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Effects of the different nitrogen, phosphorus and carbon source on the growth and glycogen reserves in *Synechocystis* and *Anabaena*

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The effects of different nutrient sources (nitrogen, phosphorus and carbon) on the growth status and glycogen reserves in *Synechocystis* sp. strain PCC 6803 and *Anabaena* sp. strain PCC 7120 were studied. The two cyanobacteria grew well on suitable nutrient sources, for example, nitrate (sodium nitrate), phosphate (two potassium hydrogen phosphate) or inorganic (sodium carbonate, dicarbonate) and organic (sodium acetate, glucose) carbon. On the contrary, the growth rate decreased markedly when grown on ill-suited nutrient sources, for example, ammonium (ammonium chloride), organic nitrogen (urea), pyrophosphate (sodium pyrophosphate) and organophosphate (D-Glucose,6-(dihydrogen phosphate) sodium salt (1:2), adenosine-triphosphate). The yield of phycocyanin and chlorophyll a was higher when grown on suitable than ill-suited nutrient sources, whereas, the activities of superoxide dismutase (SOD) and peroxidase (POD) were higher on ill-suited than suitable nutrient sources, and the glycogen reserves presented the same variation tendences as peroxidase. These results indicate that the nutrient sources used in this study were involved in regulation of (1) the contents of pigments and glycogen, (2) energy and electron transport efficiencies of photosynthesis, and (3) activity level of SOD and POD in *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120.

Key words: Nitrogen, phosphorus, carbon, growth, glycogen reserves.

INTRODUCTION

With the intensification of human activities in lakes or reservoirs, large quantities of nutrients were input the water bodies through a variety of ways, for example, non-point source nutrient pollution (Ribbe et al., 2008) and wastewater effluent (Oehmen et al., 2007), the nutrient levels in water has been observed worldwide over the last few years. Nutrients over-enrichment may initiate eutrophication which was defined by the International Organization for Economic Cooperation and Development (OECD), with eutrophic lakes or reservoirs frequently being dominated by cyanobacteria during considerable periods of time (Vieira et al., 2005).

Cyanobacteria are photosynthetic bacteria, which are widespread in water ecosystem. Their mass accumulation, known as cyanobacterial blooms, frequently occurs not only in freshwater but also in marine environment with durative Waterblooms of cyanobacteria (that is *Microcystis*, *Anabaena*, *Synechocystis*, *Nostoc*, *Nodularia,*

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etc) are becoming recognized as a great threat to the aquatic environment because of the secondary metabolites that they can produce (that is hepatotoxin, neurotoxin, skin irritant, etc), which have been responsible for the death of wild and domestic animals in different countries worldwide and would also represent a health hazard for Humans (Chen et al., 2009). Cyanobacterial blooms may consume dissolved oxygen in water and lead death of other hydrobionts (that is fishes, zooplanktons, benthic animals, etc). Although several studies on the influence of environmental factors like hydrology, light, pH, temperature and nutrient availability on cyanobacterial bloom formation have been carried out, the reasons for cyanobacterial bloom formation are not always clear (Schreiter et al., 2001). Nutrient bioavailability in aquatic ecosystems is affected by nutrient supply, which can be altered by external factors, such as meteorological changes and anthropolo-gical influence (Schreiter et al., 2001). These nutrients do not always exist in forms accessible to microorganisms (Schreiter et al., 2001). Nitrogen (Martiny et al., 2009; Howarth et al., 2011), phosphorus (Ernst et al., 2005; Smith et al., 2011) and carbon (Waal et al., 2009; Paerl and Paul, 2012) availability are three of the key factors implicated in cyanobacterial proliferation and can be limiting factors in aquatic environments. Cyanobacteria present highly uptake efficiency and retention mecha-nisms for three nutrient sources: bicarbonate, nitrate, and phosphate (Herrero et al., 2001; Ritchie et al., 2001; Badger and Price 2003).

Synechocystis sp. strain PCC 6803, a unicellular freshwater cyanobacterium, has been a model organism for gene disruption analysis, DNA microarray analysis and proteomic analysis (Ikeuchi and Tabata, 2001; Hihara et al., 2001; Oliveira and Lindblad, 2009), since its genome sequence was determined in 1996 (Kaneko et al., 1996). *Anabaena* sp. strain PCC 7120, a strain of cyanobacterial genera found in blooms, has also been a model organism since its genome sequence was determined in 2001 (Kaneko et al., 2001). In this study, the two strains were isolated and the effects of different nutrient sources (nitrogen, phosphorus and carbon) on the yield of light harvesting pigments, electron transport efficiency and glycogen reserves were studied.

