Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © 2009 PHYCOSPECTRUM - ISSN: 2229 – 6905

Influence of chemical and environmental factors on the growth performance of *Spirulina platensis* strain SZ100

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Abstract
The cyanobacterium *Spirulina platensis* has been widely used by humans due to its high nutritional value and medicinal uses as well as animal feed. Our study investigated the influence of concentration of Zarrouk media, replacing high concentration of sodium nitrate with urea, pH levels, light intensities and temperature regimes under indoor and outdoor conditions on the growth rates of this microalga. We found that the half concentration of Zarrouk media was optimum for the cultivation of this alga under both indoor (1.77 O.D.) and outdoor conditions (1.74 O.D.). Lower concentration of urea fertilizer (0.15 gl^{-1}) can be used as alternative inexpensive effective nitrogen source compared to the high concentration of sodium nitrate (2.5 gl^{-1}) in the growth media. The optimization of the physiological growth rates of this algal strain was maintained at moderate alkalinity of 7 , 8 and 9 pH, (0.95, 1.92, and 1.49 O.D.), light intensities of 1500-2500 lux (1.68-1.96 O.D.), and temperature regimes of between 25°C and 30°C (1.83-1.88 O.D.). Therefore, this algal strain can be cultivated in large-scale open system using the available natural ponds under the suitable environmental conditions of Malaysia (24-35°C).

Keywords *Arthrospira* (*Spirulina*) *platensis*, indoor and outdoor cultures, light intensity, pH, sodium nitrate, temperature, urea

Introduction

Arthrospira (*Spirulina*) *platensis* is multicellular filamentous cyanobacterium that can colonize freshwater, brackish lakes, some marine and alkaline habitats (Vonshak 1997; Colla *et al*. 2007; Rodrigues *et al*. 2011; Madkour *et al*. 2012). It deserves special emphasis as high single cell protein for human food, animal and fish feed and to the capacity to produce numerous bioactive compounds such as vitamins, essential amino acids, minerals, polysaturated fatty acids (gamma-linolenic fatty acid), biopigments (carotenes, phycobiliprotein, phycocyanin, chlorophyll-a) and antioxidants (Kamat 1995; Abu *et al*. 2007; Pandey and Tiwari 2010; Pandey *et al*. 2010; Madkour *et al*. 2012; Thirumala 2012). It is used as an alternative healthy food for malnutrition, in growth stimulation through thyroid hormone synthesis, in protection against cancer, and enhancing milk secretion of mothers with lactation problems (Richmond 1986a; Ogbonda *et al*. 2007). The microalgal biotechnology and industries have been receiving increasing interest and extensively utilized during the last decades (Costa *et al*. 2003; Celekli *et al*. 2009; Thirumala 2012). With the increase in human population, there is evident search for alternative food and feed sources through exploiting biotechnological techniques for cultivation of microalgae (Venkataraman *et al*. 1981; Celekli *et al*. 2009; Jitendra *et al*. 2012). Many researches have been carried out since 1950s using many algal genera such as *Spirulina*, *Chlorella*, *Dunaliella*, and *Scenedesmus* to fulfill these purposes (Pulz and Gross 2004). The physiological growth and large-scale production of *Spirulina* depend on many physical, environmental and nutritional factors. These include nutrients availability and composition, pH levels, light and temperature regimes under both in indoor and outdoor conditions (Costa *et al*. 2003; Abu *et al*. 2007; Colla *et al*. 2007, Madkour *et al*. 2012). They were reviewed by many researchers. The challenging cultivation of *S. platensis* depends on nutrients availability. Although nitrates are commonly used in media (Paoletti *et al*. 1975; Richmond 1986; Rodrigues *et al*. 2010, 2011), many studies supported the benefit of using alternative cheaper nutritional sources such as ammonia (Bezerra *et al*. 2008; Saccano *et al*. 2007; Rodrigues *et al*. 2010) and urea which provides higher cell growth with higher chlorophyll contents and 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 media of different cost and composition were used for cultivation of *Spirulina* such as Zarrouk media, Rao media, CFTIR media, OFERR media, Revised media, and Bangladesh media (Cola *et al*. 2007; Jitendra *et al*. 2012; Madkour *et al*. 2012; Thirumala 2012). The pH is one of the factors that play evident role in the metabolic activities of microalgae (Richmond 1986b; Rafiqual *et al*. 2005; Ogbonda *et al*. 2007). Change in pH strongly affects biomass production of microalgae (Celekli and Dönmez 2006, 2009). It not only influence dissociation and chemistry of media, but also affects physiology of cell and biomass production (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). Light on the other hand, is influencing photosynthetic microorganisms and represents 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 physiological growth of this alga was investigated by

