 11) were adjusted using 40% (w/v) sodium hydroxide and 1.0 N hydrochloric acid. Four replicates were inoculated with 15 ml of 0.2 O.D. pure inoculum of algal strain. The inoculated flasks were incubated at 30°C in growth chamber of lighting provided through continuous light from white cool florescent lamp of 2500 lux and were then shaken on rotatory shaker at 140 rpm in addition to hand shaken three times per day at morning, midday and night.

Effect of light and temperature on *Spirulina* growth

To investigate the effect of light intensity on the growth of *S. platensis* strain UTEXLB2340, the algal culture room was adjusted at 30°C with various light intensity of 1500 and 2500 lux of intermittent light and dark cycle of 12 h each. Similarly, other three sets of growth chambers were set at 30°C and adjusted to 500, 1500, and 2500 lux with continuous white cool light intensity. For this test, 150 ml of Zarrouk media were added to 250 ml conical flasks, inoculated with 15 ml of algal inoculum (0.05 O.D.) and the pH was maintained at 9 in all cultures. The inoculated flasks were covered with cotton wool and aluminum foil and were then shaken using rotary shaker at 140 rpm for 20 days. The algal growth rate as an optical density was measured every 4 days using UV spectrophotometer at 560 nm. To evaluate the impact of temperature on the growth of this microalga strain, thermostatic controlled growth chambers were set at different temperature regimes (20, 25, 30, 35, and 40°C). For this, 150 ml of Zarrouk media were added to 250 ml conical flasks and inoculated with 15 ml from 0.2 O.D. algal inoculum with pH adjusted to 9 and incubated under continuous light illumination of 2500 lux. The inoculated flasks were then vigorously shaken manually at three times a day in the morning, midday, and night for 20 days.

Measurement of the physiological growth rates

The effect of growth conditions, media concentrations, urea and sodium nitrate ingredients, different pH levels, light intensities and temperature regimes on *S. platensis* strain on physiological growth rates and biomass production were measured as optical densities using UV spectrophotometer at wavelength of 560 nm.

Statistical analyses

For comparison between different concentrations of Zarrouk media, urea and sodium nitrate, different pH levels, light intensities, and temperature regimes, Duncan's multiple range test and one way ANOVA were used with $p<0.05$. The analysis was conducted with statistical package software SPSS of version 11.0.

RESULTS AND DISCUSSION

Influence of indoor, outdoor conditions and media on growth

The growth and biomass production of *Spirulina* depends on many physico-chemical factors such as nutrients availability, pH, light, and temperature. For economic reasons, the open-air system is predominating in the large-scale commercial cultivation of these types of organisms (Celekli et al., 2009). On the other hand, closed system is being very expensive and often difficult to scale up (Borowitzka, 1999). Cost and composition of media are challenging factors for viable and mass production of cyanobacteria (Jitendra et al., 2012). Different media were used for cultivation of *Spirulina* such as Zarrouk media, CFTIR media, OFERR media, and Bangladesh media (Belay et al., 1993; Jitendra et al., 2012). Zarrouk media have been successfully served as standard media (SM) for cultivation of *Spirulina* (Madkour et al., 2012). Higher growth rates and lipids content of *Spirulina* grown on Zarrouk media compared to cultivation under nitrogen starvation (Olguin et al., 2001; Colla et al., 2007). In the present study, the growth rates of *S. platensis* strain UTEXLB2340 on full, half, and quarter concentrations of Zarrouk media significantly ($p<0.05$) increased with incubation time and the maximum growth was obtained on 20^{th} day and 16^{th} day of cultivation under outdoor and indoor conditions, respectively (Table 1). The full and half concentrations of Zarrouk media displayed significantly ($p<0.05$) higher and optimum growth of *Spirulina* compared with quarter concentration. Therefore, from economic point of view, the half concentration of this media is evidently suitable for cultivation of this *Spirulina* strain in both indoor and outdoor conditions of Malaysia as suggested in our earlier reports (Fagiri et al., 2013). This supports the basic aim of providing simple and inexpensive media to decrease the cost of the large-scale production under open-air system as suggested by many researchers (Madkour et al., 2012; Jitendra et al., 2012). These intensions were implemented by substituting all the ingredients of Zarrouk media with cheaper and locally available commercial fertilizers and chemicals (Madkour et al., 2012).