MATERIALS AND METHODS

Bacterial strains, media and growth media

Axenic cells of *Synechocystis* sp. strain PCC 6803, a glucose-tolerant strain, and *Anabaena* sp. strain PCC 7120 provided by the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China, were grown in BG11 medium buffered by N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES, 20 mM, pH 8) in Erlenmeyer flasks at 30°C under continuous light of 50 µmol photons·m^{-2}·s^{-1}. The flasks were bubbled with a continuous stream of 0.03% (v/v) CO_2 in air, and shaken at 150 revolutions per minute (Sokolenko et al., 2002). Growth of the *Synechocystis* sp. strain PCC 6803 and *Anabaena* sp. strain PCC 7120 was monitored by measuring the absorbance at 730 nm (A_{730}) (Wang et al., 2011) and 750nm (A_{750}) (Neunuebel and Golden, 2008) by a Lambda 25 (Perkin-Elmer) spectrophotometer, respectively. *Anabaena* sp. strain PCC 7120 is catenarian and interlaced which is unfaithful to calculate the number related to A_{750}, however, *Synechocystis* sp. strain PCC 6803 is unicellular which is easy to calculate the cell number related to A_{730}, and the relationship between A_{730} and cell number of *Synechocystis* sp. PCC 6803 is, cell number = $6.87124\times10^6 + 9.46733\times10^7\times A_{730}$. We used A_{750} and cell number to present the growth status of *Anabaena* sp. strain PCC 7120 and *Synechocystis* sp. PCC 6803, respectively. For different nitrogen sources conditions, cells were grown in BG11 medium in which $NaNO_3$ (sodium nitrate) was replaced by $NaNO_2$ (sodium nitrite), CON_2H_4 (urea) and NH_4Cl (ammonium chloride) with 247.03 mg·L^{-1} of nitrogen, respectively.

For different phosphorus sources conditions, cells were grown in BG11 medium in which K_2HPO_4 (two potassium hydrogen phosphate) was replaced by $Na_4P_2O_7$ (sodium pyrophosphate), $C_{10}H_{16}N_5O_{13}P_3$ (adenosine-triphosphate) and $C_6H_{11}Na_2O_9P$ (D-glucose,6-(dihydrogen phosphate), sodium salt (1:2)) with 5.43mg·L^{-1} of phosphorus, respectively. For different carbon sources conditions, cells were grown in BG11 medium in which Na_2CO_3 (sodium carbonate) was replaced by $NaHCO_3$ (dicarbonate), CH_3COONa (sodium acetate) and $C_6H_{12}O_6$ (glucose) with 2.26 mg·L^{-1} of carbon, respectively. In all cases, cells grown in BG11 medium were harvested by centrifugation at 6000 × g, washed, and then re-suspended in BG11 medium replaced by different nutrient sources.

Physiological and biochemical characterization

Chlorophyll *a* (Chl *a*) content of the cultures was determined spectrophotometrically in 90% (v/v) acetone extracts following the study of Andrés et al. (2010). The concentration (*C*) of phycocyanin (PC) was determined through the following equation (Soni et al., 2008): C (PC, mg· mL^{-1}) = (A_{615}–0.474×A_{652})/5.34, where A_{615} and A_{652} are the absorbances at 615 and 652 nm, respectively (Liao et al., 2009).

For determining the glycogen content, *Synechocystis* and *Anabaena* were grown to a chlorophyll concentration of 5-8 mg·L^{-1}, and the cells from 30 to 40 ml culture collected by centrifugation. The resulting pellet was suspended in 500 µL H_2SO_4 (2.5%), and then boiled for 40 min. After centrifugation at 9000 × *g* for 5 min, the content of the reducing sugars in the supernatant was assayed with 3,5-dinitrosalicylic acid (DNS) (Valladares et al., 2007), and the amounts expressed per cell for *Synechocystis* sp. PCC 6803 and per Chl *a* for *Anabaena* sp. PCC 7120.

