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Full Length Research Paper

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.

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MATERIALS AND METHODS

Microalga *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 (Phlips 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 $NaHCO_3$, 8.06 Na_2CO_3, 1.00 K_2HPO_4 5.00 $NaNO_3$, 2.00 K_2SO_4, 2.00 NaCl, 0.40 $MgSO4.7H_2O$, 0.02 $CaCl_2.2H_2O$, 0.02 $FeSO_4.7H_2O$, 0.16 $EDTANa_2$ and micronutrient elements (0.001 $ZnSO_4.7H_2O$, 0.002 $MnSO_4.7H_2O$,

Table 1. Main parameters of growth, lipid and protein content of *S. platensis* at different $NaNO_3$ concentration in the growth medium.

$NaNO_3$ (gL^{-1})	OD	Biomass (gL^{-1})	Lipid (%)	Protein (%)
5 (Control)	1.02 ± 0.1^{a}	1.12 ± 0.06^{a}	5.78 ± 0.3^{c}	67.4 ± 0.4^{a}
2.5 (50% N)	0.98 ± 0.1^{a}	1.10 ± 0.06^{ab}	13.66 ± 0.2^{b}	53.5 ± 0.3^{b}
0 100% N)	0.98 ± 0.02^{a}	1.00 ± 0.02^{b}	17.05 ± 0.7^{a}	5.6 ± 0.4^{c}

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

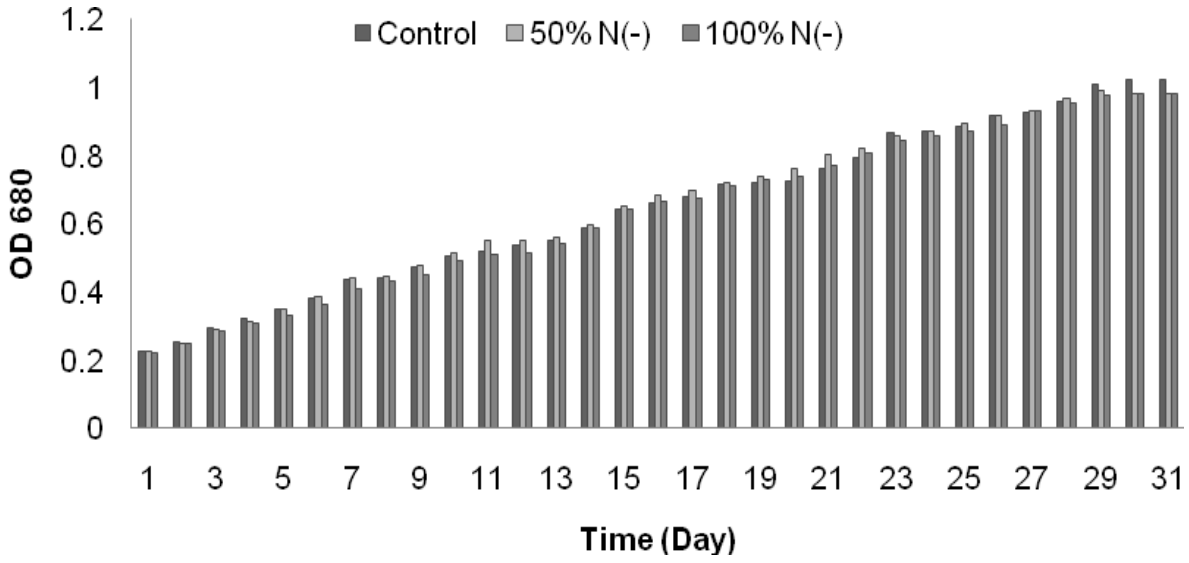

Figure 1. Optical density of *S. platensis* at different nitrogen concentrations.

0.01 H_3BO_3, 0.001 $Na_2MoO_4.2H_2O$, 0.001 $Co(NO_3)_2.6H_2O$, 0.00005 $CuSO_4.5H_2O$, 0.7 $FeSO_4.7H_2O$, 0.8 $EDTANa_2$) were added 10 mL to 1 L.

