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The effect of pH, dark – light cycle and light colour on the chlorophyll and carotenoid production of *Spirulina* sp.

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Abstract

Spirulina sp., multicellular filament algae, is helically coiled. This is a rich nutrition microalgae with protein, carbohydrate, vitamin, chlorophyll and carotenoid. Many researches and applications of *Spirulina* sp. have been studied by interested scientists, especially, pigment production. The purpose of this research was determination the effect of pH, dark – light cycle and light colour on the growth and pigment production of *Spirulina* sp. The results showed that pH 9, white light and continuous illumination 24/24 hours were appropriate conditions for biomass increasing, chlorophyll a, chlorophyll b and carotenoid production in *Spirulina* sp. At pH 9, biomass and carotenoid were highest in day 8 (0.16g/ 50mL and 1.43 μg/mL, respectively). The highest production of chlorophyll a and chlorophyll b were collected in day 12 (2.72 μg/mL and 3.35 μg/mL, respectively). The growth of *Spirulina* sp. was slow at green colour and limited at red colour. Compared to 12/24 hour illumination, growing algae under continuous illumination 24/24 hour was higher 1.08 times in biomass, 2.36 times in chlorophyll a, 1.2 times in chlorophyll b and 1.7 times in carotenoid content. When white light was applied, continuous illumination 24/24 hour and aeration, pH remained from 10 to 10.8, then decreased in day 16 and 20 and no significant differences between treatments during the experiment. Besides, *Spirulina* 's shape recovered quickly in appropriate conditions.

Keywords: biomass, carotenoid, chlorophyll a, chlorophyll b, *Spirulina* sp.

1. Introduction

Microalgae have been chosen as food for many years (1). *Spirulina* sp. contains bio - elements such as beta - carotene, vitamin E, carotenoid, chlorophyll and phycocyanin pigment which can prevent oxidation and cancer. About the structure, the width is 6 - 12 μm, length is 0.5 - 1 μm, cylinder cell. The algae can change from curly to helically coil based on hydration and dehydration of oligopeptide in peptidoglycan (2). *Spirulina* sp. dry biomass contains 60 - 70% protein, more than 40% essential amino acid but small nonessential amino acid, sulphur such as methionine and cysteine (3). Beside that *Spirulina* also contain vitamin A, B1, B2, B3, B12 and minerals such as iron, phosphorous, magnesium and calcium…(4). Moreover, chlorophyll is a photosynthesis pigment which only find in autotrophic organisms or algae, chlorophyll content depends on biomass production (5). Carotenoid is

provitamin A which prevents natural oxidation (6). The accumulation and isomer of β-carotene were controlled by light intensity and quality (7). Temperature plays an important role in the growth of algae, biomass production, protein and chlorophyll concentration (4). According to Dylan (8), *Spirulina* sp. grows well at pH from 9 to 11. High pH leads to prevent the infection of other green algae (9).

Objectives: Determination of pH concentration, dark - light cycle and light colours appropriate for biomass growth rate, chlorophyll and carotenoid production in *Spirulina* sp.

2. Materials and methods

2.1 Materials: *Spirulina* sp. was received from Microbiology Laboratory of Biotechnology Research and Development Institute, Can Tho University, Can Tho City, Vietnam. Chemicals in Zarrouk media (10), acetone, alcohol 90, alcohol 70.

2.2 Methods

Increasing the biomass of *Spirulina* sp. in order to have enough microalgae for further experiments at white light (wave length 0.4 – 0.76 μm) illumination time 24/24 under light bulb (23W, 220-240V).

Biomass collection: Algae biomass was collected by Whatman filter – paper. 50 mL of algae was taken out, dried at 75°C in 24 hours, weighted and determined biomass.