For determining the activity of superoxide dismutase (SOD) and peroxidases (POD), *Synechocystis* and *Anabaena* were grown to a chlorophyll concentration of 5 to 8 mg·L^{-1}, and the cells from 80-100 ml culture collected by centrifugation at 8000 × *g* for 10 min. The resulting pellet was suspended in KPB (50 MM, pH 7.0), and then broken for 20 min in a ultrasonic broken instrument. After centrifugation at 9000 × *g* for 10 min, the activity of SOD in the supernatant was tested by measuring the inhibition of pyrogallic acid autoxidation, while the activity of POD was detected by measuring the decrease of the absorbance at 470 nm at 25°C for 5 min (Marklund and Marklund, 1974; Prestamo et al., 2001; Liao et al., 2009). The enzyme unit of SOD is defined that the pyrogallic acid autoxidation is inhibited by 50% in 1 ml of enzyme reaction liquid, while the enzyme unit of POD is defined that the absorbance at 470 nm changes by 0.01 in 1 min. The enzyme activities expressed per cell for *Synechocystis* sp. PCC 6803 and per Chl *a* for *Anabaena* sp. PCC 7120.

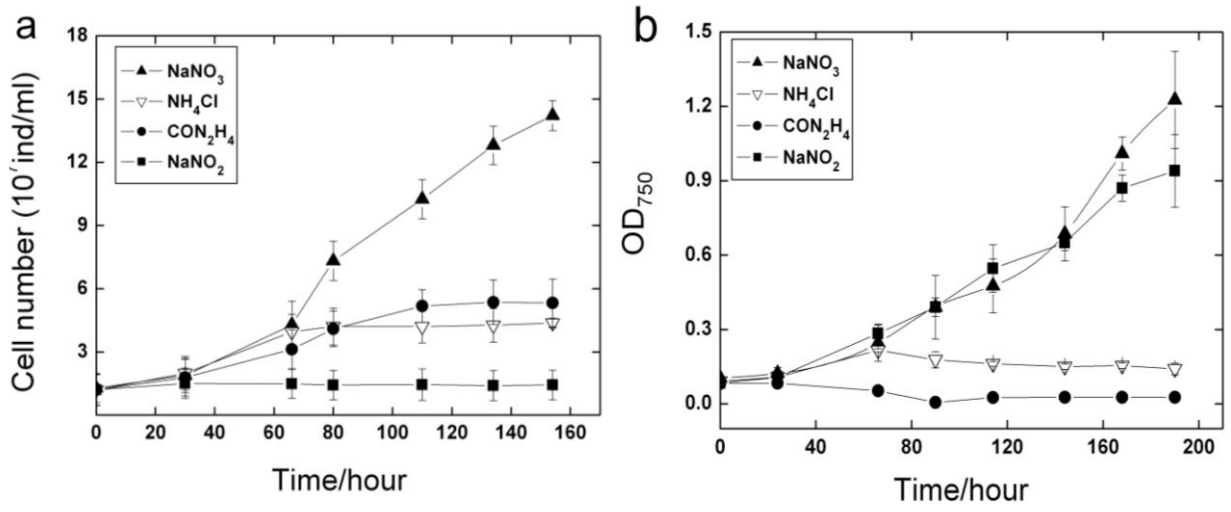

Figure 1. Growth curves of *Synechocystis* PCC 6803 (a) and *Anabaena* sp. PCC 7120 (b) under grown in different nitrogen sources. Results are from 3 independent repetitions of the experiments.

Table 1. The contents of chlorophyll a (Chl *a*), phycocyanin (PC) and glycogen and the activities of superoxide dismutase (SOD) and peroxidase (POD) in *Synechocystis* PCC 6803 under grown in different nitrogen sources.

Substrate	Chl *a** (10^{-8}μg/ind cell)	PC* (10^{-10}mg/ind cell)	glycogen* (10^{-10}mg/ind cell)	SOD* (10^{-9}U/ind cell)	POD* (10^{-8}U/ind cell)
$NaNO_3$	7.24 ± 0.29	3.88 ± 0.19	1.06 ± 0.31	5.00 ± 1.01	1.62 ± 0.34
NH_4Cl	2.90 ± 0.03	1.96 ± 0.02	1.61 ± 0.08	16.92 ± 0.36	5.54 ± 1.42
urea	1.84 ± 0.47	1.45 ± 0.23	2.01 ± 0.06	13.13 ± 0.07	5.40 ± 0.20
$NaNO_2$	0.81 ± 0.49	0.24 ± 0.00	2.39 ± 0.24	24.09 ± 1.62	9.15 ± 1.06

*Chl *a*, C-PC, glycogen, SOD and POD contents were measured when the cells grew in different nitrogen sources after 154 h. Results are from 3 independent repetitions of the experiments.

