The effects of nitrogen deficiencies on the lipid and protein contents of *Spirulina platensis*

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Nitrogen deficiencies were studied in *Spirulina platensis* (Cyanophyceae) with the aim of determining the effects of the 50 and 100% deficient nitrogen on the lipid and protein contents of the cell under laboratory conditions. *S. platensis* cultures were grown in *Spirulina* medium and kept at the constant room temperature of 26 ± 2°C, illuminated with fluorescent lamps at an irradiance level of 80 µmol m⁻² s⁻¹ with photoperiod 16:8 (L:D) and aerated continuously. In the *Spirulina* biomass harvested at the stationary phase, 67.4, 53.5, 5.6% protein and 5.78, 13.66, 17.05% lipid were recorded for the groups of control, 50% N(-) and 100% N(-), respectively. The highest lipid content and 1.00 gL⁻¹ dry-weight were recorded from the culture to which treated 100% N(-).

**Key words:** *Spirulina platensis*, lipid, nitrogen deficiencies, protein.

**INTRODUCTION**

Microalgae photosynthetic microorganisms, are able to use the solar energy combining water with carbon dioxide to create biomass. Many countries studied about microalgal lipid for the biodiesel sources in recent years. Microalgae can be cultured throughout the year, because it has a simple reproducing system, use the water most effective, do not need rich soil and a source of fat for biofuels, as such interest on it became increased.

*Spirulina platensis* is widely used in many countries as a health food due to its protein content and biochemical substances for immune system. The optimum temperature for *Spirulina* growth lies in the range of 30 to 35°C (Richmond, 1992). It is known that, the environmental conditions, especially culture temperature, greatly influence the composition and physiological state of phytoplankton (Reynolds, 1984) and in particular, change fatty acids (FA) metabolism, vitamins and carotenoids contents in the cells (Cohen, 1999).

Hundreds of microalgal strains capable of producing high content of lipid have been screened and their lipid production metabolism have been characterized and reported (Sheehan et al., 1998). Several studies have shown that, the quantity and quality of lipids within the cell can vary as a result of changes in growth conditions (temperature and light intensity) or nutrient media characteristics (concentrations of nitrogen, phosphates and iron) (Illman et al., 2000; Liu et al., 2008). The aim of this study was to compare lipid production of *S. platensis* at different N concentrations in culture medium.

**MATERIALS AND METHODS**

Microalgae *S. platensis* was used in this study. The starter culture was obtained from Ben Gurion University of the Negev, The Jacob Blaustein Institute for Desert Research, Israel. Microalga *S. platensis* cultures were kept at a constant room temperature of 26 ± 2°C and illuminated with fluorescent lamps (Philips TLM 40W/54RS) at an irradiance level of 80 µmol m⁻² s⁻¹ with photoperiod 16:8 (light:darkness, L:D). The irradiance was measured by a Radiation Sensor LI-COR (LI-250). *S. platensis* were grown in 8-L glass jar in a batch culture system with an initial biomass concentration 0.43 gL⁻¹ and the culture was continuously aerated.

The cultures were grown in *Spirulina* medium. The medium consists of the following composition (gL⁻¹): 18.6 NaHCO₃, 8.06 NaCO₃, 1.00 KH₂PO₄, 5.00 NaNO₃, 2.00 K₂SO₄, 2.00 NaCl, 0.40 MgSO₄.7H₂O, 0.02 CaCl₂, 0.02 FeSO₄.7H₂O, 0.16 EDTANa and micronutrient elements (0.001 ZnSO₄.7H₂O, 0.002 MnSO₄.7H₂O, 0.001 CuSO₄.5H₂O, 0.002 CoCl₂.6H₂O, 0.002).
Table 1. Main parameters of growth, lipid and protein content of *S. platensis* at different NaNO$_3$ concentration in the growth medium.

<table>
<thead>
<tr>
<th>NaNO$_3$ (gL$^{-1}$)</th>
<th>OD</th>
<th>Biomass (gL$^{-1}$)</th>
<th>Lipid (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (Control)</td>
<td>1.02 ± 0.1$^a$</td>
<td>1.12 ± 0.06$^a$</td>
<td>5.78 ± 0.3$^c$</td>
<td>67.4 ± 0.4$^a$</td>
</tr>
<tr>
<td>2.5 (50% N)</td>
<td>0.98 ± 0.1$^a$</td>
<td>1.10 ± 0.06$^{ab}$</td>
<td>13.66 ± 0.2$^b$</td>
<td>53.5 ± 0.3$^b$</td>
</tr>
<tr>
<td>0 100% N)</td>
<td>0.98 ± 0.02$^a$</td>
<td>1.00 ± 0.02$^b$</td>
<td>17.05 ± 0.7$^a$</td>
<td>5.6 ± 0.4$^c$</td>
</tr>
</tbody>
</table>

Different letters between the lines indicate significant difference at 5% by Duncan multiple range test.