Chlorophyll and carotenoid extraction (11): 2 mL of algae was transferred from treatments to eppendorf, centrifuged at 6000 rpm in 10 minutes, washed the algae twice by distilled water and extracted by 2 mL acetone 80%. Then, the extraction of chlorophyll and carotenoid production was measured by spectrophotometer (at wave length 663 nm, 646 nm and 470 nm). The calculation of chlorophyll and carotenoid were calculated based on Lichtenthaler Welburn (12).

Study the effect of pH (8, 9, 10 and 11), light colour (blue, green, red, white) and illumination time (12/24 hour and 24/24 hour) on pH changing in the culture, biomass, chlorophyll and carotenoid production in *Spirulina* sp. Next experiment was done to observe the recovery of *Spirulina*. Small fragments of algae were supported in the appropriate culture in 0.5 liter of media which adjusted pH value to 9, continuous aeration, inoculation ratio in 20% and 24/24 illumination light. Followed in 5 continuous days, the growth of *Spirulina* was determined by microscopy and the biomass increasing.

2.3 Data analysis method

Microsoft Office Excel and SPSS software were used for data analysis.

3. Results and discussion

3.1 The effect of pH on *Spirulina* sp. chlorophyll and carotenoid production

The results showed that biomass, chlorophyll and carotenoid production of all treatments were highest in day 8 and pH 9 was the better condition for the growth of *Spirulina*. In day one, algae were shocked when transferred from pH 9 to pH 11 and they settled down to the bottle bottom. However, they grew again in day 4 in case of green colour. In all treatments, biomass increased from day 0 to day 8 and decreased to day 20. The highest biomass in day 4 was 0.14 g/ 50 mL at pH 10, comparing to 0.12 g/ 50 mL and 0.11 g/ 50 mL at pH 8 and 9, respectively. In day 8, treatment at pH 9 had the highest biomass (0.16 g/ 50 mL) and significant difference at 5% according to Duncan test among treatments (Table 1). These results were similar to the research about the effect of pH on biomass (13).

Table 1. The effect of pH on *Spirulina* sp. biomass, chlorophyll and carotenoid production

	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Biomass (g/50mL)						
pH 8	0.08	0.12^{b}	0.13^{ab}	0.12^{b}	0.11^{ab}	0.09^{b}
pH 9	0.08	0.11^{bc}	0.16^{a}	0.14^{a}	0.13^{a}	0.12^{a}
pH 10	0.08	0.14^{a}	0.14^{ab}	0.10^{b}	0.07^{bc}	0.07^{c}
pH 11	0.08	0.10^{c}	0.11^{b}	0.08^{c}	0.06^{c}	0.05^{d}
	ns					
Chlorophyll a (µg/mL)						
pH 8	0.02	0.63^{b}	2.10^{a}	1.89^{b}	1.90^{b}	0.93^{b}
pH 9	0.02	0.76^{b}	2.40^{a}	2.72^{a}	2.72^{a}	1.62^{a}
pH 10	0.02	1.10^{a}	0.94^{b}	0.78^{c}	0.65^{c}	0.54^{c}
pH 11	0.02	0.35^{c}	0.87^{b}	0.67^{c}	0.74^{c}	0.71^{bc}
	ns					
Chlorophyll b (µg/mL)						
pH 8	0.07	0.85^{c}	3.26	3.10^{a}	3.26^{a}	2.45^{a}
pH 9	0.05	2.60^{a}	3.35	3.35^{a}	3.35^{a}	2.71^{a}
pH 10	0.06	2.46^{ab}	2.35	0.93^{b}	0.63^{b}	0.51^{b}
pH 11	0.05	1.48^{bc}	2.38	0.39^{b}	0.49^{b}	0.32^{b}
	ns		ns			
Carotenoid (µg/mL)						
pH 8	0.02	0.45	1.13^{a}	0.77^{a}	0.90^{a}	0.58^{ab}
pH 9	0.03	0.51	1.43^{a}	1.32^{a}	1.08^{a}	0.79^{a}
pH 10	0.03	0.36	1.03^{a}	1.11^{a}	0.52^{b}	0.36^{bc}
pH 11	0.03	0.33	0.21^{b}	0.77^{a}	0.40^{b}	0.26^{c}
	ns	ns				

Note: mean values with different subscripts within a column are statistically different at the 95% confidence level, ns: not significantly different

During experiment, pH fluctuated from 10 to 10.18, decreasing in day 16 and 20 (10.04 to 10.08) compared to day 12 (10.11 to 10.18) and there was no significant difference between treatments. Another study also showed that pH was small change and remained from 9.98 to 10.01 during algal living (14).