RESULTS

Effects of nitrogen sources

The cell number of *Synechocystis* PCC 6803 increased logarithmically at the end of 154 h when grown on $NaNO_3$ as nitrogen source, however, the cells stopped increasing at 80 h on NH_4Cl and urea, especially, the cell number changed scarcely on $NaNO_2$ (Figure 1a). The cell density of *Anabaena* sp. PCC 7120 increased logarithmically at the end of 190 h when grown on $NaNO_3$ and $NaNO_2$, whereas, the cells stopped increasing at 64 h on NH_4Cl, especially, the cell number changed scarcely on urea (Figure 1b).

The contents of chlorophyll a (Chl *a*), phycocyanin (PC) and glycogen and the activities of superoxide dismutase (SOD) and peroxidase (POD) in *Synechocystis* PCC 6803 cells grown on different nitrogen sources were measured at the end of 154 h (Table 1). The both pigments presented the same variation tendency as the growth curve, for example, the contents of Chl *a* were 1.5, 2, and 8 fold more abundant, respectively, on $NaNO_3$ than NH_4Cl, urea and $NaNO_2$, and the contents of PC were 1, 1.7, and 15.5 fold more abundant, respectively. On the contrary, the contents of glycogen were 34.2, 47.3 and 55.6% less abundant, respectively, on $NaNO_3$ than NH_4Cl, urea and $NaNO_2$, similarly, the enzyme activities of SOD were 70.4, 61.9 and 79.2% less abundant, respectively, and the enzyme activities of POD were 70.8, 70.0 and 82.3% less abundant, respectively.

The contents of Chl *a*, PC and glycogen and the activities of SOD and POD in *Anabaena* sp. PCC 7120 cells grown on different nitrogen sources were measured at the end of 190 h (Table 2). The contents of PC were 0.7 and 1 fold more abundant, respectively, on $NaNO_3$ or $NaNO_2$ than NH_4Cl and urea, which presented the same variation tendency as the growth curve. On the contrary, the contents of glycogen were 96.7 and 99.1% less abundant, respectively, on $NaNO_3$ or $NaNO_2$ than NH_4Cl and urea, similarly, the enzyme activities of SOD were 96.6 and 99.4% less abundant, respectively, and the enzyme activities of POD were 99.3 and 99.5% less

Table 2. The contents of PC and glycogen and the activities of SOD and POD in *Anabaena* sp. PCC 7120 under grown in different nitrogen sources.

Substrate	PC* (μg /μg Chl *a*)	Glycogen* (μg/μg Chl *a*)	SOD* (U/μg Chl *a*)	POD* (U/μg Chl *a*)
$NaNO_3$	11.00 ± 0.20	3.00 ± 0.09	0.43 ± 0.13	0.46 ± 0.07
NH_4Cl	6.40 ± 0.50	90.0 ± 51.0	11.81 ± 3.28	74.58 ± 3.24
urea	5.60 ± 4.00	353 ± 14.0	71.90 ± 17.85	110.78 ± 47.45
$NaNO_2$	11.00± 2.90	2.40 ± 0.40	0.37 ± 0.11	0.49 ± 0.04

*Chl *a*, C-PC, glycogen, SOD and POD contents were measured when the cells grew in different nitrogen sources after 190 h. Results are from 3 independent repetitions of the experiments.