many researchers (Richmond 1986; Richmond and Grobbelaar 1986; Torzillo *et al*. 1986, 1991; Torzillo and Vonshak 1994; Bocci *et al*. 1987; Boussiba *et al*. 1988; Pandey *et al*. 2010). In lower temperature, the photoinhibition effect is more accentuated resulting in both low final cell concentrations and productivity (Richmond and Grobbelaar 1986; Bocci *et al*. 1987; Boussiba *et al*. 1988; Pandey *et al*. 2010). Photoinhibition is a reduction of the photosynthetic activities caused by exposure to high light intensity (Soletto *et al*. 2008). However, these studies cover the cultivation under natural illumination; whereas such temperature effect and light intensity are related to climatic variances occurred by the seasons of the years 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). For economic reasons, the culture system predominating in the large-scale commercial production of these types of organisms is the open-air system, whereas closed systems being very expensive and often difficult to scale up (Borowitzka 1999; Costa *et al*. 2003). Nonetheless, indoor cultivation of *Spirulina* sp. under controlled conditions which may facilitate checking of the simultaneous effect of such variables on the cell growth (Danesi *et al*. 2011). Therefore, the present study was undertaken to evaluate the physiological growth of *S. platensis* strains (SZ100) under indoor and outdoor conditions using different concentrations of commercial grade chemicals of Zarrouk media or supplemented with cheap urea fertilizer and sodium nitrate at different pH levels, light intensities, and temperature regimes. This will help in developing microalgae industry and exploiting several hundred natural ponds available in the study area.

Materials and methods

Microorganism strain and cultivation media

Cyanobacteria strain of *S. platensis* namely SZ100 was supplied by obtained Dr. Phang Siew Moi of the Institute of Advanced Studies, University of Malaya, Malaysia. This strain was previously obtained from Dr. Vonshak Ben Gurion of the University of the Negev, the Jacob Blaustein Institute for Desert Research, Microalgal Biotechnology, Sede Boker Campus, 84990, Israel. The strain was inoculated in 2 liters Erlenmeyer flask with one liter of sterilized Zarrouk medium (1966) containing (gl^{-1}): NaHCO3 8.0, NaNO3 2.5, NaCl 1.6, KCl 1.0, K2HPO4 0.5, MgSO4.7H2O 0.2, (NH2)2CO 0.1, EDTA-Na2 0.08, CaCl2.2H2O 0.04 and FeSO4.7H2O 0.01. The media was sterilized at 121°C under pressure of 15 Lb/in^2 for 20 minutes. 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

The algal inoculum was prepared by adding aliquot from the stock culture 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 levels and temperature regimes (Canizores and Domigues 1995).

Effect of outdoor, indoor conditions and media on growth

The outdoor experiment was carried out in Plexiglas tanks of 45 × 30 × 30 cm in size. 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 with cement roof with the western side covered by perforated folding cover to maintain light intensity in a sunny day of about 100,000 lux 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 15L of Zarrouk media of full, half, and quarter concentrations of the ingredients. The media were inoculated with 1.5L 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 fluorescent 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 algal inoculum (0.05 O.D.), stopped by cotton wool, covered by aluminum foil, sealed with sealing film and were then shaken on rotatory shaker (140 rpm).

Effect of urea and pH on growth

For testing the efficient of urea as inexpensive nitrogen source, 0.15 gl^{-1} urea was used and compared with 2.5 gl^{-1} sodium nitrate (NaNO3) 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. To determine the effect of pH on the growth and biomass production of this strain, 150 ml Zarrouk media in 250 Erlenmeyer conical flask with different pH (7, 8,

9, 10, 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 algal inoculum. The inoculated flasks were incubated at 30°C in growth chamber of lighting provided through continuous light from white cool fluorescent lamp of 2500 lux and were hand shaken three times per day at morning, midday and night.