RESULTS AND DISCUSSION

In this study, the effects of nitrate concentrations on the lipid and protein contents and biomass productivity of *S. platensis* were investigated. Nitrogen (N) limiting conditions were in fact reported to significantly increase the lipid fraction of many microalgae (Illman et al., 2000). For this purpose, in this study, N concentration was reduced to fifty and hundred percent rate in *Spirulina* medium described in method. The effects of N deficiency on *S. platensis* growth is summarized in Table 1. Optical density was not significantly affected at different concentrations of N (p > 0.05). Figures 1 and 2 show the optical densities and biomass production of *S. platensis*.

A decrease in the concentration of N in the medium resulted in a significant change in cell composition, favoring the accumulation of lipid components and decrease protein content in *S. platensis* during the batch growth. While lipid content increased in the cell, the protein content was significantly affected at different concentrations of N (p > 0.05). Figures 1 and 2 show the optical densities and biomass production of *S. platensis*. The procedure was repeated three times until the entire lipid was extracted. Total protein was determined by Kjeldahl method (AOAC, 1998). SPSS statistical package programme was used to compare means (Version 12.0, SPSS, Chicago, IL) (Zar, 1999).
content decreased with lack of N (Table 1). Numerous studies on chemical and/or metabolism changes in cyanobacteria induced by light, N, or other nutrients have been reported (Tandeau de Marsac and Houmard, 1993). The reduction of the concentration of nitrate in the growth medium increased the lipid fraction in *S. platensis*, the lack of NaN₃ limited the protein biosynthesis (Guillard, 1973). In this study, the N deficiency (50% N (-) and 100% N (-)) in *Spirulina* culture medium increased total lipid ratio (13.66 and 17.05%) and caused a reduction in protein (53.5 and 5.6%).

Tedesco and Duerr (1989) indicated that, lack of N in *S. platensis* culture medium increased total lipid ratio. Olguin et al. (2001) cultured *S. platensis* to determine the lipid amount in two different culture mediums (Zarrouk and complex mediums) and different light intensities (66 and 144 µmol photon m⁻² s⁻¹). The researchers found that, 28.6% lipid ratio in complex medium which contained 10 times less of N and at the low light intensity. Two green algae (*Chlorella vulgaris* and *Scenedesmus obliquus*) and four blue green algae (*Anacystis nidulans*, *Microcystis aeruginosa*, *Oscillatoria rubescens* and *Spirulina platensis*) were grown in 81 batch cultures at different N levels. In all the algae increasing N levels led to an increase in the biomass (from 8 to 450 mgL⁻¹), in protein content (from 8 to 54%) and in chlorophyll. At low N levels, the green algae contained a high percentage of total lipids (45% of the biomass) (Piorreck et al., 1984).

Bulut (2009), studied with *C. vulgaris* in laboratory conditions, five different nutrient media (lack of 50% N, 100% N, 50% N-P, 50% P and nitrite addition as a N source) and investigated lipid and protein content and biomass of *C. vulgaris*. While, the high lipid content of 35.6% was reported with the group to treated deficiency of 100% N and the lowest protein content of 13.01% determined at the same group. The lowest biomass of the 0.12 gL⁻¹ were found in the group which contained nitrite.

Thomas et al. (1984) studied with *P. tricornutum* in N sufficient and deficient mediums. N sufficient cells contained 55% protein, 10% carbohydrate, 20% lipid and 12% ash in dry weight. N deficiency changed these values to 25, 15, 22, 16 and 5-0%, respectively. During extreme deficiency, lipid content was as high as 30% of the dry weight, but lipid yield did not increase because overall cellular yield was decreased. However, in this study, while *S. platensis* biomass of 1.12 gL⁻¹ was obtained from the control culture, in 50 and 100% N deficient cultures, 1.10 and 1.00 gL⁻¹ *Spirulina* biomass was reported, respectively. In the study, it was shown that although lipid increased, *Spirulina* biomass was not a drop more.

Zhila et al. (2005) cultured *Botryococcus braunii* in 75% reduced N medium with 1% CO₂ at 10:14 light-dark period during the 20 days and observed the growth of the algae and lipid composition. When the culture of 75% reduced N was compared with the control group, it was observed that, the biomass decreased from 6.8 to 2.9% and the lipid ratio increased to 21%. In the other study, *Neochloris oleoabundans* was cultured in photobioreactor system in N deficient medium and the biomass and lipid levels were examined. While 16.5 g m⁻²/day biomass and 23% lipid were recorded in the control group, low biomass and 37% of lipid were obtained in the culture to which applied N was deficient (Pruvost et al., 2009).

*S. platensis* is a protein rich and low fat microalgae species. *Spirulina* large-scale culture and harvesting are easy. The species is resistant to contamination, although, the fat content is low, biomass is very well productive. In the study carried out, it was determined that the stress of

Figure 2. Dry weight of *S. platensis* at different nitrogen concentrations.
N deficiency increased the lipid content from 5.78 to 17.05%, however, biomass productivity did not fall significantly. For the feasible biodiesel studies with microalgae, the issue should be determining algae species which are resistant to changing environmental condition with high biomass and high lipid productivity. There are some microalgae species having more lipid but their low biomass productivity will restrict large-scale culture for biodiesel.

REFERENCES


