Original Research Article

Effect of Nutrients and Antioxidant Enzyme Activities from *Microcystis aeruginosa*

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A B S T R A C T

The fresh water microalgae *Microcystis aeruginosa* has been examined for its chlorophyll-α and antioxidant enzymatic activities in different nitrate and phosphate concentrations. In the experiment, *M. aeruginosa* were cultured 28 days at 18°C, 25°C and 30°C in various light intensity. The maximum chlorophyll-α content was 33.79 (mg/L) in phosphate (460 µM) at 30°C. Superoxide dismutase (SOD) was significantly higher of 66.53% in phosphate (980 µM) at 30°C. DPPH radical scavenging activity of 78.16 % in the nitrate (329.5µM) at 18°C. The results showed that the maximum chlorophyll-α recorded at 30°C from N:P, P and N respectively. DPPH increased with nitrate concentration during the lower temperature and the SOD increased with phosphate in highest temperature. Chlorophyll-α content significantly compared with 18°C and 30°C (P < 0.05).

**Keywords**

*Microcystis aeruginosa*, Nitrate; phosphate, Chlorophyll-α, DPPH radical-scavenging activity, Superoxide Dismutase activity.

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**Introduction**

*Microcystis aeruginosa* is a major primary producer and a common toxic cyanobacterium, playing an important role in the aquatic environment (Chen *et al.*, 2016). *Microcystis aeruginosa* is commonly observed in highly eutrophic lakes (Watanabe and Oishi, 1985). In recent years, surface cyanobacterial bloom occurred frequently in most large shallow lakes in China, such as Lake Taihu (Zhang *et al.*, 2009), Lake Dianchi (Shen *et al.*, 2004) and Lake Chaohu (Liu, 2007), especially in summer, resulting from eutrophication caused by human activities. There are many studies on the factors that lead to water bloom and close relation between water bloom and concentration of nutrients, particularly phosphorus level was reported (Hecky and Kilham, 1988). Some researches indicated that the decline of cyanobacterial bloom was accompanied with the decrease in dissolved phosphorus (Chen *et al.*, 2005).

Algae are generally abundant in eutrophic water bodies, in these aquatic environments it is commonly the case that high level nitrate and nitrite exist simultaneously, furthermore the concentration of nitrate usually is higher than nitrite (Okafor and Ogbonna, 2003 and Li *et al.*, 2009). The chlorophyll a concentration in some lake
areas was higher than 130 lg L-1 (Kong et al., 2009). It is certain that toxic Microcystis blooms are harmful to human health in South China (Chen et al., 2009b). Super Oxide Dismutase (SOD) is a kind of active substance derived from the organisms, which can eliminate the harmful substances produced in the process of metabolism.

In many cyanobacteria, the greatest proportion of SOD activity in cells is due to Fe-SOD, whose concentration has been shown to increase when cyanobacteria are transferred from low to high levels of photosynthetically active radiation (Grilli Cailoa & Canini, 1993). Therefore, the objective of this study was to determine the effect of N:P concentrations in different temperatures, light intensity, Chlorophyll-a content and antioxidant enzyme response of Microcystis aeruginosa.

Materials and Methods

Algal collection & cultivation

The fresh water cyanobacteria M. aeruginosa were isolated from Lotus lake-Shantou, South China by micro-pipette methods and cultured in BG-11 medium (Rippka et al., 1979) with the modified N: P in five different concentrations; light: dark = 12 h: 12 h; 850 lux (18°C), 1600 lux (25°C), and 2300 lux (30 °C). The species were identified using the light microscope and the stock cultures were maintained. All experiments were carried out in triplicate, totally forty five conical flask (250 ml) were used.

The effect of NaNO₃ and K₂HPO₄ (µM/ml) composition was variable. In nitrate117.7µM, 175µM, 235µM, 282.4µM and 329.5µM; phosphate 110µM, 230µM, 460µM, 750µM and 980µM; N:P propositions 117.7-110µM, 175-230µM, 235-460µM, 282.4-750µM and 329.5-980µM.

Preparation of enzyme extracts

Antioxidant enzyme activity assays was performed with biomass obtained from 10 mL of Microcystis aeruginosa culture, by homogenizing in 0.1 M phosphate buffer (pH 6.5) containing 1% (w/v) polyvinylpyrrolidone (PVP), and centrifuging the homogenate at 10,000 x g and 0°C for 10 min. The supernatant was used for total proteins measurement and antioxidant enzyme activity assays (Mathias Ahii Chia et al., 2015).

Chlorophyll ‘a’

5ml of M.aeruginosa biomass was concentrated and centrifugation (4000rpm) of 8ml culture at ambient temperature for 5 min. The supernatant was discarded and the pellet was re-suspended and macerated in 5 ml of 90% acetone and kept it 14 hours incubation. Again centrifuged 10 min and the absorbance of the supernatant were measured at 750nm, 664nm, 647nm, 630nm (GB 17378.7, 2007).

