Investigating the effect of different concentrations of nitrate and phosphate on the quantity of mycosporine like amino acids production and growth in *Spirulina platensis*

Sara Saadatmand*, Morteza Zamani

*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

**Key words:** Nitrate, phosphate, mycosporine like amino acids, chlorophyll a, carotenoids.

[http://dx.doi.org/10.12692/ijb/6.5.63-69](http://dx.doi.org/10.12692/ijb/6.5.63-69)  Article published on March 09, 2015

**Abstract**

The present study invested on the effect of different concentrations of \( \text{NaNO}_3 \) (1.5, 2, 2.5 gl\(^{-1}\)) and \( \text{K}_2\text{HPO}_4 \) (0.04, 0.05, 0.06 gl\(^{-1}\)) on chlorophyll a, carotenoids, fresh and dry weight measures, and the quality of mass absorption in *Spirulina platensis* alga. Findings revealed a significant increase in the amount of chlorophyll a and carotenoids in cultivation setting by increasing \( \text{NaNO}_3 \) and \( \text{K}_2\text{HPO}_4 \), especially at 2.5 gl\(^{-1}\) \( \text{NaNO}_3 \) and 0.06 gl\(^{-1}\) \( \text{K}_2\text{HPO}_4 \) concentrations. More findings also revealed that nitrate or phosphate supplemented growth medium had reducing effects on fresh weight; but, adversely affected the dry weight. Here, there was an increase in concentration of \( \text{NaNO}_3 \) and \( \text{K}_2\text{HPO}_4 \), especially for 2.5 gl\(^{-1}\)nitrate and 0.05 gl\(^{-1}\)phosphate concentration samples. It should also be mentioned that the highest dry mass measures were obtained in 2.5 gl\(^{-1}\) \( \text{NaNO}_3 \), though mass absorption in *Spirulina platensis* was at the highest rate in 2 gl\(^{-1}\) nitrate and 0.06 gl\(^{-1}\) phosphate concentration samples. Supplementary findings also indicated that quantities of mycosporine like amino acids (MAAs) increased significantly by increasing nitrate and phosphate concentrations. Collectively, it could be concluded that the altered composition of cultivation medium, especially macro elements like nitrate and phosphate, may affect the composition of quasi-mycosporine like amino acids by changing nitrogen assimilation, photosynthetic pigments content, which leads to probably higher photosynthesis and stimulating shikimate pathway.

*Corresponding Author:* Sara Saadatmand ✉️ s_saadatmand@srbiau.ac.ir
Introduction
Spirulina cyanobacteria is an orderly twisted mycosporine string bacteria with no pre-specified, if existed, width partitions (Desikachary, 1995). It is also the richest known green food in the world with no cholesterol and just a little amount of fat and carbohydrate. The existing pigments in *Spirulina* improve the body immune system and incorporate to its health care (Barsanti *et al.*, 2006). The growing importance of *Spirulina* has led to its growing production with commercial purposes in the last 20 years. Although a few countries use some methods to grow it directly in lakes (Belay, 2002), the commercial algae is usually produced in huge pools and under controlled circumstances. Nowadays, it has reached to a mass production of 57000 tons all over the world (FAO, 2006). The alga could be found at different areas with fresh and sea water, and also used as a nourishing food for goldfish and the ones kept as a pet. Although its nourishing level depends on the circumstances in which it grows (Belay, 2002).

Nitrogen is one of the most important and highly effective mediums used to improve *Spirulina* algae growth. Here, the photosynthesis rate strongly depends on the nitrogen rate. As a result, lack of nitrogen decreases photosynthesis and accordingly chlorophyll synthesis. It also is the second prevalent element in *Arthrospira* sp. biomes (Faintuch, 1989). cyanobacteria use mineral nitrogen, and specifically nitrates and ammonium, to improve its growth. Although they could grow up in organic nitrogen too (Faintuch, 1989). Many studies conducted investigating the effect of different nitrogen sources in *S. platensis* cultivation setting, among which, Faintuch (1989) proposed nitrate as a source producing the best results in biology mass production.

Phosphor is another important food resource for alga which determines its primary production (Mastert & Grobbelaar, 1987). Indeed, the phosphor main role is keeping mass production at a high level for microalgae (Vonshark, 1997). Quasi-mycosporine like amino acids are classified as secondary metabolites (Yadav *et al.*, 2011) and play different roles in a cell such as; protecting it against UV rays, antioxidant molecules and osmotic regularizes (Yadav *et al.*, 2011; Rastogi *et al.*, 2010). mycosporine like amino acids, as mentioned before, need nutritious materials to be produced. As a result, a decreasing nitrogen rate and its absence in the environment where alive beings live, leads to a drop in amino acids production. Subsequently, a fall in NO$_3$ concentration decreases the amino acids production as well (Klisch and Donat, 2008). In this study we investigated the effect of the different concentrations of NaNO$_3$ and K$_2$HPO$_4$ on the photosynthetic pigments, fresh and dry weight and the quality of MAAs mass absorption in *Spirulina platensis* alga for estimated MAAs production in the different conditions.