Like biomass, chlorophyll at pH 9 was highest at day 12 and 8 (chlorophyll a: 2.72 µg/mL, chlorophyll b: 3.15 µg/mL) and significant difference at 5% according to Duncan test among treatments (Table 1). According to Pandey (15), pH 9 was better condition for chlorophyll accumulation (among pH from 7 to 12).

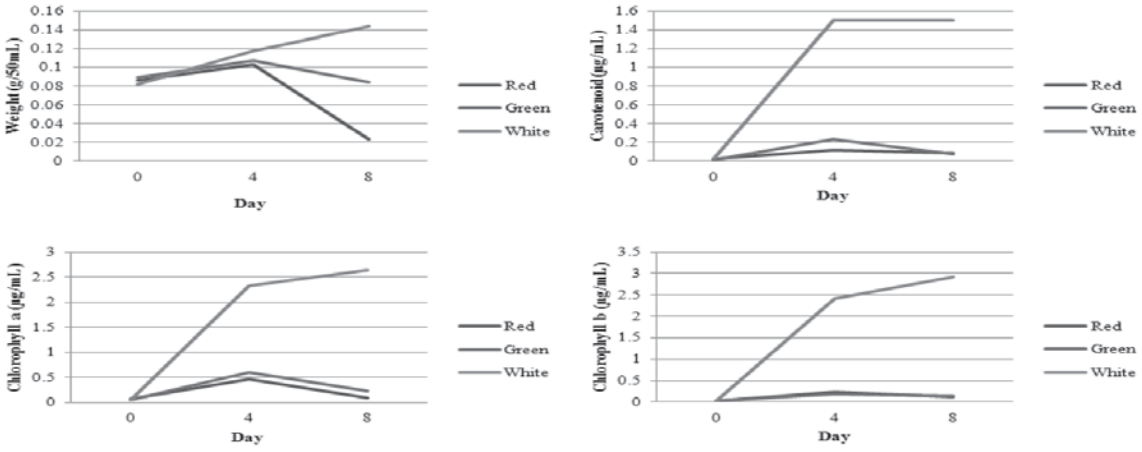

Figure 1. Biomass, chlorophyll and carotenoid in *Spirulina* under different light colours

Highest carotenoid was achieved in day 8 with 1.13 µg/mL for treatment pH 8 and 1.43 µg/mL at pH 9. While in day 12, pH 10 and 11 these numbers were 1.11 µg/mL and 0.77 µg/mL, respectively (Table 1). For all treatments, carotenoid decreased in day 16 and 20 because of lack of nutrition in culture and high density of algae.

3.2 The effect of light colour on *Spirulina* sp. chlorophyll and carotenoid production

Light affected directly on the photosynthesis. So this was a strong factor which controlled the growth of algae. In this experiment, 3 different colours (green, red and white) had significantly different effects in all treatments. In this case, *Spirulina* grew slowly under green light and limited under red light (Figure 1).

The results of this experiment showed that light played the most important role on *Spirulina* growth. Algae were broken into very small fragments in red and green light condition. *Spirulina* also grew to day 4 so that further experiment should be carried out to check the effect of light colour on 5 continuous days. In this study, white, green and blue light were chosen. As the results obtained before, *Spirulina* grew well at white light. From day 0 to day 4, biomass in three treatments showed that the difference is not significant while pigments had significant difference. From day 1 to day 3, algae biomass increased under blue and white light. It rose slowly under green colour from day 2 because biomass was similar in first 4 days (Figure 2). In day 5, highest biomass was collected at white light (0.13 g/ 50 mL) while these numbers were 0.09 g/ 50 mL and 0.08 g/ 50 mL for blue and green light, respectively.