Effect of urea and sodium nitrate on *Spirulina* growth

Various studies focused on the use of a cheaper nitrogen sources and locally available fertilizers for cultivation of microalgae (Rodrigues et al., 2011; Madkour et al., 2012). In the biotechnological process, the cultivation media components are responsible for higher costs (Danesi et al., 2011). The cost of the nutrients is considered the second to labour as major factors influencing the cost of *Spirulina* biomass production (Vonshak, 1997). Therefore, production of *Spirulina* with reduced cost is necessary when considering large-scale cultivation for industrial purposes. The growth of *Spirulina* was best when using urea compared to potassium nitrate (KNO_3) (Danesi et al., 2011). Therefore, urea is cheap effective alternative which significantly provides higher cell growth (Piorreck et al., 1983; Stanca and Popovici, 1996; Volkmann et al., 2008). In contrast, cultures supplemented with urea showed slow growth rates and higher rates when compared to

Table 1. Effect of Zarrouk media concentrations on the physiological growth rates of *S. platensis* strain UTEXLB2340 under outdoor and indoor conditions.

Time (day)	Outdoor conditions			Indoor conditions		
	Concentrations of Zarrouk medium			Concentrations of Zarrouk medium		
	Full	Half	Quarter	Full	Half	Quarter
0	0.03Fa	0.04Fa	0.05Fa	0.03Fa	0.05Ea	0.04Ea
4	0.17Ea	0.20Ea	0.16Ea	0.32Ea	0.26Da	0.26Da
8	0.32Db	0.42Da	0.40Da	0.65Da	0.60Ca	0.45Cb
12	0.59Cb	0.69Ca	0.61Cab	0.86Cb	1.12Aa	0.68Ac
16	0.78Bb	0.86Ba	0.70Bc	1.06Ab	1.14Aa	0.60Bc
20	1.04Aa	0.99Aa	0.89Ab	0.99Ba	0.94Ba	0.56Bb

Within rows and columns, means followed by different lower case and upper case letters differ significantly (p<0.05) according to the Duncan's multiple range test, respectively.

Table 2. Effect of urea and sodium nitrate as ingredients in Zarrouk media on the growth physiology of *S. platensis* strain UTEXLB2340.

Growth rate (optical density)		
Time (day)	Urea (0.12g/L)	Sodium nitrate (2.5 g/L)
0	0.03Fa	0.03Ea
4	0.18Eb	0.32Da
8	0.45Db	0.65Ca
12	0.81Ca	0.80Ba
16	1.15Ba	1.01Ab
20	1.56Aa	1.06Ab

Within rows and columns, means followed by different lower case and upper case letters differ significantly (p<0.05) according to the Duncan's multiple range test, respectively.

Zarrouk media, and ammonium nitrate media, respectively (Madkour et al., 2012).

The growth parameters in urea containing media showed a significant increase urea concentration. Although, urea has been know as an excellent nitrogen source and successfully metabolized by algae, *Spirulina* could most efficiently utilize ammonia nitrate compared to urea (Bezerra et al., 2008; Rodrigues et al., 2010). However, the inhibition effect was less marked with urea due to enzymatic hydrolysis of this compound by urease enzyme (Converti et al., 2006). On the other hand, the concentration of sodium nitrate in Zarrouk media (2.5 gl^{-1}) can be reduced without loss of productivity, as an important cost-saving factor in large-scale cultivation of *S. platensis* (Colla et al., 2007).

