International Core Journal of Engineering Volume 8 Issue 6, 2022
ISSN: 2414-1895 DOI: 10.6919/ICJE.202206_8(6).0047

Effects of Nitrogen Enrichment on Growth, Photosynthesis Pigments and Chlorophyll Fluorescence Parameters of Ulva Lactuca

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Abstract

Taking Ulva lactuca as the research object, the relative growth rate, photosynthetic pigment content, and chlorophyll fluorescence parameters of the photosynthetic organism with nitrogen enrichment were measured, to determine the function of nitrogen enrichment on the photosynthetic mechanism. The results showed that the fraction of OEC, ETo/RC, Fv/Fo, φPo, PIabs, φEo, ψo were significantly higher than those of control group ($p<0.05$). And Wk, Mo, DIo/RC and φDo were lower than those of control group ($p<0.05$), respectively. Nitrogen supplementation promoted the activities of donor side, recipient side and reaction center of Ulva lactuca on photosynthetic system II (PSII). In conclusion, nitrogen enrichment at 200 μmol/L can effectively improve the growth rate and photosynthetic performance of Ulva lactuca, thus promoting its photochemical activity.

Keywords

Ulva Lactuca; Nitrogen Enrichment; Growth; Photochemical Activity.

1. Introduction

Macroalgae is the main species of mariculture in China, showing a great demand for nitrogen and other nutrients in the growth process, while releasing oxygen. In addition, the photosynthetic capacity, biological yield and economic value of macroalgae are relatively high, and the large-scale cultivation technology is quite mature at the present stage. Ulva lactuca (Ulva lactuca L.), commonly known as sea cabbage, sea green vegetables, sea lettuce, or green vegetables, is a kind of large marine economic algae belonging to the Chlorophyta phylum, Ulvophyceae, Ulvales, Ulvaceae, Ulva. This seaweed is about 10-30 cm wide and up to 40 cm long. It is widely distributed and has the characteristics of high unit output and nutrition absorptivity [1, 2]. Nowadays, it has been used in the study of sewage purification [3]. Ulva l. also maintains rapid growth when environmental conditions are suitable and is often an important part of intertidal productivity [4]. Therefore, studying the ecological conditions of Ulva l. is of great significance to effectively utilize and control Ulva l..

Nitrogen nutrition is the basis for the growth and reproduction of marine plant, and play an important role in regulating phytoplankton population dynamics and community structure [5, 6]. Recent studies

ISSN: 2414-1895 DOI: 10.6919/ICJE.202206_8(6).0047

have shown that nitrogen enrichment can improve the growth rate and photosynthetic pigment content of algae, promote the photosynthetic performance [7, 8]. Nitrogen in the sea water is one of the important nutrient factors that affects the macroalgae productivity [9, 10]. Algae growing in most seas use nitrate as the main nitrogen source, macroalga can absorb the nitrogen nutrient in environment quickly. Algae can remove excess nutrients in water and reduce water pollution [3, 11, 12]. The efficiency and stability of nutrient uptake in this process depend on the biomass, productivity and competitiveness of the macroalgae [13]. In addition, it can also realize resource utilization of aquaculture pollutants. At the same time, the combination of macroalgae and marine environment can better deal with the environmental balance.

At present, there have been some studies on the growth, enrichment and photosynthesis of macroalgae caused by environmental factors such as temperature, light and salinity. However, we found that these studies on photosynthetic characteristics only analyzed the effects of nutrients on apparent photosynthesis of macroalgae. The specific effects of nitrogen nutrients on PSII of macroalgae were not analyzed from the energy level and PSII structure level. In order to improve the high-value utilization of Ulva l. and other macroalgae, and explore the feedback and influence relationship between nitrogen addition and photosynthetic characteristics of Ulva l., the condition factors of nitrogen addition were set up, along with the chlorophyll fluorescence parameters of Ulva l. PSII deep-going analysis, in order to effectively utilize and control Ulva l..

