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Response of photosynthetic apparatus of *Isochrysis galbana* to different nitrogen concentrations

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A B S T R A C T

The chlorophyll fluorescence parameters of *Isochrysis*. *galbana* at different nitrogen levels were determined by using chlorophyll fluorescence with the aim of better understanding the operative status of photosynthetic system II (PSII) and electron transport chain in oleaginous microalgae. In this experiment, the growth and photosynthetic efficiency were restricted in N-deprived *I. galbana*, which were mainly presented in significant decrease of cell numbers and chlorophyll content, the dramatic decline of Fv/Fm, P.I., ΦPSII and rETR, and commensurate increase of NPQ and W_k. The growth of algal cells gradually increased with rising nitrogen concentration, especially reached the top in N-1.76 mM L_1 medium, but were inhibited in N-3.52 mM L_1 medium. The results suggested that N-deprivation and N-deficiency could inhibit the growth and photosynthesis of *I. galbana*, but excessive nitrogen could also restrict the growth of algal cells. The nitrogen concentration plays an important role in the algal growth and photosynthesis.

1. Introduction

The oleaginous microalgae are considered to be a potential renewable resource to produce biodiesel (Chiaramonti et al., 2017). *Isochrysis galbana* is a marine unicellular phytoflagellate of the order *Chrysomonadales*, which is an important alternative for solving the energy problem, because it is rich in total lipid and saturated fatty acids (Silitonga et al., 2017). The research purpose was to define optimal growth conditions of *I. galbana*. The effects of different nitrogen concentrations on the growth of the alga were studied.

Nutrient salt plays an important role in the growth and lipid accumulation of algal cells during microalgal culture. Nitrogen is one of the most important nutrients to maintain the microalgal growth, and the microalgal photosynthesis is closely related to the absorption and assimilation of nitrogen (Peccia et al., 2013; Converti et al., 2009). Hence, optimization of nitrogen concentration in the culture medium is very crucial to improve the oil yield of microalgae.

Lower or excessive nitrogen concentration can affect the growth of algal cells (Ördög et al., 2016). Young (Young and Beardall, 2003) found that, the photosynthetic capacity of *Dunaliella salina* was decreased in N-deprived medium, which was especially presented in the marked decline of Fv/Fm. Furthermore, the chlorophyll content and photosynthetic capacity of algal cells can also decrease in N-restricted condition. When the nitrogen source is insufficient, the proteins synthesis of algal cells, especially including the photosynthetic enzymes and pigment molecules, can obviously decrease, which can result in the slower growth, lower chlorophyll content and photosynthetic efficiency of algal cells (Fan et al., 2014). Lippemeier (Lippemeier et al., 2003) found that the specific growth rate of *Heterosigma akashiwo* was proportional to the nitrogen concentration in a certain range, but the growth rate of algal cells was slowed down when nitrogen concentration exceeded this range. Some studies have also shown that excessive nitrogen concentration isn't conducive to the absorption and utilization of nutritive salts in microalgae, thus inhibiting the growth of algal cells (Ramsundar et al., 2017).

The light energy normally absorbed by plants is mostly used in photochemical reactions, and the parts that cannot be used will dissipate in the form of heat or fluorescence (Goltsev et al., 2016). The photosynthesis of plants can be directly reflected by the fluorescence dynamical characteristics of chlorophyll, and the chlorophyll fluorescence parameters can be used to analyze the mechanism of environmental factors on photosynthesis of plants more quickly and non-destructively (Kalaji et al., 2016). At present, the chlorophyll fluorescence analysis has been widely used in some studies about the stress in higher plants (Lippemeier et al., 2003), but is very few in algae. Studies have shown that the environmental stresses were significantly associated with the suppression of fluorescence parameters in plants (Kalaji et al., 2014). Hence, according to the changes of chlorophyll fluorescence

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Table 1
The growth and chlorophyll fluorescence parameters of *Isochrysis. galbana* at different nitrogen levels in sixth day.

$NaNO_3$(mM)	Cell density ($\times 10^6$/ml)	Chl(mg/l)	Fv/Fm	P. I.	RC/CS_o
0	5.58 ± 0.07	0.68 ± 0.12	0.34 ± 0.01	0.01 ± 0.001	24.93 ± 0.67
0.44	13.55 ± 1.70	1.68 ± 0.13	0.55 ± 0.02	0.13 ± 0.02	68.19 ± 2.78
0.88	14.03 ± 0.88	2.34 ± 0.39	0.54 ± 0.04	0.14 ± 0.06	102.7 ± 15.16
1.76	15.25 ± 1.13	3.16 ± 0.11	0.55 ± 0.01	0.13 ± 0.01	139.2 ± 7.06
3.52	12.83 ± 0.32	2.10 ± 0.12	0.52 ± 0.02	0.11 ± 0.02	105.7 ± 13.8

$NaNO_3$(mM)	ΦPSII	rETR	NPQ	Wk
0	0.21 ± 0.01	9.00 ± 0.22	1.48 ± 0.22	0.82 ± 0.02
0.44	0.31 ± 0.03	12.98 ± 1.11	0.87 ± 0.23	0.73 ± 0.01
0.88	0.29 ± 0.05	12.30 ± 2.17	0.79 ± 0.33	0.73 ± 0.01
1.76	0.34 ± 0.01	13.63 ± 0.67	0.49 ± 0.06	0.67 ± 0.01
3.52	0.25 ± 0.01	10.39 ± 0.28	0.88 ± 0.22	0.73 ± 0.01

parameters, the available information about the photosynthesis apparatus of microalgae under different stresses can be obtained.