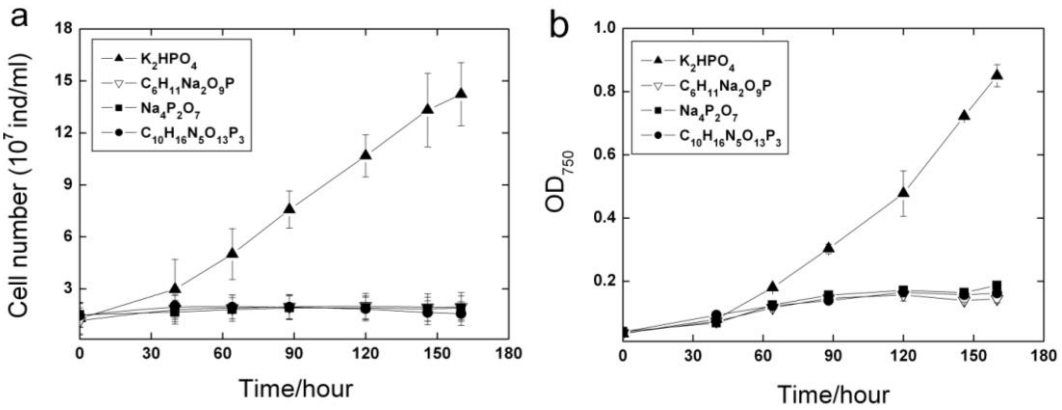

Figure 2. Growth curves of *Synechocystis* PCC 6803 (a) and *Anabaena* sp. PCC 7120 (b) under grown in different phosphorus sources. Results are from 3 independent repetitions of the experiments.

Table 3. The contents of Chl *a*, PC and glycogen and the activities of SOD and POD in *Synechocystis* PCC 6803 under grown in different phosphorus sources.

Substrate	Chl *a** (10^{-8}μg/ind cell)	PC* (10^{-10}mg/ind cell)	Glycogen* (10^{-10}mg/ind cell)	SOD* (10^{-9}U/ind cell)	POD* (10^{-8}U/ind cell)
K_2HPO_4	7.15 ± 0.98	7.44 ± 1.87	1.27 ± 0.10	1.10 ± 0.09	2.11 ± 0.17
$C_6H_{11}Na_2O_9P$	0.89 ± 0.12	1.32 ± 0.05	6.98 ± 1.05	4.40 ± 0.33	15.68 ± 1.52
$Na_4P_2O_7$	0.94 ± 0.05	1.99 ± 0.10	8.46 ± 0.42	4.52 ± 0.23	31.95 ± 2.09
$C_{10}H_{16}N_5O_{13}P_3$	1.44 ± 0.07	1.70 ± 0.09	12.11 ± 0.61	5.15 ± 0.26	25.01 ± 6.74

*Chl *a*, C-PC, glycogen, SOD and POD contents were measured when the cells grew in different phosphorus sources after 160 h. Results are from 3 independent repetitions of the experiments.

abundant, respectively.

Effects of phosphorus sources

The cell density of *Synechocystis* PCC 6803 and *Anabaena* sp. PCC 7120 increased logarithmically at the end of 160 h when grown on K_2HPO_4 as phosphorus source, however, the cell number of *Synechocystis* PCC 6803 changed scarcely (Figure 2a) and the cells of *Anabaena* sp. PCC 7120 stopped increasing at 40 h (Figure 2b) on $C_6H_{11}Na_2O_9P$, $Na_4P_2O_7$ and $C_{10}H_{16}N_5O_{13}P_3$.

The contents of Chl *a*, PC and glycogen and the activities of SOD and POD in *Synechocystis* PCC 6803 cells grown on different phosphorus sources were measured at the end of 160 h (Table 3). The both two pigments presented the same variation tendency as the growth curve, for example, the contents of Chl *a* were 7, 6.6, and 4 fold more abundant, respectively, on K_2HPO_4 than $C_6H_{11}Na_2O_9P$, $Na_4P_2O_7$ and $C_{10}H_{16}N_5O_{13}P_3$ as phosphorus source, and the contents of PC were 4.6,

Table 4. The contents of PC and glycogen and the activities of SOD and POD in *Anabaena* sp. PCC 7120 under grown in different phosphorus sources.

Substrate[1]	PC* (μg /μg Chl *a*)	glycogen* (μg/μg Chl *a*)	SOD* (U/μg Chl *a*)	POD* (U/μg Chl *a*)
K_2HPO_4	9.00 ± 0.30	7.20 ± 0.30	0.35 ± 0.03	0.65 ± 0.31
$C_6H_{11}Na_2O_9P$	6.00± 2.00	19.3 ± 1.90	1.41 ± 0.06	8.55 ± 0.78
$Na_4P_2O_7$	8.00 ± 0.40	15.0 ± 0.75	1.48 ± 0.07	8.59 ± 0.43
$C_{10}H_{16}N_5O_{13}P_3$	7.00 ± 0.35	25.0 ± 1.25	1.27 ± 0.06	7.74 ± 0.39