In the present results, it is evident that the use of low concentration of urea (0.12 gl^{-1}) and high concentration of sodium nitrate (2.5 gl^{-1}) significantly (p<0.05) increased the growth rates of *Spirulina* strain with time and maximum growth was obtained with urea (1.56 O.D.) compared to sodium nitrate (1.06 O.D.) after 20 days of cultivation (Table 2). These findings support the effective use of inexpensive urea as alternative to sodium nitrate in large-scale commercial cultivation of this microalga as concluded by many authors (Converti et al., 2006; Colla et al., 2007; Vonshak, 1997; Volkman et al., 2008). Therefore, in similar studies, KNO3 was replaced by urea (Piorreck et al., 1983; Danesi et al., 2011) whereas the use of higher concentrations of sodium nitrate (1.875 and 2.500 gl^{-1}) showed no increase in the algal growth and level of protein (Colla et al., 2007) and increase lipids (Manabe et al., 1992). Therefore, in this study the high concentration of sodium nitrate can be reduced or replaced by urea in Zarrouk media as an important cost-saving in large-scale cultivation (Colla et al., 2007).

Effect of pH on *Spirulina* growth

pH is one of the limiting factors which affect the metabolic activities of the microalgae (Richmond, 1986b; Rafiqual et al., 2005; Ogbonda et al., 2007). It affects the physiological growth and biomass production (Celekli and Dönmez, 2006; 2009). The cyanobacterium massively grows in tropical and subtropical bodies of water which have pH of up to 11 (Kim et al., 2007; Ogbonda et al., 2007; Celekli et al., 2009). The optimization of the growth of *S. platensis* culture was recorded at pH 9-10 (Soundarapandian and Vasanthi, 2008; Pandey et al.,

Table 3. Effect of different pH levels on the growth physiology of *S. platensis* strain UTEXLB2340.

Growth rate (optical density)					
Time (day)	**pH 7**	**pH 8**	**pH 9**	**pH 10**	**pH 11**
0	0.04Fa	0.06Ea	0.06Fa	0.05Fa	0.05Ca
4	0.19Ec	0.28Db	0.41Ea	0.39Ea	0.39ABa
8	0.59Dd	0.71Cc	1.34Aa	0.81Ab	0.37Ae
12	1.19Ab	1.76Aa	1.10Bc	0.67Bd	0.34Ae
16	0.79Bc	1.52Ba	0.92Cb	0.53Cd	0.30Ae
20	0.68Ca	0.74Ca	0.70Da	0.42DEb	0.28ABc

Within rows and columns, means followed by different lower case and upper case letters differ significantly (p<0.05) according to the Duncan's multiple range test, respectively.

2010; Thirumala, 2012). In these investigations, inoculation of *S. platensis* at different levels of pH (7, 8, 9, 10, and 11) for 20 days showed that the physiological growth rates were significantly (p<0.05) increased up to 12th day and eventually declined towards the end of the cultivation time (Table 3). The highest growth rates were recorded at pH 8 (1.76 O.D.), followed by pH 7 (1.19 O.D.) and evidently reduced pH 9 (1.10 O.D.) and pH 10 (0.42 O.D.) and pH 11 (0.28 O.D.). Similar high biomass production was obtained at pH 8.5 and temperature of 35°C, whereas at pH values of 9, 9.5, and 10 the highest biomass production occurred at 30°C (Ogbonda et al., 2007) and at moderate pH 7-9 (Fagiri et al., 2013). It was also reported that pH 9 and temperature of 32°C as optimal conditions for biomass production by this microalga (Rafiqul et al., 2005). The same author reported the pH 10 and 37°C as optimal conditions for *Spirulina fusiformis*. Here the optimum conditions for our strained ranged between 7-8 at ambient condition (30°C) as concluded in similar studies under controlled temperature of 30-35°C (Rafiqul et al., 2005; Ogbonda et al., 2007; Soundarapandian and Vasanthi, 2008; Pandey et al., 2010; Thirumala, 2012). Thus, low alkalinity is required for optimal growth of this strain where solubility of CO_2 and other mineral compounds was affected by pH (Ogbonda et al., 2007). Therefore, pH conditions will help in avoiding the auto inhibition effect with increased pH on the cell growth (Richmond, 1986).