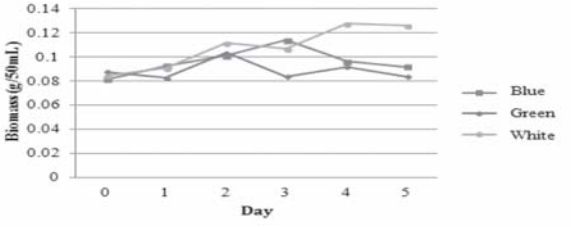

Figure 2. Biomass production in *Spirulina* under different light colours

In treatments controlled with white light, chlorophyll a accumulated rapidly from day 2 (1.02 µg/mL) to day 3 (2.15 µg/mL) while day 4 (2.42 µg/mL) and day 5 (2.71 µg/mL) the accumulation of the pigment were slower (Figure 3). Madhyastha and Vatsala (16) demonstrated that white light was better condition for chlorophyll accumulation in *Spirulina* compared to blue and green light.

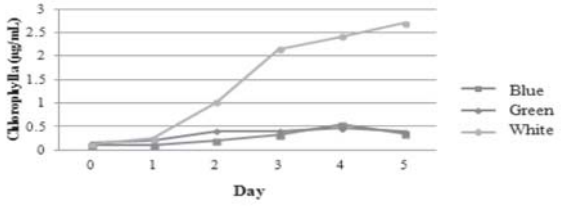

Figure 3. Chlorophyll a in *Spirulina* under different light colours

Clorophyll b production of *Spirulina* under white light was 3.35 μg/mL in day 4 while this number was 0.27 μg/mL for green and 0.26 μg/mL for blue light (Figure 4).

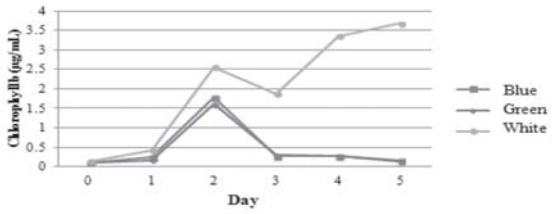

Figure 4. Chlorophyll b in *Spirulina* under different light colours

Spirulina did not synthesize carotenoid under green and blue light from day one (Figure 5). In treatment applying white light, carotenoid grew up fast from day 2 (0.77 μg/mL) to day 4 (1.24 μg/mL).

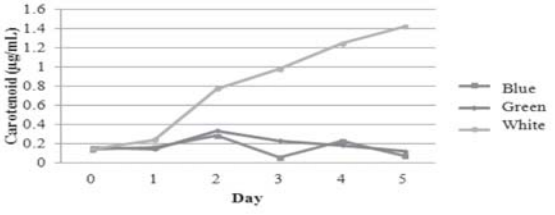

Figure 5. Carotenoid production in *Spirulina* under different light colours

pH value in this study had no significant difference for all treatments and remained at 9.8 - 9.9 in day 4 and 5 (Figure 6). These results were similar to previous experiments in this study. It could be concluded that pH did not change much in algal growth.

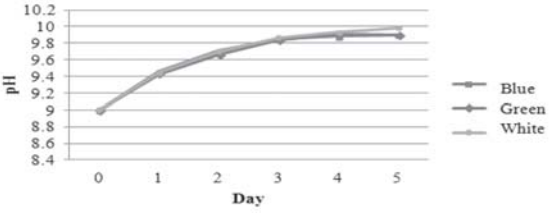

Figure 6. The effect of colour light on pH of algal culture

3.3 The effect of dark – light cycle on *Spirulina* sp. chlorophyll and carotenoid production

In sufficient light regime (24/24 hour of illumination) *Spirulina* grew better than 12/24 hour of illumination. After 4 days, biomass was similar between two treatments but pigments of *Spirulina* were significantly different. In day 5, both biomass and pigments of *Spirulina* at 24/24 hour of illumination were higher than 12/24 hour of illumination (1.08 times in biomass, 2.36 times in chlorophyll a, 1.2 times in chlorophyll b and 1.7 times in carotenoid) (Figure 7).