*Chl *a*, C-PC, glycogen, SOD and POD contents were measured when the cells grew in different phosphorus sources after 160 h. Results are from 3 independent repetitions of the experiments

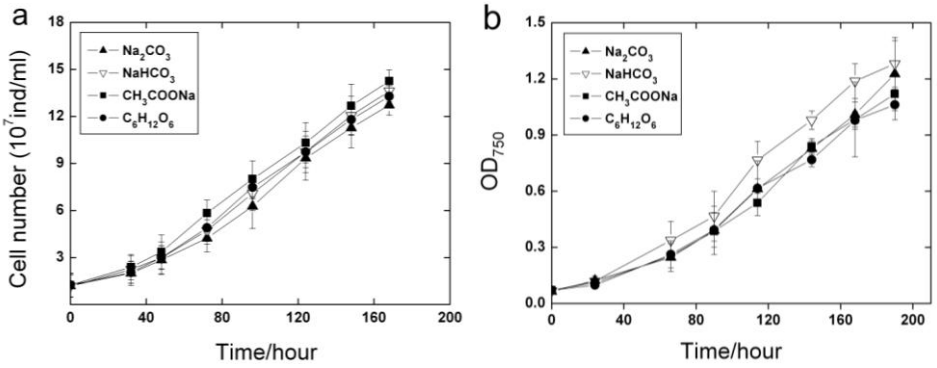

Figure 3. Growth curves of *Synechocystis* PCC 6803 (a) and *Anabaena* sp. PCC 7120 (b) under grown in different carbon sources. Results are from 3 independent repetitions of the experiments.

2.7, and 3.4 fold more abundant, respectively. On the contrary, the contents of glycogen were 81.8, 85.0 and 89.5% less abundant, respectively on K_2HPO_4 than $C_6H_{11}Na_2O_9P$, $Na_4P_2O_7$ and $C_{10}H_{16}N_5O_{13}P_3$, similarly, the enzyme activities of SOD were 75.0, 75.7 and 78.6% less abundant, respectively, and the enzyme activities of POD were 86.5, 93.4 and 91.6% less abundant, respectively.

The contents of Chl *a*, PC and glycogen and the activities of SOD and POD in *Anabaena* sp. PCC 7120 cells grown on different phosphorus sources were measured at the end of 160 h (Table 4). The contents of PC were 0.5, 0.1, and 0.3 fold more abundant, respectively, on K_2HPO_4 than $C_6H_{11}Na_2O_9P$, $Na_4P_2O_7$ and $C_{10}H_{16}N_5O_{13}P_3$ as phosphorus source, which presented the same variation tendency as the growth curve. On the contrary, the contents of glycogen were 62.7, 52.0 and 71.2% less abundant, respectively, on K_2HPO_4 than $C_6H_{11}Na_2O_9P$, $Na_4P_2O_7$ and $C_{10}H_{16}N_5O_{13}P_3$, similarly, the enzyme activities of SOD were 75.2, 76.4 and 72.4% less abundant, respectively, and the enzyme activities of POD were 92.4, 92.4 and 91.6% less abundant, respectively.

Effects of carbon sources

The cell density of *Synechocystis* PCC 6803 and *Anabaena* sp. PCC 7120 increased logarithmically at the end of 168 h when grown on different carbon source used in this study. Whereas, insignificant differences were observed between these different tests with different carbon sources (Figure 3a, 3b).

The contents of Chl *a*, PC and glycogen and the activities of SOD and POD in *Synechocystis* PCC 6803 and *Anabaena* sp. PCC 7120 cells grown on different carbon sources were measured at the end of 168 h (Tables 5 and 6). The both pigments presented the same variation tendency as the growth curve except the *Synechocystis* PCC 6803 cells grown on $C_6H_{12}O_6$, however, the contents of glycogen and the enzyme activities of SOD and POD presented the contrary variation tendency compared with the growth curve.

DISCUSSION

Light-harvesting is achieved in cyanobacteria not only through chlorophyll-protein complexes, but also through phycobilisomes (PBS) that are mainly associated with photosystem II (PSII) (Joshua et al., 2005; Arteni et al., 2009). PBSs are composed of phycobiliproteins that can comprise up to 50% of the total cellular proteins, and of linker proteins that are relevant for their organization, attachment to the membrane, and fine-tuning of the light absorption and energy transport (Miller et al., 2008).