Effect of light and temperature on growth

To investigate the effect of light on the growth of *S. platensis* strain SZ100, 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 hours 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, 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 time 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 the average physiological growth rates and biomass production of *S. platensis* strain were measured as optical densities using UV spectrophotometer at wavelength of 560 nm.

Statistical analyses.

For comparison between the effects of different concentrations of Zarrouk media, urea and sodium nitrate, different pH levels, light intensities, and temperature regimes on the growth rates of this microalga strain, Duncan's multiple range test and one way ANOVA were used with $p<0.05$. The analysis was carried out using 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* depend on nutrients availability, pH, light, and temperature. The open-air system is predominating in the large-scale commercial cultivation of this microalga (Costa *et al*. 2003; Celekli *et al*. 2009). Closed system is 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). For cultivation of *Spirulina* different growth media were used such As Zarrouk media, CFTIR media, OFERR media, and Bangladesh media (Belay *et al*. 1993; Jitendra *et al*. 2012). Zarrouk media served as standard media (SM) for cultivation of this microalga (Zarrouk 1966; Madkour *et al*. 2012). Higher growth rates and lipids content of *Spirulina* grown on Zarrouk media were observed and compared to growth under nitrogen starvation (Olguin *et al*. 2001; Colla *et al*. 2007). In this investigation, the growth rates of *S. platensis* strain SZ100 on standard full, half, and quarter concentrations of Zarrouk media under both indoor and outdoor conditions significantly ($p<0.05$) increased with incubation time and the maximum growth was obtained at the end of cultivation period under outdoor and indoor conditions, respectively (Table 1). The full and half concentrations of Zarrouk media display significantly ($p<0.05$) higher and optimum growth of *Spirulina* compared to the quarter concentration of Zarrouk media. The highest growth rates were obtained in half concentration of the media under outdoor condition (1.74 O.D.) and full concentration under indoor conditions (1.77 O.D.). Therefore, from economic point of view, the half concentration of Zarrouk media is evidently suitable for cultivation of this microalga strain in outdoor conditions of Malaysia. This will reduce the cost for large-scale production under open-air system as concluded in similar studies (Madkour *et al*. 2012; Jitendra *et al*. 2012; Costa *et al*. 2003). The hope is to substitute 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 growth

Various studies were evaluating the use of cheaper nitrogen sources and locally available fertilizers for cultivation of microalgae (Rodrigues *et al*. 2011; Madkour *et al*. 2012). The components of the cultivation media are responsible for higher costs (Danesi *et al*. 2011). The cost of the nutrients is second to labor as major factors influencing the cost of 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 (KNO3) (Danesi *et al*. 2011). Although, KNO3 is the commonly used as nitrogen source for cultivation of this microalga, it was

replaced with cheap urea which is an effective alternative and 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 when compared to Zarrouk media, and ammonium nitrate media (Madkour *et al*. 2012). The growth parameters in urea containing media showed a significant associated with increasing urea concentration. Although, urea has been known as an excellent nitrogen source and successfully metabolized by algae, *Spirulina* could most efficiently utilize ammonia nitrate compared to urea (Baldia *et al*. 1991). 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 ($2.5gl^{-1}$) can be reduced without loss of productivity, as an important cost-saving factor in large-scale cultivation of microalga (Colla *et al*. 2007). In this result, it is evident that the use of low concentration of urea ($0.15\ gl^{-1}$) 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 (1.77 O.D.) compared to sodium nitrate (1.68O.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 growth