2. Materials and Methods

2.1 Experimental Materials

Ulva l. was collected from Nanao Island, Shantou city, Guangdong Province. The algae with good and similar growth condition were selected and brought back to the laboratory at 4℃. Sterilized seawater filtered by 0.22μm membranes was used to clean the surface sediment, miscellaneous algae and other objects several times. The cleaned algae were placed in seawater culture medium (adding 100 μmol/L NaNO3 and 20 μmol/L NaH2PO4) for 3 days to adapt to the environment (12h light/dark period, temperature 20℃, light intensity 100 μmol·photons m-2s-1), and filled with air pump for 24h.

2.2 Experimental Methods

Ulva l. with the same shape, size and healthy status were selected again after the temporary cultivation as experimental materials. Ulva l. was divided into control group (group CK, nitrogen concentration 10 μmol/L) and nitrogen-enrichment treatment group (group N+, nitrogen concentration 200 μmol/L) according to nitrogen nutrient. The nitrogen concentration of 200 μmol/L was obtained by adding NaNO3 and NH4Cl (NO3- : NH4+ = 4:1) to natural seawater. There were 3 replicates in each group, and other conditions were the same. Ulva l. was cultured in 20 ℃ light incubator for 9 days. The cultured water was changed every 3 days and various indexes were measured.

2.2.1 Relative Growth Rates

The fresh weight of Ulva l. was measured regularly during cultivation. The relative growth rate (RGR) was calculated. Calculate the relative growth rate of Ulva l. according to the following formula:

$$RGR(\%\ d\text{-}1\ ) = 100\% \times (\ln Mt - \ln M0)/t \quad (1)$$

M0 and Mt are the fresh weight (g) of Ulva l. on day 0 and t, respectively. t is the test time (d).

2.2.2 Photosynthetic Pigment Contents

The contents of chlorophyll a (Chl a) and carotenoid (Car) of Ulva l. were measured according to Wellburn et al.[14].

The calculation formula is as follows:

$$\text{Chl a (mg /g)} = [16.29 \times (A665 - A750) - 8.54 \times (A652 - A750)] \times V/1000 \times Fw \quad (2)$$

ISSN: 2414-1895 DOI: 10.6919/ICJE.202206_8(6).0047

$$Car\ (mg/g) = [7.6 \times [(A480 - A750) - 1.49 \times (A510 - A750)]] \times V/1000 \times Fw \quad (3)$$

In the formula, A480, A510, A652, A665 and A750 represent the absorbance values with wavelengths of 480, 510, 652, 665 and 750 nm respectively, FW represents the fresh weight of algae, and V represents the volume of methanol.

2.2.3 Chlorophyll Fluorescence Parameters

Transient changes in chlorophyll a fluorescence were measured using plant efficiency analyzer (PEA, Hansha, UK). The light intensity of the instrument can reach 3000 μmol m-2s-1, focusing on the area to be measured (diameter of about 4 mm). Before the experiment, the samples need to be dark for about 20 min, so that the samples get full dark adaptation. The picking point rate of the instrument was recorded once every 10 μs before 2 ms and once every 1 ms after 2 ms, respectively, to collect and record the fluorescence signal of the first 3 s, thus reflecting the instantaneous change of chlorophyll a fluorescence. By analyzing the chlorophyll fluorescence parameters (Table.1), we can know the reaction mechanism of photosynthetic apparatus to nitrogen enrichment environment.

2.2.4 Statistics and Analysis

Excel 2010 and Origin 2022 were used to process and plot the experimental data. One-way ANOVA or T-test was used for statistical analysis, $p < 0.05$ was set as significant level.

3. Results and Discussion

3.1 Growth

The growth status of macroalgae is expressed by relative growth rate. With the change of time, the growth rate of Ulva l. under indoor natural conditions and the growth rate of Ulva l. in group N+ increased firstly and then decreased (Fig. 1). Compared with the group CK, the growth rate of Ulva l. in nitrogen enrichment condition was significantly higher than that in relative stress stage ($p < 0.05$).