In the experiment, N was added 50% (2.5 gL^{-1}) and 100% (0 gL^{-1}) missing according to amounts in *Spirulina* medium. In the control culture, original *Spirulina* medium was used. All the applications were repeated triplicate. *S. platensis* filamentous concentration was determined daily by optical density measurements at 680 nm (Costa et al., 2003) by a UV–vis spectrophotometer (Shimadzu, UV mini 1240 model). Dry weight was determined by the filtering of certain volumes of algal culture through Whatman GF/C glass fibre. Algae biomass was dried at 105°C for two hours and weighed (Boussiba et al., 1992). All measurements were carried out in triplicate.

For lipid and protein analyses, samples of microalgae were collected in the stationary phase. *S. platensis* cells were separated from the medium by centrifugation at 7500 rpm for 10 min, using the centrifuge model Hereaus Suprafuge 22. Biomass was dried at 55°C for 2 h, pulverized in a mortar and stored at -20°C for later analysis. Dry extraction procedure according to Zhu et al., (2002) as a modification of the wet extraction method by Bligh and Dyer (1959) was used to extract the lipid in microalgal cells. Dry biomass for lipid extraction in the mix of chloroform:methanol (2:1, v/v) was kept overnight. About 120 ml of solvents were used for each gram of dried sample in each extraction step. The solid phase was separated carefully using filter paper (Advantec filter paper, no. 1, Japan) twice to provide complete separation. The solvent phase was evaporated in a rotary evaporator under vacuum at 60°C. 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).

RESULTS AND DISCUSSION

In this study, the effects of nitrate concentrations on the lipid and protein contents and bimass 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* media 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

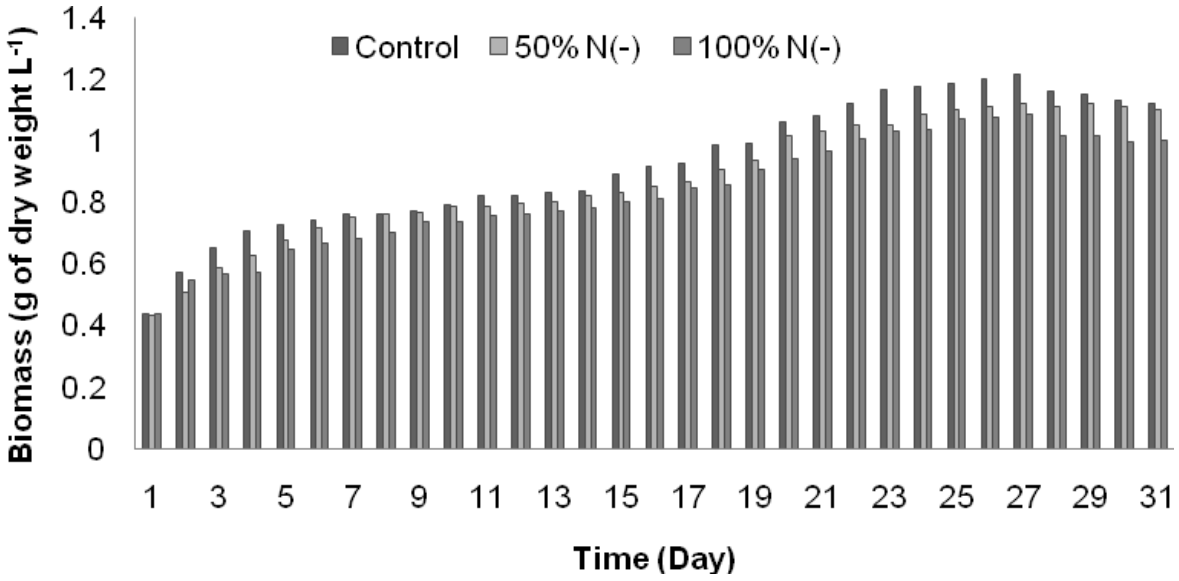

Figure 2. Dry weight of *S. platensis* at different nitrogen concentrations.

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 $NaNO_3$ 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 defficiency 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 oleoabundas* 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 microlga 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

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.

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