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Effects of reduced carbon supply and sunlight on photosynthetic and antioxidant activities of *Gracilariopsis lemaneiformis*, and subsequent changes of these activities under recovery conditions with different salinities

Heng Jiang[a,b], Dinghui Zou[a,c,*], Binbin Chen[a]

[a] School of Environment and Energy, South China University of Technology, Guangzhou 510006, China
[b] Dongting Lake Research Center, Hunan Hydro & Power Design Institute, Changsha 410007, China
[c] The Key Lab of Pollution Control and Ecosystem Restoration in Industry Clusters, Ministry of Education, China

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ABSTRACT

Gracilariopsis lemaneiformis usually suffers reduced carbon supply and irradiance due to increasing density. Moreover, salinity also frequent changes at cultivation field. *G. lemaneiformis* thalli were cultured under ambient carbon (390 μatm) and decreased carbon (20 μatm) supply, with ambient sunlight and decreased sunlight conditions, and subsequent cultivation of the above four thalli under ambient carbon and sunlight with ambient (32) and low (21) salinity. The study aimed to investigate how the decreased carbon supply and sunlight conditions affected the photosynthesis and antioxidant activity, and the effects of salinity on their changes of these algae under the recovery conditions. Decreased carbon in culture reduced the maximal quantum yield of photosystem II (F_v/F_m) and non-photochemical quenching (NPQ). The F_v/F_m, NPQ, superoxide dismutase (SOD) and catalase (CAT) activity in the thalli grown at ambient sunlight condition were greater than the thalli grown at decreased sunlight. However, reduced carbon and lowered light had no synergistic inhibitory effect on photosynthetic and antioxidant activities of the algae. After the thalli were continuous cultured, the photosynthetic activity and antioxidant enzyme activity were similar between the two salinities. The results indicated that the effects of decreased carbon and sunlight on the photosynthetic and antioxidant activities of *G. lemaneiformis* were reversible and their recovery was also achieved under low salinity. Therefore, we suggest that it was a possible new model for *G. lemaneiformis* maricultivation that some algal fragments were leaved in polyethylene rope as "seeds" for the next batch of *G. lemaneiformis* during the algal harvest.

1. Introduction

Generally, severe low dissolved inorganic carbon (Ci) concentrations in seawater would be of frequent occurrence, especially under the conditions of slow water exchanges, high standing stock and large seaweeds density (Friedlander and Levy, 1995; Israel and Friedlander, 1998; Richards et al., 2011; Zou, 2014). As carbon decreasing, photosynthetic activity of algae was reduced, which was thought to stress condition for some seaweeds (Mercado et al., 1999; Zou, 2014; Jiang et al., 2016, 2017). Seaweeds thalli also usually experience low irradiance as light was sharply attenuated by seawater and the sinking of thalli to deeper water, and self-shading due to high stocking density. Moreover, tidal change also imposes an excess of light energy on seaweeds. As algae cells contain a considerable amount of water, this will be an important target resulting in the production of reactive oxygen species (ROS), such as superoxide, peroxides, hydroxyl radicals and singlet state oxygen. During periods of elevated physiological stress, ROS formation can escalate rapidly. ROS scavenging enzymes and substrates (i.e. antioxidants) in addition to tocopherols and polyamines are present within algal cells as protective agents against the damages commonly associated with ROS (Sampath-Wiley et al., 2008).

Gracilariopsis lemaneiformis is source of a number of natural products with bioactivities, such as soluble sulfated polysaccharides and phycoerythrin (Wang et al., 2016). It is used for agar extraction due to its good-quality gel (Yang et al., 2015; Zou et al., 2004; González-Leija et al., 2009), and can potentially be converted into energy (Meinita et al., 2013). Moreover, the cultivation of the species can be an effective bioremediation measure for eutrophication control in coastal waters (Fei, 2004). *G. lemaneiformis* fragments transplanted on offshore rafts and fixed in a layer of a polyethylene rope in January. The algal weight of per cluster was 3–5 g. The biomass of *G. lemaneiformis* can reach up to ca. 5 kg m^{-2}, and all algal thalli in polyethylene rope were taken away,

* Corresponding author at: School of Environment and Energy, South China University of Technology, Guangzhou 510006, China.
E-mail address: dhzou@scut.edu.cn (D. Zou).