3.2 Photosynthetic Pigment Contents

The physiological and biochemical regulation ability of macroalgae and its ability to cope with environmental changes can be reflected by the changes in the pigment content of algae. The changes of Chl a and Car contents in group N+ were significantly increased after day 3 ($p < 0.05$) (Fig. 2). Compared with the group CK, the pigment content of Ulva l. increased under the condition of nitrogen-enrichment addition. Meanwhile, nitrogen nutrient had obvious influence on the pigment content of Ulva l..

3.3 Chlorophyll Fluorescence Parameters

3.3.1 PSII Acceptor Side

As shown in Fig. 3, at each specific time point, the fraction of OEC in group N+ was significantly increased compared with group CK ($p < 0.05$). It showed an increasing trend with the extension of time, while Wk was decreased compared with the control group, respectively ($p < 0.05$).

3.3.2 PSII Reaction Center

Among the four types of energy, except for the energy captured for electron transport per unit reaction center (ETO/RC), the energy in group N+ decreased compared with the group CK. The light energy absorbed by antenna pigment per unit reaction center (ABS/RC), and the energy used for heat dissipation per unit reaction center (DIO/RC) in group N+ were significantly lower than those in group CK ($p < 0.05$) (Fig. 4). The enrichment of nitrogen nutrients also affected the absorption of light energy by Ulva l. But at the same time, the energy consumed by the group CK was much higher than that of group N+. Moreover, the energy used for electron transfer in group CK was significantly lower than that in group N+ ($p < 0.05$). It can be seen that in the case of enrichment of nitrogen nutrients, the consumption of light energy absorbed by Ulva l. in the form of heat energy is effectively reduced.

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In addition, compared with group CK, φPo, Fv/Fo and PIabs in group N+ were significantly increased ($p < 0.05$). The φDo of the treatment group was negatively correlated with the cultivation time, which was lower than that of the control group, respectively ($p < 0.05$). It also reflects that the addition of nitrogen nutrients in the environment can reduce the heat dissipation energy of Ulva l., increase the utilization rate of light energy absorbed by Ulva l., and promote the energy utilization rate.

3.3.3 PSII Donor Side

ψo and φEo in group N+ increased firstly and then decreased, and were significantly increased compared with control group ($p < 0.05$) (Fig. 5). In the mean time, Mo was significantly lower than that of group CK ($p < 0.05$), and gradually decreased with the extension of time.

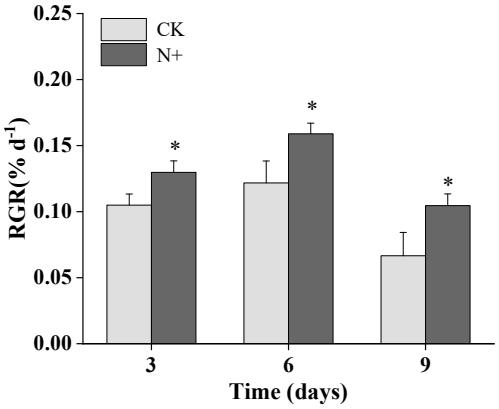

Fig. 1 Nitrogen enrichment effects on the relative growth rate of Ulva l.

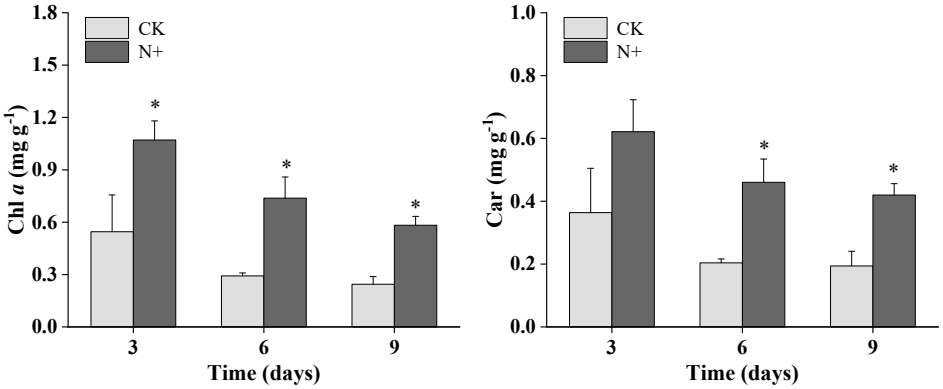

Fig. 2 Nitrogen enrichment effect on chlorophyll a and carotenoid contents of Ulva l.