Influence of light and temperature on *Spirulina* growth

In literature, the interaction between illumination and temperature on the growth of *S. platensis* was extensively studied showing that at low temperature, the photo inhibition effect is more accentuated and results in low cells concentrations and productivities (Danesi et al., 2011). However, these studies covered the cultivation under natural illumination where the temperature effect and light intensity associated with seasonal climatic changes and the photoperiod of the year (Samuelsson et al., 1985; Jensen and Knutsen, 1993). Various studies were investigating the optimal light intensity and temperature for the growth of *S. platensis* under different climatic conditions. For almost all algal species, growth increases nearly proportional with light intensity when it is lower than the lower saturation point, while above saturation point, significant light inhibition may occur (Ogbonda and Tanaka, 2000; Yuan et al., 2011). The algal cultures produce small amounts of biomass when grown in darkness or light intensity below 300 µmolm^{-2}s^{-1} (Wang et al., 2007). On the contrary, higher light intensities could harvest more algal biomass. The growth of *S. maxima* was inhibited at light intensity higher than 25 to 30 Klux (300-360 µmolm^{-2}s^{-1}). The growth of *S. platensis* reached the maximum at light intensity exceeding 150-200 µmolm^{-2}s^{-1} (Vonshak, 1997). At 5 Klux light intensity, the dry weight of *S. maxima* was 0.72 g/500 ml (Pandey and Tiwari, 20109), and the dry weight of *S. platensis* was 0.85 g/500 ml (Pandey et al., 2010). The best was achieved at light intensity of 60 µmolm^{-2}s^{-1} (Danesi et al., 2011). It is also inclined to drop with light intensity below 5 Klux. The dry weight of *S. platensis* is 0.91 g/500 ml at 5 Klux light intensity (Pandey et al., 2009). In the present investigations, incubation of *S. platensis* strain on Zarrouk media under different light intensities regimes of 500, 1500, and 2500 lux continuous light and 1500 and 2500 lux intermittent light (12 light/12 dark) for 20 days, showed that the growth rates were significantly (p<0.05) increased with time and the maximum growth rates achieved at the end of cultivation time (Table 4). The best growth rates were obtained with continuous light of 2500 lux (1.71 O.D.), and 1500 lux (0.99 O.D.), followed by intermittent light of 2500 lux (1.33 O.D.), whereas continuous light of 500 lux and intermittent light of 1500 lux displayed the lowest growth rates of 0.71 and 0.58 O.D., respectively. Therefore, the optimal light intensity for this algal strain is continuous light of 1500-2500 lux and 2500 lux of intermittent light as suggested in similar studies on different strain of *Spirulina* (Fagiri et al., 2013). It is noticeable in this study that the growth rates of this strain increases with increase in light intensities as suggested by Pandey et al. (2010). However, in similar studies growth was inhibited at light intensity higher than 25-30 Klux (Vonshak, 1997), or in darkness below 300 µmolm^{-2}s^{-1} (Wang et al., 2007), or light intensity above 5 Klux (Pandey et al., 2010). Nonetheless, the best growth rates were recorded at light of 60 µmolm^{-2}s^{-1} (Danesi et

Table 4. Effects of continuous and intermittent light intensities on the growth rate of *S. platensis* strain UTEXLB2340.

Growth rate (optical density)					
Time (day)	**500 Cont.[a]**	**1500 Cont.[a]**	**2500 Cont.[a]**	**1500 Int.[b]**	**2500 Int.[b]**
0	0.02Ea	0.02Ea	0.02Fa	0.02Ea	0.02Fa
4	0.11Dd	0.32Da	0.15Ec	0.18Dbc	0.21Eb
8	0.17De	0.65Ca	0.50Db	0.31Cd	0.39Dc
12	0.29Ce	0.86Bb	1.00Ca	0.52Bd	0.60Cc
16	0.49Be	1.06Ab	1.50Ba	0.66Ad	0.91Bc
20	0.71Ae	0.99Ac	1.71Aa	0.58Bd	1.32Ab

Within rows and columns, means followed by different lower case and upper case letters differ significantly (p<0.05) according to the Duncan's multiple range test, respectively. Light intensity (lux): Cont.[a], Continuous light; Int.[b], intermittent light.