The pH is one of the limiting parameters which affect the metabolic activities of the microalgae (Richmond 1986b; Rafiqual *et al*. 2005; Ogbonda *et al*. 2007) and affects the physiological growth and biomass production (Celekli and Dönmez 2006, 2009). These microalgae massively grow 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 optimum growth of *S. platensis* culture was recorded at pH 9-10 (Soundarapandian and Vasanthi 2008; Pandey *et al*. 2010; Thirumala 2012). In the present 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 (1.92 O.D.), followed by pH 9 (1.49 O.D.) and pH 7 (0.95 O.D.) and evidently reduced at pH 10 (0.84 O.D.) and pH 11 (0.27 O.D.). Similarly high biomass production was obtained at pH 8.5 and temperature of 35°C, whereas at pH 9, 9.5, and 10 the highest biomass production occurred at 30°C (Ogbonda *et al*. 2007). The pH 9 and temperature of 32°C were optimal conditions for biomass production of this microalga (Rafiqul *et al*. 2005). The same author reported the pH 10 and 37°C as optimal conditions for *Spirulina fusiformis*. In our study, the optimum pH levels for this strain ranged between 7 to 9 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). Therefore, moderate alkalinity is required for optimal growth of this strain where solubility of CO2 and other mineral compounds affected by pH (Ogbonda *et al*. 2007). This level of pH conditions will help in avoiding the autoinhibitor effect of increased pH on the algal cell growth (Richmond 1986).

Influence of light and temperature on growth

The interaction between illumination and temperature on the growth of *S. platensis* was extensively studied. At low temperature, the photoinhibition effect is more accentuated and results in low cells concentration and productivity (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). 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 amount of biomass when grown in darkness or light intensity below 300 $\mu molm^{-2}s^{-1}$ (Wang *et al*. 2007). On the contrary, the higher light intensities could harvest more algal biomass. The growth of *S. maxima* was inhibited at light intensity higher than 25-30 Klux (300-360 $\mu molm^{-2}s^{-1}$). The growth of *S. platensis* reached the maximum at light intensity exceeding 150-200 $\mu molm^{-2}s^{-1}$ (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 $\mu molm^{-2}s^{-1}$ (Danesi *et al*. 2011). It is also inclined to drop with light intensity below 5Klux. 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 were achieved at the end of cultivation time (Table 4). The best growth rates were obtained with continuous light of 2500 lux (1.96 O.D.), and 1500 lux (1.77 O.D.), followed by intermittent light of 2500 lux, whereas continuous light of 500 lux revealed the lowest growth rate (0.36 O.D.) (Table 4). Therefore, the optimal light intensity for this algal strain is intermittent light of 1500 lux and 2500 lux

whether continuous or intermittent. It is noticeable in this study the growth rates of this strain increase with increasing in light intensities as suggested by Pandey *et al*. (2010). However, in similar studies, the 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 intensity of 60 µmolm^{-2}s^{-1} (Danesi *et al*. 2011).

The growth of *S. platensis* was optimum 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 light (Ogbonda *et al*. 2007). Under laboratory controlled conditions, Richmond (1986b) reported an optimum growth temperature for *Spirulina* of 35-37°C, and 39°C for outdoor cultivation. Thermotolerant strain of *Spirulina* grows best at 35-40°C (Vonshak *et al*. 1982). In the present study (Table 5), Cultivation of *Spirulin*a strain at different levels of temperature (20, 25, 30, 35, and 40°C) showed significantly ($p<0.05$) higher growth rates at 25°C (1.83 O.D.) at the end of the experiment and followed by 30°C (1.88 O.D.) after 12 days of cultivation. However, the growth rates and biomass production were significantly reduced ($p<0.05$) above 30°C (35-40°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 temperature of between 25-35°C was optimum for cultivation of this cyanobacteria strain as concluded by many researchers (Ogbonda *et al*. 2007; Thirumala 2012). This temperature is the average level in the outdoor conditions of Malaysia (24-35°C).