Materials and methods
Preparing the pure samples
*Spirulina platensis* cyanobacteria was prepared from fishery investigation institution, agriculture ministry, Iran.

*Spirulina* cultivation and care
BG11 environment was applied to cultivate *Spirulina platensis*. Cultivation mediums were kept in the cultivation setting at 25˚C to 30˚C, while being radiated by 2500 incubation light for 12 hours a day. For the other half a day, they were kept in darkness. Three different concentrations of nitrate (1.5, 2, 2.5 gl$^{-1}$ NaNo$_3$) and phosphate (0.06, 0.05, 0.04 gl$^{-1}$ K$_2$HPO$_4$) were investigated as six experimental groups. Each treatment was repeated six times with the incubation period of 25 days.

Growth analysis
 Samples were studied for fresh and dry weight. The sum weigh of the fresh alga equals: [wet filter paper weight plus the whole alga-wet filter paper weight] and the sum weigh of the dry alga equals: [dry filter paper weight plus the whole alga-dry filter paper weight].

To measure the dry weight, samples were kept inside the laboratory environment for 24 hours and then
they were weighing.

**Chlorophyll a and Carotenoids analysis**

Chlorophyll a and Carotenoids were measured based on the method proposed by Lichtenthaler and wellburn (1983). To this end, 0.03g alga was homogenized in 10ml of %80 acetone. Then, 20ml of the smooth solution was investigated by visible UV spectrophotometer for its absorption rate at 470 and 663 wavelengths, and the final substance concentration was reported based on (μg/g cyanobacteria). The following equations were used to measure Chlorophyll a and Carotenoids:

\[
\text{Chlorophyll} \ a (\mu g/ml) = 12.21 (A_{665}) - 2.81 (A_{646})
\]

\[
\text{Carotenoids} (\mu g/ml) = (1000A_{470} - 3.27[chl \ a] - 10.4[chl \ b])/297
\]

**Mycosporine like amino acids extraction by spectroscopy**

2ml of mature cyanobacteria cells was collected by a centrifuge at 3000g for 10 minutes. Then, 2ml of 100% pure methanol was added to the solution and incubated at 4°C for 24 hours. The mixture then was centrifuged at 5000g for 15 more minutes and the supernatant was used as the extracted methanol substance. And finally, the methanol substance spectrum was measured at 250-500 nanometers by UV/visible spectrophotometers (Sinha et al., 1995).

**Data analysis**

The collected data was analyzed by one way ANOVA and Duncan testing method using SPSS (ver. 18).

**Results**

According to figure 1, as the concentration of nitrate and phosphate increased in cultivation setting, comparing to the control group samples, the fresh weight of *Spirulina* cyanobacteria decreased significantly. Although the dry weight measurement brought about exact opposite results; due to that, an increase in nitrate and phosphate concentration led to an increase in dry weight too. This was also apparent in nitrate application phase and especially at 2.5ml⁻¹ nitrate concentration, comparing to the groups where phosphate was applied (figure 2). Based on figure 3, mass absorption also improved by increasing nitrate and phosphate concentration as well. Accordingly, as nitrate and phosphate concentration increased, cyanobacteria biomass increased too, but its water absorption potential decreased. cyanobacteria mass weight growth in the environment also led to an increase in chlorophyll a and carotenoids concentration. As there was an increase in nitrate and phosphate concentration, chlorophyll a and carotenoids also increased significantly. For chlorophyll a and KH₂PO₄ sample at 0.06g/L concentration, the growth was faster comparing to the other samples (figures 4&5). Mycosporine like amino acids absorption diagram also proved that, as comparing to the other samples, the 0.06g/L K₂HPO₄ sample increased mycosporine like amino acids concentration.

![Fig. 1. Fresh weight-NaNO₃ and K₂HPO₄ different samples for Spirulina platensis at P≤0.05.](image1.png)

![Fig. 2. Dry weight- NaNO₃ and K₂HPO₄ different samples for Spirulina platensis.](image2.png)