Table 5. Effect of different temperature regimes on the growth rate of *S. platensis* strain UTEXLB2340.

Time (day)	**Growth rate (Optical density)**				
	20°C	**25°C**	**30°C**	**35°C**	**40°C**
0	0.04Ca	0.06Fa	0.06Fa	0.06Ea	0.05Ca
4	0.25Ab	0.15Ec	0.41Ea	0.44Da	0.15Bc
8	0.17Bc	0.50Db	1.34Aa	1.36Ba	0.22Bc
12	0.15BDe	1.00Cc	1.13Bb	1.98Aa	0.42Ad
16	0.12BDe	1.50Bb	0.92Cc	2.01Aa	0.36Ad
20	0.10Bd	1.71Aa	0.70Db	0.71Cb	0.18Bc

Within rows and columns, means followed by different lower case and upper case letters differ significantly (p<0.05) according to the Duncan's multiple range test, respectively.

al., 2011).

The optimization of the growth of *S. platensis* was noticed at 35°C with maximum biomass production (Soundarapandian and Vasanthi, 2008; Thirumala, 2012). The highest biomass concentration of 4.4 mg/ml was obtained at 30°C without limiting effect of lighting (Ogbonda et al., 2007). Under laboratory controlled conditions, the optimum growth temperature for *Spirulina* was reported at 35 to 37°C, and 39°C for outdoor cultivation (Richmond, 1986b), and 25-30°C for indoor cultivation (Fagiri et al., 2013). Thermotolerant *Spirulina* grows at 35 to 40°C (Vonshak et al., 1982). In the present study (Table 5), cultivation of *Spirulina* strain at different levels of temperature (20, 25, 30, 35, and 40°C) showed significantly (p<0.05) higher growth rates at 35°C (2.01 O.D.) after 16 days of cultivation, followed by 25°C (1.71 O.D.) at the end of the cultivation period (20 days) and at 30°C (1.13 O.D.) after 12 days. However, the growth rates and biomass production were significantly reduced (p<0.05) above 35°C (40°C) and below 25°C (20°C). This is may be attributed to photoinhibition effect of temperature and light (Jensen and Knusten, 1993). Therefore, the optimum temperature for the cultivation of this cyanobacteria strain in the range of 25 to 35°C as concluded by many researchers (Richmond, 1986b; Ogbonda et al., 2007; Soundarapandian and Vasanthi, 2008; Thirumala, 2012). This temperature is the average level in the outdoor conditions of Malaysia (30°C).

Conclusions

S. platensis strain UTEXLB2340 cultivations were carried out in different concentrations of Zarrouk media under outdoor and indoor conditions and at 0.12 gl^{-1} of urea and 2.5 g/l sodium nitrate, different pH levels, light intensities, and temperature regimes. The growth of this microalga strain was best using half concentration of Zarrouk media under both indoor and outdoor conditions. The maximum growth rate was obtained at low concentration of urea (0.12 gl^{-1}) compared to 2.5 gl^{-1} of sodium nitrate commonly used as nitrogen source in Zarrouk media. The pH of between 7-8 and low alkalinity were optimum ranges for achieving highest growth rates as well as light intensities of 1500-2500 lux and temperature in the range of 25 to 35°C. Therefore, from economic point of view, it is feasible to cultivate this strain of cyanobacteria under the outdoor conditions of Malaysia (24-35°C) using half concentration of Zarrouk media and replacing sodium nitrate with effective, inexpensive and available urea fertilizer. This will help in introducing the biotechnology of microalgae industry and exploiting the available natural ponds and resources for large-scale commercial cultivation. However, further studies were needed to evaluate the use of

alternative and environment friendly growth media and other natural products.

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