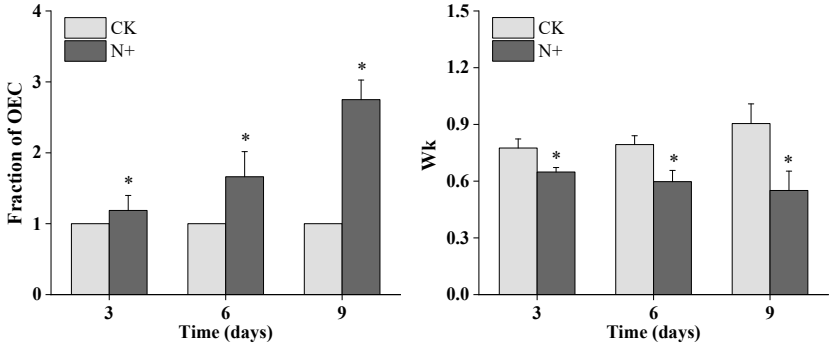

Fig. 3 Nitrogen enrichment effect on PSII acceptor side of Ulva l.

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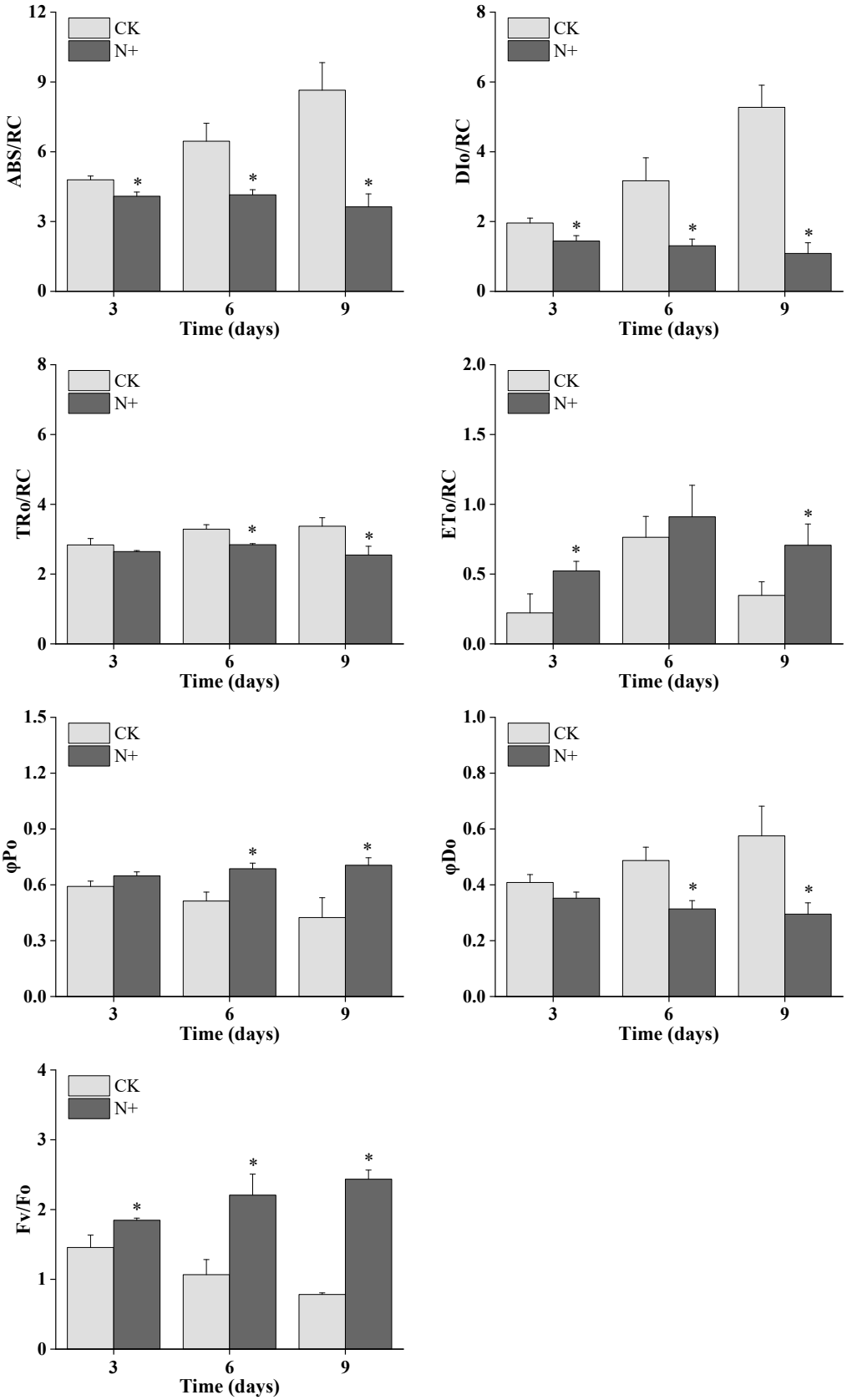