Table 5. The contents of Chl *a*, PC and glycogen and the activities of SOD and POD in *Synechocystis* PCC 6803 under grown in different carbon sources.

Substrate	Chl *a** (10^{-8}μg/ind cell)	PC* (10^{-10}mg/ind cell)	glycogen * (10^{-10}mg/ind cell)	SOD* (10^{-9}U/ind cell)	POD* (10^{-8}U/ind cell)
Na_2CO_3	6.24 ± 0.06	4.39 ± 0.13	1.97 ± 0.27	3.52 ± 0.48	5.88 ± 0.80
$NaHCO_3$	6.60 ± 0.37	4.62 ± 0.27	1.55 ± 0.05	3.29 ± 0.04	5.25 ± 2.48
CH_3COONa	6.80 ± 0.57	4.97 ± 0.36	1.52 ± 0.34	2.52 ± 0.13	3.09 ± 0.85
$C_6H_{12}O_6$	6.25 ± 0.31	4.30 ± 0.21	2.34 ± 0.12	3.93 ± 0.15	6.72 ± 1.61

*Chl *a*, C-PC, glycogen, SOD and POD contents were measured when the cells grew in different carbon sources after 168 h. Results are from 3 independent repetitions of the experiments.

Table 6. The contents of PC and glycogen and the activities of SOD and POD in *Anabaena* sp. PCC 7120 under grown in different carbon sources.

Substrate	PC* (μg /μg Chl *a*)	Glycogen* (μg/μg Chl *a*)	SOD* (U/μg Chl *a*)	POD* (U/μg Chl *a*)
Na_2CO_3	9.50 ± 0.40	3.10 ± 0.50	0.53 ± 0.03	0.43 ± 0.10
$NaHCO_3$	9.60 ± 0.80	3.20 ± 0.90	0.49 ± 0.03	0.32 ± 0.06
CH_3COONa	9.20 ± 0.10	4.90 ± 2.10	0.58 ± 0.02	0.54 ± 0.03
$C_6H_{12}O_6$	8.60 ± 2.80	4.90 ± 0.90	0.60 ± 0.06	0.79 ± 0.17

*Chl *a*, C-PC, glycogen, SOD and POD contents were measured when the cells grew in different carbon sources after 190 h. Results are from 3 independent repetitions of the experiments.

Duysens (1951) and French et al. (1956) found that the absorption energy from phycoerythrin could excite relevant fluorescence of phycocyanin and chlorophyll a, whereas, the absorption energy from phycocyanin or chlorophyll a could not excite relevant fluorescence of phycoerythrin, and then the energy transport order was put forward as phycoerythrin (λ_{abs}=565 nm) → phycocyanin(λ_{abs} = 620 nm) → chlorophyll a. Thus, it can be seen that the contents of phycocyanin and chlorophyll a were important indicators to scale photosynthesis efficiency and growth status of cyanobacteria. In this study, the contents of phycocyanin and chlorophyll a in *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 cells grown on different nutrient sources presented the same variation tendency as the growth curve. The results indicated that the energy transport in photosynthesis and growth status of cyanobacteria were not influenced when grown on suitable nutrient sources, for example, nitrate (sodium nitrate) as nitrogen source, phosphate (two potassium hydrogen phosphate) as phosphorus source and inorganic (sodium carbonate, dicarbonate) and organic (sodium acetate, glucose) carbon source. On the contrary, the energy transfer in photosynthesis was inhibited and the growth rate decreased markedly when grown on ill-suited nutrient sources, for example, nitrite for *Synechocystis* sp. PCC 6803, ammonium (ammonium chloride) and organic nitrogen (urea) as nitrogen sources, pyrophosphate (sodium pyrophosphate), organophos-phate (D-glucose,6-(dihydrogen phosphate) sodium salt (1:2), and adenosine-triphosphate) as phosphorus sources, for *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120. Nitrite has been found to cause severe inhibition to the growth of many bacteria (Zhou et al., 2011), in this study, it is likely inhibitory for *Synechocystis*, but not to *Anabaena*. It is described that nitrogen or phosphours starvation cause bleaching and stops growth in *Synechococcus* (Gorl et al., 1998; Gillor et al., 2002). In our study, the cells of *Synechocystis* and *Anabaena* may not effectively absorb the nutrient of the cultures when using the ill-suited phosphorus sources, causing the phosphorus starvation. For *Synechocystis* may be the same reason for the nitrogen starvation, whereas, for *Anabaena* is different, because this strain is diazotrophic and fix atmospheric N_2, it cannot be nitrogen starved. Perhaps the effect observed in this study is due to the toxicity of ammonium or urea (Dai et al., 2008).