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at harvest time. Then the farmers proceed to the next batch of cultivation. The number of harvests could be up to 3 times at most during algal maricultivation. *G. lemaneiformis* is cultivated in the bay, usually experiences decreased Ci as slow water exchanges and increased density. The seaweed specie also experiences different irradiance as self-shading and/or tide. Our previous study found that reduced carbon supply decreased the photosynthetic pigment contents, nitrogen uptake rate and photosynthetic activity of *G. lemaneiformis*, and the chlorophyll *a* and carotenoids contents of the lowered sunlight grown-algae were decreased (Jiang et al., 2017). Considering that *G. lemaneiformis* experiences freed low carbon and low light conditions resulted from anthropogenic or natural origins in fields during mariculture and *G. lemaneiformis* usually suffers low salinity because of freshwater inputs from rivers and rainfall, we cultured *G. lemaneiformis* outdoor with decreased carbon supply and lowered sunlight conditions, and subsequent continuous cultured under ambient carbon supply and sunlight with low salinity in the present study. We specially focus on: 1) biochemical components, 2) photosynthetic characteristic, and 3) antioxidant enzyme activity. This study aimed to examine how the decreased carbon supply and sunlight conditions affected the photosynthesis and antioxidant activity of the maricultured seaweed, and the effects of salinity on the physiological indicators changes of these algae under the recovery conditions.

2. Materials and methods

2.1. Algae material

Thalli of *Gracilariopsis lemaneiformis* were collected on 17 December 2016 from a cultivation field at the Shen'ao bay, Nan'ao Island, Shantou, China (23°20'N, 116°40'E). Thalli were artificially cultivated by means of the pole-system. The thalli were gently rinsed to remove sediments and epiphytes. Only unwounded and healthy thalli were selected. Samples were transported to the laboratory (Guangzhou, China) in a plastic bucket with some seawater (temperature ca. 4 °C), and were maintained in plexiglass aquaria with synthetic seawater (distilled water containing NaCl, KCl, $CaCl_2$, $MgSO_4$, salinity ca. 32) at 20 ± 0.5 °C. The seawater was supplemented 20 μM $H_2PO_4^-$ and 200 μM NO_3^- to avoid the possible nutrients limitation. The seaweeds received an irradiance of about 100 μmol photons $m^{-2} s^{-1}$ (PAR, LD cycle 12 h: 12 h). The seawater was continuously aerated and renewed every day. The thalli were used for experimental treatments after two days of above laboratory maintenance (Zou, 2014). The irradiance was quantified by means of a quantum sensor (QSL2100, San Diego, CA USA).

2.2. Culture treatments

The culture media were synthetic seawater (salinity ca. 32). The thalli were cultured at outdoor with two levels of inorganic carbon (Ci) availability and two levels of irradiance. For the treatment of decreased carbon supply (DC), the culture media was aerated continuously with ambient air through 5 M NaOH solution (the CO_2 concentration in the air was ca 20 μatm; Jiang et al., 2017). As for the treatment of ambient carbon supply (AC), the culture media were aerated continuously with ambient air (the CO_2 concentration in the air was ca. 390 μatm). During early culture period, the mean values of pH were 8.11 and 8.94 in the AC and DC supply seawater, respectively. The estimated mean concentrations of dissolved CO_2 and HCO_3^- were 0.7 and 712.8 μM in the DC supply seawater, 13.6 and 2078.3 μM in the AC supply seawater, respectively (Table 1). The treatment of decreased sunlight (DL) here means that the culture vessel were wrapped with sunshade net, and the treatment of ambient sunlight (AL) here means that the culture, nothing wrapped culture vessel. The irradiance intensity of DL treatment was about 20% of ambient sunlight in the culture vessel within daytime. For all of the treatments, the culture seawater was supplemented 20 μM $H_2PO_4^-$ and 200 μM NO_3^- to avoid the possible nutrients limitation. Three replicate cultures were maintained at each growth treatment.