Fig. 4 Nitrogen enrichment effect on PSII reaction center of Ulva l.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202206_8(6).0047

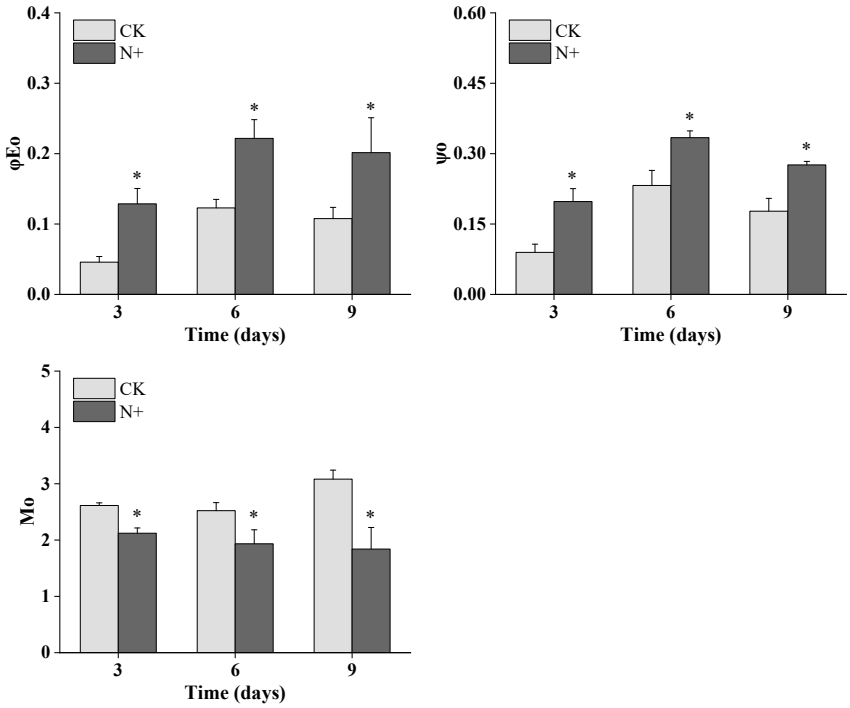

Fig. 5 Nitrogen enrichment effect on PSII donor side of Ulva l.