Active oxygen species, including superoxide (O_2^-), hydrogenperoxide (H_2O_2), and the hydroxyl radical (OH•), are byproducts of both aerobic respiration and oxygenic photosynthesis in all organisms that carry out these processes (Tichy and Vermaas, 1999). Photosynthesis of cyanobacteria is carried out by the energy and electron transport between photosystems, the transfer progress can be considered as PS II $\xrightarrow{e}$ photosystem I (PS I) $\xrightarrow{e}$ NADPH (Ferreira et al., 2004). The major site of O_2^- production in the photosynthetic electron transport chain is at the reducing side of PS I : particularly under conditions when NADPH utilization is suboptimal and NADP levels are low, O_2 rather than NADP may occasionally

accept an electron from PS I (Asada, 1994). Because of the relatively high reactivity of active oxygen species with proteins and membranes, efficient scavenging O_2^- is important to prevent photooxidative damage to the organism. O_2^- is efficiently scavenged by superoxide dismutase (SOD), the activity of the enzyme that can catalyze O_2^- to O_2 or H_2O_2 is sufficient to limit O_2^- which can induce damages of cellular components. Redundant H_2O_2 can be catalyzed to O_2 or H_2O by peroxidase (POD) (Schutzendubel et al., 2001; Bernroitner et al., 2009). Increased activity of SOD and POD was found in many organisms (Bai and Wang, 2002; Choudhary et al., 2006; Sabatini et al., 2009). These observations are also supported by the results in this study, the enzyme activities of SOD and POD in *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 increased observably when grown on ill-suited nutrient sources and presented the contrary variation tendency compared with the growth curve. The electron transport in photosynthesis and physiological functions of cyanobacteria could be carried out unimpeded on suitable nutrient sources, whereas, the yield of phycocyanin and chlorophyll a decreased evidently on ill-suited nutrient sources, which played as external stress factors, restricting the electron transport between photosystems and causing accumulation of O_2^- around the cell membrane. Therefore, when grown on ill-suited nutrient sources, more SOD should be synthesized to clear the redundant O_2^- and protect the cellular components, and then the production of O_2^- by catalytic reaction, H_2O_2, was cleared by POD to prevent lipid peroxidation of the cell membrane.

The major carbon- and energy-reserve compound accumulated by cyanobacteria during photoautotrophic growth is generally a glycogen-like polyglucan (Shively, 1988; Philippis et al., 1992). It is an α-D-(1→4)-glucan polymer containing about 10% α-(1→6)-branch linkages (Yoo et al., 2002). This polymer is accumulated massively in cyanobacterial cells whenever balanced growth is hampered by a particular nutrient deficiency (Philippis et al., 1992; Eriksen, 2008). Once conditions for balanced growth are re-established, accumulated glycolgen is rapidly broken down to yield energy and carbon for cell metabolism. It has therefore been suggested that glycogen acts as a dynamic reserve with the dual function of storage product and of buffer substance able to separate the process of carbon supply from its subsequent utilization in other biosynthetic pathways (Philippis et al., 1992; Cherchi and Gu, 2010). In this study, the contents of glycogen in *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 were observably higher when grown on ill-suited than suitable nutrient sources, and presented the contrary variation tendency compared with the growth curve. These phe-nomena, which were similar to the findings described in the study of Philippis et al. (1992), could be explained as that, the biosynthesis yield of phycocyanin and chloro-phyll a decreased when grown on ill-suited nutrient sources, causing interrupt of the normal photosynthesis and growth, and then, the glycogen in cell was accumulated, whereas, the growth resumed balanced and the accumulative glycogen was rapidly broken down to yield energy and carbon for cell metabolism when grown on suitable nutrient sources.

In conclusion, three primary actions of different nitrogen, phosphorus and carbon sources used in this study can be envisioned from the data obtained in this work. The nutrient sources are involved in regulation of (1) the contents of pigments and glycogen, (2) energy and electron transport efficiencies of photosynthesis, and (3) activity level of SOD and POD in *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120. These regulatory actions may be helpful to understand the influence of nutrient availability on cyanobacterial bloom formation.

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