In this research, the cell density, chlorophyll content and chlorophyll fluorescence parameters of *I. galbana* were measured, and the effect of nitrogen concentration on the growth, PSII primary photochemical reaction and the photosynthetic electron transport of *I. galbana* were analyzed, which can not only reveal the variation of the growth and photosynthetic reaction of algal cells at different nitrogen concentration, but also provides a strong basis for the lipid synthesis of microalgae.

2. Materials and methods

2.1. Algal strain and growth conditions

Isochrysis galbana IOAC724S was obtained from the algal collection, Institute of Oceanology, Chinese Academy of Sciences, which was considered as a potential oilproducing microalgae (Liu et al., 2014). The *I. galbana* was cultivated by the two-stage culture method. The algal cells were first cultured in L_1 liquid medium (Guillard and Hargraves, 1993) at 26 °C (100 μmol photons $m^{-2} s^{-1}$, 14:10 h light/dark photoperiod), and manually shaken 6–8 times daily. When the cells density reached to 8×10^6 cells ml^{-1}, they were harvested by centrifugation (25 °C, 4000 rpm, 5 min). The cell pellets were efficiently washed three times with N-free L_1 medium. Then, the cell pellets were equally divided into five groups and inoculated separately in L_1 medium of different nitrogen concentrations (0 mM, 0.44 mM, 0.88 mM, 1.76 mM and 3.52 mM $NaNO_3$). The common nitrogen concentration in L_1 medium is 0.88 mM (control group). The algal cultures were sampled and analyzed every 2 days.

2.2. Cell density and chlorophyll content

The cell density was determined by using the hemocytometer under the light microscope. The algal chlorophyll was extracted by the methanol and measured by the photospectrometer. The chlorophyll concentration was calculated by the equation: Chlorophyll (mg/l) = $444 \times A_{666nm} + 19.71 \times A_{653nm}$ (Guillard and Hargraves, 1993; Song et al., 2013).

2.3. Chlorophyll fluorescence induction dynamics

The chlorophyll fluorescence transients of *I. galbana* in L_1 medium of different nitrogen concentrations were measured with a Handy PEA fluorometer (Hansatech Instruments, Norfolk, UK) according to Strasser (Strasser, 1995). All measurements were performed after 15 min dark adaptation at room temperature (Song et al., 2013).

The determination of the rapid light curves was set with fourteen light intensity gradients. After the correction, the following order was 10, 20, 30, 50, 100, 150, 200, 300, 500, 700, 1000, 1300, 1600 and 2000 μmol $m^{-2} s^{-1}$. The measurement of chlorophyll fluorescence was automatically performed according to the setup procedure (Zhang et al., 2011; Schreiber et al., 1997). In any photosynthetic active radiation (PAR), the fluorescence is Fs before opening the saturated pulse, and the maximum fluorescence is Fm′ when the saturated pulse is opened, so the variable fluorescence is ΔF = Fm′-Fs. The effective quantum yield of photosynthetic system II (PSII) in the state of light adaptation is elicited, that is ΦPSII = ΔF/Fm = (Fm′-Fs)/Fm′. According to the ΦPSII and PAR, the relative electron transfer rate (rETR) can be calculated, that is rETR = ΦPSII × PAR × 0.5 × 0.84 (Garrote-Moreno et al., 2015; Krall and Edwards, 1992).

The absorbance coefficient of higher plants is 0.84. The absorption coefficient of microalgae can't be determined, so the absorption coefficient of higher plants is used to calculate the relative electron transfer rate.

2.4. Analysis

Based on the obtained data, the mean and standard deviation per group were calculated, and one-way analysis of variance was conducted by using the SPSS 19.0 software. $P < 0.05$ indicates that the two groups are different, $P < 0.01$ indicates the two groups have significant difference.

3. Results

3.1. The effect of different nitrogen concentrations on the cell density and chlorophyll content of I. galbana

Cell density of *I. galbana* increased gradually in L_1 medium of different nitrogen concentrations during the 6-days culture period. With the increase of $NaNO_3$ concentration from 0 mM to 3.52 mM, the cell numbers were separately 5.58×10^6 cells/ml, 13.55×10^6 cells/ml, 14.03×10^6 cells/ml (control group), 15.25×10^6 cells/ml, 12.83×10^6 cells/ml (Table 1). In the eighth day, the cell numbers of N-deprived *I. galbana* decreased to 4.85×10^6 cells/ml, which was far lower than the control group (Fig. 1A, $P < 0.01$). The cell numbers of *I. galbana* was stationary at about 13.3×10^6 cells/ml in N-1.44 mM L_1 medium, which was obviously lower than the control group (Fig. 1A, $P < 0.05$). In N-1.76 mM L_1 medium, the cell numbers increased to 21.05×10^6 cells/ml that is much higher than control group (Fig. 1A, $P < 0.01$); But when the $NaNO_3$ concentration reached to 3.52 mM, the cell numbers fell to 19.32×10^6 cells/ml, which was no obvious difference with the control group (Fig. 1A). So the algal cells had the highest growth rate in N-1.76 mM L_1 medium.

When the $NaNO_3$ concentration were 0 mM and 0.44 mM,