Table 1. Relevant ChlorophyII fluorescence parameters

Position	ChlorophyII fluorescence parameters	Meaning	Formula
PSII acceptor side	W_k	The extent to which the oxygen release complex (OEC) is destroyed	$W_k=(F_k-F_0)/(F_J-F_0)$
	Fraction of OEC	The ratio of oxygen complexes	[1 - (V_k / V_j)] treated / [1 - (V_k / V_j)] control
PSII reaction center	ABS / RC	Unit reaction center antenna pigment absorption of light energy	(M_O / V_j) / [1 - (F_O / F_m)]
	DIo / RC	Unit reaction center for heat dissipation of energy	ABS / RC - TRo / RC
	TRo / RC	Energy captured per unit reaction center to reduce Q_A	M_O / V_J
	ETo / RC	Energy captured per unit of reaction center for electron transport	(M_O / V_j) / [1 - V_j]
	φPo	Maximum photochemical efficiency of the initial photochemical reaction	1-F_O/F_M=TR_O/ABS=F_v/F_m
	φDo	Quantum yield used for heat dissipation	1 - Po = Fo / F_m
	F_V / F_O	The potential activity of PSII	
PSII donor side	φEo	Quantum production used for electron transport	(1-F_O/F_M)•ψ_O =ET_O/ABS
	ψo	The ratio of acceptor energy to total energy used to push electron-transport excitons downstream of Q_A in the electron transport chain	ETo / TRo = 1 - V_j
	Mo	The maximum rate at which Q_A is restored	
Comprehensive performance	PIabs	The performance index based on absorbed light energy reflects the comprehensive performance of PSII	RC/ABS • [φPo/(1-φPo)] • [ψ_O/(1 - ψ_O)]

ISSN: 2414-1895 DOI: 10.6919/ICJE.202206_8(6).0047

4. Discussion

In recent years, more and more studies have been conducted on the effects of eutrophication on plants and their nutrient absorption characteristics. Nitrogen is responsible for providing nutrition foundation and reflect the effect of external environment signal in the plant growth process [15]. Previous studies have shown that appropriate increase the concentration of nitrate nitrogen in water could significantly promote the growth of Ulva prolifera, Gracilaria lemaneiformis and other macroalgae [16-19]. These studies show a moderate amount of nitrogen enrichment can promote the relative growth rate of seaweed. As the enrichment of nitrogen increases the concentration of nitrogen metabolism substrates of seaweed plants, nitrogen assimilation may be strengthened in a certain range, thus promoting their rapid growth [20]. In this study, adding proper amount of nitrogen nutrient into the culture medium can effectively promote the growth rate of Ulva l., which is consistent with the results of these studies.

The content of photosynthetic pigments in macroalgae is an important indicator to reflect the photosynthetic efficiency of algae. Photosynthetic pigments and proteins form a light-trapping pigment protein complex, which is responsible for capturing light energy [21]. Therefore, photosynthesis will be affected by the change of photosynthetic pigment content. Chl a is the reaction center pigment of most algae, reflecting the photosynthetic efficiency of algae. Car is a non-enzymatic antioxidant that plays an important role in plant responses to antioxidant stress [22, 23]. In this study, the contents of Chl a and Car in Ulva l. were significantly increased when nitrogen nutrient was added, which may be as the increase of nitrogen concentration increased the contents of pigment [15, 24, 25]. The results suggest that the increase of photosynthetic pigment content may be due to the increased turnover of key proteins and protein-pigment complexes in photosynthetic apparatus because of nitrogen enrichment [26]. The increase of photosynthetic pigment indicated that N nutrient supplementation had significant effect on Chl a and Car contents of Ulva l.

ChlorophyII fluorescence parameter is an important indicator reflecting the photosynthetic efficiency of plants. When the algae are irradiated by strong light, the light-trapping pigment in the PSII reaction center captures the light energy, and the absorbed light energy is used to promote electron transfer for photosynthetic reaction, dissipate in the form of fluorescence, or in the form of heat energy [27]. For the donor side of PSII, the higher the Wk value increased, the more serious the damage of the oxygen release complex was [28]. The Fraction of OEC increased and Wk decreased after the treatment of Ulva l. with nitrogen nutrient addition. It can be seen that the donor side of PSII is protected and the oxygen release complex is in good condition under the action of appropriate nitrogen addition. Nitrogen enrichment at this concentration did not harm the oxygen-releasing complex.