Table 1. Effect of different concentrations of Zarrouk media on the physiological growth rate (Optical Density) of *Spirulina platensis* strains SZ 100 under outdoor and indoor conditions

Time (Day)	Growth rate (Optical Density)					
	Outdoor conditions			Indoor conditions		
	Concentration of media			Concentration of media		
	Full	Half	Quarter	Full	Half	Quarter
0	0.03Fa	0.05Fa	0.04Fa	0.04Fa	0.05Ea	0.06Da
4	0.27Eb	0.63Ea	0.21Ec	0.36Ea	0.28Da	0.26Ca
8	0.63Db	0.79Da	0.49Dc	0.76Da	0.81Ca	0.63Aa
12	1.01Cb	1.27Ca	0.78Cc	1.10Ca	1.05Ba	0.64Ab
16	1.20Bb	1.46Ba	0.94Bc	1.54Ba	1.20Ab	0.44Bc
20	1.47Ab	1.74Aa	1.13Ac	1.77Aa	1.24Ab	0.22Cc

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 (0.15gl^{-1}) and sodium nitrate (2.5gl^{-1}) on the growth physiology of *Spirulina platensis* strain SZ 100 in Zarrouk medium

Time (Day)	Growth rate (Optical Density)	
	Urea	Sodium nitrate
0	0.03Fa	0.03Fa
4	0.43Ea	0.36Eb
8	0.92Da	0.76Db
12	1.36Ca	1.10Cb
16	1.54Ba	1.46Bb
20	1.77Aa	1.68Ab

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 3. Effect of pH levels on the growth physiology of *Spirulina platensis* strain SZ100

Time (Days)	Growth rate (Optical Density)				
	pH				
	7	8	9	10	11
0	0.03Da	0.03Fa	0.03Fa	0.03Fa	0.04Ca
4	0.12Cc	0.15Ebc	0.27Ea	0.18Eb	0.13Bc
8	0.52Ba	0.58Da	0.59Ca	0.57Ca	0.28Ab
12	0.95Ac	1.42Bb	1.49Aa	0.84Ad	0.27Ae
16	0.92Ac	1.92Aa	1.16Bb	0.76Bd	0.23Ae
20	0.52Bb	0.66Ca	0.51Db	0.47Db	0.20Ac

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 4. Effects of continuous and intermittent light intensities on the growth rate of *Spirulina platensis* strains SZ100

Time (Day)	Growth rate (Optical Density)				
	Light intensity (LUX)				
	500cont.	1500cont.	2500cont.	1500int.	2500int.
0	0.03Ea	0.03Fa	0.03Fa	0.03Fa	0.03Fa
4	0.24Bc	0.36Ea	0.29Eb	0.14Ed	0.28Eb
8	0.25Bc	0.76Da	0.75Da	0.54Db	0.70Da
12	0.19Ce	1.10Cc	1.55Ca	0.85Cd	1.15Cb
16	0.33Ae	1.54Bb	1.77Ba	1.09Bd	1.47Bc
20	0.36Ae	1.77Ab	1.96Aa	1.23Ac	1.68Ad

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 5. Effect of temperature regime on the growth rate of *Spirulina platensis* strain SZ100

Time (Day)	Growth rate (Optical Density)				
	Temperature (°C)				
	20	25	30	35	40
0	0.06Ba	0.06Fa	0.06Fa	0.05Fa	0.07Da
4	0.22Aab	0.29Ea	0.32Ea	0.27Ea	0.20b
8	0.10Be	0.75Dc	1.18Da	0.95Cb	0.28Bd
12	0.07Be	1.55Cb	1.88Aa	1.49Ac	0.48Ad
16	0.17Ad	1.77Ba	1.74Ba	1.16Bb	0.30Bc
20	0.17Ad	1.83Aa	1.59Cb	0.51Dc	0.14Cd

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.

Conclusions

In these investigations we have concluded that half concentration of Zarrouk media can be used for cultivation of this microalgal strain under both indoor and outdoor conditions. The high concentration of sodium nitrate (2.5 gl^{-1}) in Zarrouk median can be replaced by inexpensive and low concentration of urea fertilizer (0.15 gl^{-1}) for the growth of this microalga. The optimal growth rates of this *Spirulina* strain were obtained at pH levels of between 7-9, temperature regimes of 25-30ºC and light intensities of 1500-2500 lux. Therefore, this microalga can be cultivated for large scale and biomass production under outdoor conditions of numerous natural ponds of Malaysia (24-35ºC).

Acknowledgements

This research was funded by the grant from the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia Dr. Phang Siew Moi of Institute of Advanced Studies, UM Malaysia supplied the algal strain.

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