**Discussion**

Nourishment conditions is one of the key elements
affecting growth and production in microalga environments (Vanshak and Richmond, 1988; Faintuch et al., 1991). Variations in nitrogen source and its quantity in cultivation setting restrict the microalga growth and changes the pigments and their chemical compounds (Mostert and Grobbelaar, 1987). Based on the results, as long as NaNO₃ and K₂HPO₄ concentrations increase in cultivation setting, chlorophyll a and carotenoids concentrations increase significantly. Chlorophyll concentration in S. platensis also increases by increasing nitrogen concentration in cultivation setting (Rangel – Yagui et al., 2004; Maddour et al., 2012; Raoof et al., 2006; Rodrigues et al., 2010). Findings are also at the same line with that of the other previously conducted studies working on the same research project. S. platensis chlorophyll magnitudes depend highly on combination of the cultivation setting, and specially type and concentration of nitrogen (Rodrigues et al., 2010; Rangel – Yagui et al., 2004). Increasing phosphate concentration led to a significant growth in chlorophyll a production by S. platensis (Celeklı et al., 2009; Raoof et al., 2006; Lodi et al., 2005). Based on the results brought about in some studies, the produced carotenoid extent in S. platensis wasn’t affected significantly by variant growth conditions (Madkour et al., 2012). Although Madkour and et al. (2012) proved that in a dim light condition together with nitrogen, the carotenoid extent increased, incorporating to prove the present study results regarding carotenoid production growth in the mentioned conditions. Our findings also showed that the dry weight and mass absorption in Spirulina platensis alga developed significantly as a result of an increase in NaNO₃ and K₂HPO₄ concentration. A majority of the dry weight was reached at 2.5 g l⁻¹ concentration of NaNO₃. cyanobacteria biomes production is a highly complicated process with a number of effective elements that for a proper growth, needs some key combinations as nitrogen and phosphor (Mkadkour et al., 2012). The assimilated nitrogen at first is used by micro-organisms as a resource to help the cell growth, and then the rest is being saved in the form of organic materials such as protein (Rodrigues et al., 2010). Results of the present study were in the same line with the research carried out by Mastert and Grobbelaar (1987), proving that the highest Spirulina growth was presented at 2.5 g l⁻¹ concentration of KNO₃, and showing the key role of nitrogen in Spirulina production (Raoof et al., 2006; Mastert and Grobbelaar, 1987).
The optimum nitrate concentration (2.5 gl⁻¹) that increases biomes production by the help of *S. platensis* might be due to the proportion of nitrate to phosphate (Bulgakov and Levich, 1999; Radmann et al., 2007; Madhyastha and Vatsala, 2007). Those cyanobacteria which at the same time are exposed to UV and background active photosynthesis radiation (PAR; 400-700nm) have some different defensive mechanisms helping them to reduce the direct or indirect effects of UV beams. One of these defensive methods is synthesizing the compositions such as mycosporine like amino acids like that absorb UV rays (Sinha et al., 2007). Findings of the present research shed some light on the fact that as nitrate and phosphate concentrations increase, mycosporine like amino acids concentration also increase as well. There are some elements affecting on MMA production. To name some, we can refer to food resources, lack of nitrogen in the environment, as well as downturn of NO₃ concentration, which undermine MMA production. On the other hand, the existence of ammonium in red algae and cyanobacteria environments increases MAA production (Klisch and Donat, 2008). Findings of the present study also proved the same fact once again, mycosporine like amino acids biosynthesis is done through shikimic acid path. It depends on glycolytic and phosphate pentose path to produce the necessary raw materials of this path; accordingly, a growing photosynthesis rate could increase mycosporine like amino acids production too (Ghosh et al., 2012). Chlorophyll a is a touchstone for intensity of microalgae cells photosynthesis; as a result, increasing chlorophyll a could be used as the optimum condition for the intensity of microalgae cells photosynthesis (Chen et al., 2011). Accordingly, increasing chlorophyll a, which brings about a higher photosynthesis rate, increases mycosporine like amino acids production. MAAs, together with playing a protective role against UV harmful rays and neutralization of their antioxidant effect, also control osmotic pressure, because in organisms of the beings living in salt-rich sea water, a high concentration of MAAs was found (Yakovleva et al., 2004). And because NaNO₃ and K₂HPO₄ salt concentrations in the experiment environment increased, the mycosporine like amino acids amino acids grew up too.

**Fig. 5.** Carotenoids- NaNO₃ and K₂HPO₄ different samples for *Spirulina platensis*.

**Fig. 6.** Mycosporine like amino acids- NaNO₃ and K₂HPO₄ different samples for *Spirulina platensis*.

**References**


http://dx.doi.org/10.1111/j.1529-8817.2007.00335.x.


Celekli A, Yavuzatmaca M. 2009. Predictive...
modeling of biomass production by Spirulinaplatensis as function of nitrate and NaCl concentrations. Bioresource Technology 100, 1847–1851.
http://dx.doi.org/10.1016/j.biortech.


http://dx.doi.org/10.1007/BFb0102299.


http://dx.doi.org/10.1016/j.biotechadv.2012.03.001.


http://dx.doi.org/10.1016/j.ejar.2012.09.00.3


http://dx.doi.org/10.1016/0144-4565(87)90061-8.

http://dx.doi.org/10.1016/j.aquaculture.2007.02.00.1


http://dx.doi.org/10.1007/s10295-010-0718-5.

http://dx.doi.org/10.1016/j.biortech.2010.01.054.