The variation of quantum yield reflected the change of energy distribution ratio of the reaction center under nitrogen addition of Ulva l.. Reef[29] found that nitrogen enrichment would increase the electron transfer rate of macroalgae. Similarly, Zhang[30] discovered that high nitrogen availability increased the linear electron transfer of photosynthesis to carboxylation and oxidation, and affected the photosynthetic system to allocate more photosynthetic electrons to photocontract. In this experiment, ABS/RC, DIo/RC, φDo decreased, while ETo/RC, FV/FO increased significantly, indicating that the addition of nitrogen nutrient at this concentration did not promote the ability of Ulva l. to absorb light energy. But it effectively increased the energy for electron transfer in the reaction center, and reduced the energy of heat dissipation. It also reflects that the nitrogen nutrient under this setting does not damage the reaction center of Ulva l. and can promote the utilization rate of light energy. [31] reported high nitrogen could increase the proportion of open of PSII reaction center and the electron transfer rate in wheat leaves, more energy into photochemical reaction process, causing the photosynthesis promotion. This is reflected in the increased activity of the PSII reaction center in this study. It's worth noting that the significant increase of φPo means that the PSII primary light energy capture ability and electron transport ability are in the best state [32], thus promoting the growth of algae. PIabs is a parameter index with the most sensitive change [33], which is mainly a

ISSN: 2414-1895 DOI: 10.6919/ICJE.202206_8(6).0047

performance index based on the absorption of light energy. PIabs values in the nitrogen-enriched group are significantly higher than those in the control group, which is consistent with the results obtained in other parameters. These parameters indicate that nitrogen nutrient supplementation can maintain the structure and function of PSII reaction center and promote the growth of Ulva l. In addition, Mo, φEo, ψo and other parameters mainly reflect the changes of PSII receptor side, and the decrease of Mo value indicates that the electron transfer between QA and QB is not hindered by nitrogen enrichment, but is promoted [34]. At the same time, the increase of ψo and φEo indicates that the energy used for electron transport in the captured light energy increases, and the ability of electron transport to the downstream becomes stronger. The changes of these parameters indicated that nitrogen had a positive effect on the PSII receptor side of the reaction center, which is a correlation between photosynthesis and growth of Ulva l. [35, 36], which was also confirmed in this experiment. There is a positive correlation between chlorophyll fluorescence parameters and relative growth rate of Ulva l..

Nitrogen is a component of plant cells, and nitrogen enrichment is closely related to the growth and metabolism of algae and photosynthesis [37]. Studies have shown that when nitrogen are insufficient in the environment, photosynthetic pigments of plants will be limited to a certain extent, which will affect light absorption and electron transfer, increase the light energy dissipated in the form of heat, and reduce the photosynthetic rate [38]. Higher nitrogen content in plants that contributes to more nitrogen invested in photosynthetic mechanisms can lead to higher photosynthesis [39]. Nitrogen enrichment increased the values of various fluorescence parameters. Basically, appropriate nitrogen enrichment increased the content and activity of nitrogen compounds such as Rubisco, a key rate-limiting enzyme of photosynthesis, and the substrate concentration of photosynthetic physiological process correspondingly[15, 40], thus stimulating the photosynthetic physiological process, and then improving the photosynthetic capacity of algae. It is also possible that nitrogen promotes photosynthesis by activating oxidative phosphorylation in cells, a biochemical process that fuels carbon sequestration [41, 42]. Based on chlorophyll fluorescence parameters, nitrogen nutrient supplementation can effectively increase the photosynthetic properties of Ulva l. and promote its photochemical activity.

5. Conclusion

The addition of appropriate does of nitrogen is beneficial to the growth of Ulva l. and increase the content of photosynthetic pigments. Meanwhile, it can also improve the activity of PSII photoreaction center and the proportion of oxygen release complex, increase the yield of PSII electron transport and promote the electron transport process, thus promoting the photosynthesis of Ulva l.

Ackowledgments

This research was funded by Special Fund for Marine Economic Development in Guangdong Province (grant number 2020035), and Rural Science and Technology to the Countryside in 2019 (grant number 32419078), the Science and Technology Planning Project of Guangzhou (grant number 201804010494; GZKTP202032).

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