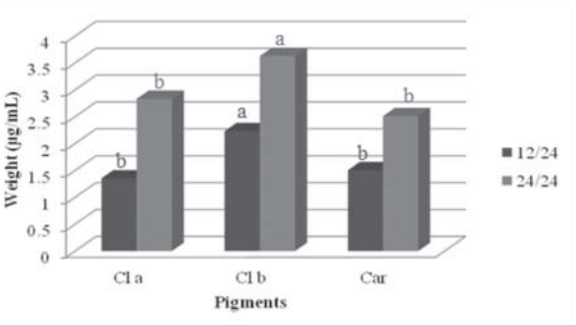

Figure 7. The effect of dark – light cycle on chlorophyll a, chlorophyll b and carotenoid in *Spirulina* at day 5

Biomass production of *Spirulina* was significantly different in day 5 (Figure 8).

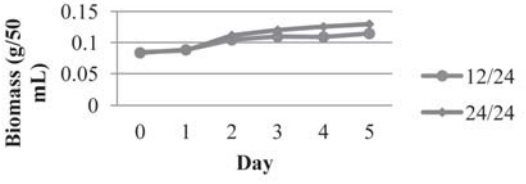

Figure 8. *Spirulina* biomass during 5 days under 12/24 and 24/24 hour illumination

For full light, biomass, chlorophyll a, chlorophyll b and carotenoid were 0.13 g/50 mL; 2.83 μg/mL; 3.63 μg/mL; 2.51 μg/mL, respectively, while these numbers were 0.11 g/50 mL; 1.35 μg/mL; 2.23 μg/mL; 1.50 μg/mL in the half day light.

At this time, algae density was very high so that they needed more light for photosynthesis. It could be explained that at high density of algae, light could not reach to all algal fibers, nutrition and carbohydrate decreased so that the photosynthesis was inhibited (17).

As from the first day, chlorophyll a has different values between two treatments. At day 5, chlorophyll a production was 1.35 μg/mL for 12/24 light hour and 2.83 μg/mL for 24/24 light hour (Figure 9). That means chlorophyll a was more affected by light than chlorophyll b and carotenoid.

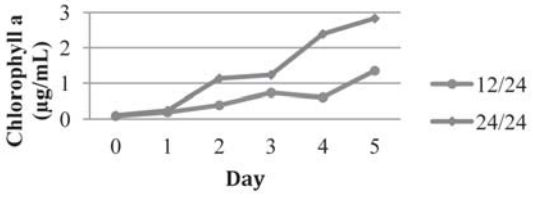

Figure 9. Chlorophyll a in *Spirulina* during 5 days under 12/24 and 24/24 hour illumination

Chlorophyll b content in the first 3 days was not significantly different but between day 3 and day 5, it dramatically increased in 24/24 light consumption (from 2.13 μg/mL to 3.63 μg/mL). At this time, the chlorophyll b production in the treatment at 12/24 hour illumination increased slowly (2.15 μg/mL–2.23 μg/mL) (Figure 10).

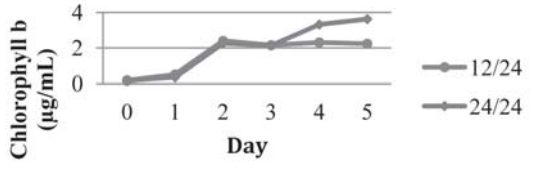

Figure 10. Carotenoid in *Spirulina* during 5 days under 12/24 and 24/24 hour illumination

Comparing to chlorophyll, there was significant difference about carotenoid production which increased day by day. At day 5, carotenoid content in 24/24 hour illumination was 2.15 μg/mL while in 12/24 hour illumination, it was 1.50 μg/mL (higher than 1.7 times) (Figure 11).