DPPH radical-scavenging activity

The scavenging effects of samples for DPPH radical were monitored according to the method of Yen and Chen, 1995. Briefly, a 2.0 ml of aliquot of test sample was added to 2.0 ml of 0.16 mM DPPH methonolic solution. The mixture was vortexed for 1 min in the dark, and its absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated using the formulae given by Duan et al., 2006. Synthetic antioxidants, Gallic acid and ascorbic acid were used as positive controls.
Superoxide Dismutase activity

SOD activity was assayed according to the method of Beauchamp and Fridovich (1971). The reaction mixture contained 0.8 mL PBS solution (50 mM, pH 7.8), 0.3 mL methionine solution (130 mM), 0.3 mL Na2EDTA solution (100 mM), 0.3 mL riboflavin solution (20 lM), 0.3 mL nitroblue tetrazolium (NBT) solution (750 lM), and 1 mL enzyme extract for a total volume of 3 mL. As SOD has the ability to inhibit the photochemical reduction of NBT, this assay utilized negative controls (silver paper wrapped around the test tube to mimic fully dark condition without any photochemical reduction of NBT), positive controls (deficiency of SOD activity in light with full photochemical reduction of NBT), and treatment groups (in light with SOD inhibition on photochemical reduction of NBT). The absorbencies of all experimental tubes were measured at 560 nm after a 20-min irradiance of 40–60 mmol photons m⁻² s⁻¹. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of photochemical reduction of NBT.

Statistical analysis

All experiments were performed in three replicates. Prism5 Statistical software was applied to analyze the data. A T-test was used to examine whether there is difference in temperature of chlorophyll -a. Differences were considered to be significant at P < 0.05.

Results and Discussion

Estimation of Chlorophyll -a

Chl-a concentration of M. aeruginosa significantly showed at 30°C higher than that at 25°C and 18°C. In phosphate concentration, the Chl-a (460 µM) showed 33.79 (mg/L) at 30°C followed by nitrate 22.95 (mg/L) at 30°C (117.7 µM) and nitrate-phosphate 19.71(mg/L) at 30°C (235-460 µM) were recorded (Fig1).

DPPH radical scavenging activity

DPPH radical scavenging activities (%) of M.aeruginosa showed significantly higher activity of 78.16 % in the nitrate at 18°C (329.5 µM) and 25°C (329.5-980 µM) followed by nitrate-phosphate 66.75% at 30°C (117.7-110 µM), phosphate 62.23% at 30°C (750 µM) and 57.69% in 30°C (230 µM) (Fig 2).

Fig.1 Effect of nitrate-phosphate, nitrate and phosphate on Chlorophyll-a of M. aeruginosa
**Fig. 2** Effect of nitrate-phosphate, nitrate and phosphate on DPPH radical scavenging assay of *M. aeruginosa*

![Figure 2](image1)

**Fig. 3** Effect of nitrate-phosphate, nitrate and phosphate on SOD activity of *M. aeruginosa* cell

![Figure 3](image2)

**Superoxide Dismutase activity**

SOD activity of *M. aeruginosa* increasing by 50.87 to 66.53% and showed significantly higher activity 66.53% in phosphate at 30°C (980 µM) followed by 54.79% at 18°C (175 µM), 50.87% in nitrate-phosphate at 18°C (117.7-110 µM) and 50.87% in nitrate at 25°C (282.4 µM) were recorded (Fig 3).

*M. aeruginosa* is a prokaryotic cyanobacterium. The chlorophyll-a content significantly decreased with increased concentrations of nitrate-phosphate. These results are consistent with those of Hu *et al.*, (2012), who studied the effect of berberine sulphate on chlorophyll-a levels in *M. aeruginosa* cells. The Chl-a concentration increased during the high temperature. This result was similar to the result of Burke, who found that a 38°C pre-incubation temperature provided maximum chlorophyll accumulation following the high temperature exposure (Burke, 2001). Adaptation of *M. aeruginosa* for high temperature is also associated with up-regulation of protective enzymes because protective enzymes can defend against free radicals (Xu *et al.*, 2006). The levels of SOD and DPPH in the *M. aeruginosa* were used in our study varied to different concentrations of nutrients (NP; P and N).
SOD activity increased in response to high concentrations of NPs (Jingxian Wang, 2007). Similarly, SOD activity increased in high concentrations of nitrate and phosphates with the temperatures at 30°C and 25°C. In the laboratory culture the high concentrations of nitrate and phosphate (25-30°C) showed the moderate values of SOD, whereas the maximum values of SOD was 18°C with the low level of nitrate-phosphate concentration. Overall, present investigation indicating that *Microcystis aeruginosa* has a strong ability to adapt to variations in environmental conditions.

In conclusion, the results of this study show that DPPH and SOD enzymes are present in the bloom-producing species *M. aeruginosa*.

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