Experimental treatments started when 20 g fresh weight (FW) algae were introduced into each of aquariums containing 10 L of synthetic seawater and cultured under outdoor conditions. The thalli were kept in suspension by continuous sparging and aeration at 0.4 L min^{-1}, which was continuously controlled using the flowmeter (Huanming LZB-3; Yuyao KingTai Instrument Co., Ltd., Yuyao, China). The seawater was renewed by every two days. The fresh medium had been pre-aerated with above air containing the appropriate [CO_2]. The algal thalli were grown under these different carbon and irradiance regimes for 18 d, and then were harvested to be used for experimental measurements.

After the four early cultivation thalli had been measured, the each algal thalli were randomly divided into two parts (ca. 10 g FW/part), and then replaced to 5 L aquariums, with two different salinity levels (21 and 32). The all aquariums were aerated continuously with ambient air, and without wrapped sunshade net. The culture mediums were adjusted into salinity 21 and salinity 32. Other conditions were the same as the conditions used for above culture. The photosynthetic and antioxidant enzyme activities of the samples were examined after 4–6 d of the recover-cultured.

2.3. Biochemical components

To determine pigments contents, about 0.1 g FW per sample were extracted in 100% methanol with mortar and pestle. The crude extracts were extracted at 4 °C in darkness for 24 h. The extract was centrifuged at 5000 × *g* for 10 min, and then the supernatant was used to determine the contents of chlorophyll *a* (*Chl a*) and carotenoid (*Car*) with an ultraviolet spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan). The concentrations of *Chl a* and *Car* were calculated spectrophotometrically using the equation by Porra (2005) and Parsons and Strickland (1963).

2.4. Chlorophyll fluorescence

Measurements of chlorophyll fluorescence were made using a portable pulse modulation fluorometer (Junior-PAM, Walz, Germany). The maximal quantum yield of photosystem II (PS II) of *G. lemaneiformis* thalli (dark-adapted for 15 min) was determined as F_v/F_m, where F_v indicated variable fluorescence ($F_v = F_m - F_o$). A saturating pulse white light (approx. 3000 μmol photons $m^{-2} s^{-1}$) was applied to gain the maximal fluorescence (F_m, indicating the fluorescence yield when all the PS II reaction centers are reduced), and the initial fluorescence (F_o, indicating fluorescence intensity with all PS II reaction centers open while the photosynthetic membrane is in the non-energized state) was obtained at a pulsed irradiance of approximately 0.1 μmol photons $m^{-2} s^{-1}$. Non-photochemical quenching (NPQ) = $F_m/F_m' - 1$, F_m' indicating the fluorescence intensity with all PS II reaction centers closed in any light adapted state (Kooten and Snel, 1990). The rapid light curves (RLCs) consisted of the fluorescence response to 8 different and increasing levels of actinic irradiance over the range of 0–820 μmol photons $m^{-2} s^{-1}$. The exposure time at each actinic irradiance was 10 s, each separated by a 0.8 s saturating flash (approx. 10,000 μmol photons $m^{-2} s^{-1}$). The parameters of the RLCs were calculated following the model equation from Jassby and Platt (1976). The parameters for the maximum relative electron transport rate ($rETR_m$) and efficiency of the electron transport (α) were obtained by referring to the study published by Ralph and Gademann (2005).

2.5. Antioxidant enzyme activities

Superoxide dismutase (SOD) activity was investigated by measuring the ability to inhibit reduction of nitro blue tetrazolium (NBT), following the means of Beauchamp and Fridovich (1971). One unit of enzyme activity was defined as the amount of enzyme required to inhibit the photoreduction of NBT by 50%, and the unit is U mg $prot^{-1}$