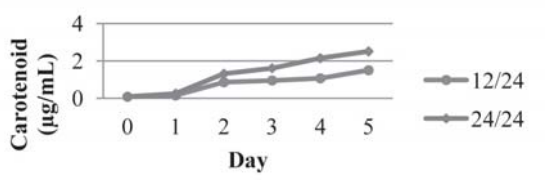

Figure 11. Carotenoid in *Spirulina* during 5 days under 12/24 and 24/24 hour illumination

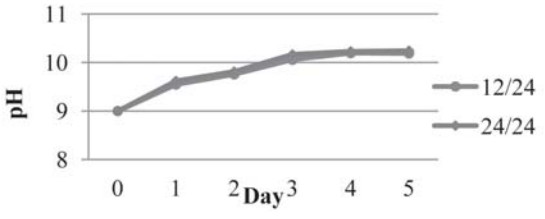

Figure 12. pH in *Spirulina* media during 5 days under 12/24 and 24/24 hour illumination

Similar to the previous results, pH increased in the first 4 days but became stable at day 5 and there was no significant difference among all treatments (Figure 12).

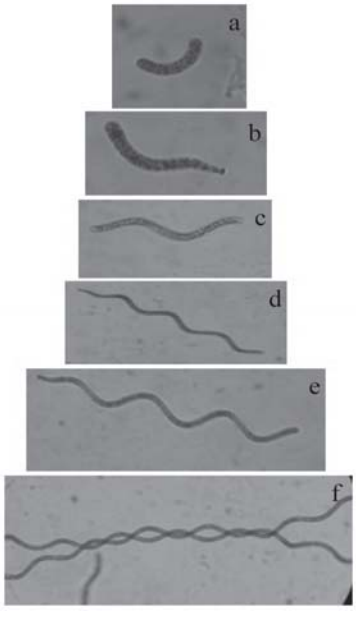

Figure 13. *Spirulina* growth during 5 days in recovery experiment of algae under microscope at magnification 400X
a: day 0; b: day 1; c: day 2; d: day 3; e: day 4; f: day 5

Table 2. *Spirulina* biomass in optimum growth culture

Day	0	1	2	3	4	5
Biomass (g/50mL)	0.070	0.076	0.081	0.103	0.114	0.118

3.4 The recovery of *Spirulina* sp.

Previous results showed that in inappropriate conditions, *Spirulina* cells were broken automatically into small fragments. These treatments were used as a source for this discovery. After 5 days in Zarrouk media, pH 9, 24/24 hour illumination and continuous aeration, *Spirulina* grew and recovered very fast. The algae fiber was longer and biomass increased day by day (Table 2).

In the first 2 days, algae biomass increased slowly from 0.07 g/50 mL (day 0) to 0.081 g/50 mL (day 2). Growth rate increased faster from day 3 to day 5 because at this time algae adapted in new environment.

Under microscope at magnification 400X, algae fiber became longer day by day and high number of twist (Figure 13). This discovery was very important in large scale production because this demonstrated that *Spirulina* could recover dramatically and quickly in suitable environment culture.

4. Conclusions and suggestions

4.1 Conclusions

pH 9, white light, 24/24 light illumination were appropriate conditions for biomass, chlorophyll and carotenoid production in *Spirulina*.

During growth of *Spirulina*, pH remained stable from 10 to 10.18.

In inappropriate conditions, *Spirulina* was broken into small fragments, and recovered quickly when it was grown in appropriate conditions.

4.2 Suggestions

More studies should be carried out to see the effect of temperature, light intensity, aeration rate on biomass and pigment production of *Spirulina* sp.

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