Exogenous carbon acquisition of photosynthesis in Porphyra haitanensis (Bangiales, Rhodophyta) under emersed state [*]

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Abstract   The photosynthetic performances of *Porphyra haitanensis* thalli were investigated in order to understand its mechanisms for exogenous carbon acquisition during emersion at low tide. The emersed photosynthesis was studied by altering the pH value in the water film on the thalli surface, treating them with carbonic anhydarase inhibitors (acetazolamide and 6-ethoxyzolamide), adjusting the CO_2 concentrations in the air, and comparing the theoretical maximum CO_2 supply rates within the adherent water film with the observed photosynthetic CO_2 uptake rates. It was found that the principal exogenous inorganic carbon source for the photosynthesis of *P. haitanensis* during emersion was atmospheric CO_2. The driving force of CO_2 flux across the water film was the CO_2 concentration gradient within it. Carbonic anhydrase accelerated both extracellular and intracellular CO_2 transport. The emersed photosynthesis of *P. haitanensis* was limited by the present atmospheric CO_2 level, and would be enhanced by atmospheric CO_2 rise that would trigger global warming.

Keywords: Porphyra haitanensis, **carbon dioxide, photosynthesis, emersion, carbonic anhydrase.**

Marine macroalgae inhabiting the intertidal zone experience a periodic alternation between immersion and emersion with the change in the ebb and flow of the tides. When the tide is high, intertidal macroalgae are submersed and photosynthesize in seawater as most other marine algae. While it is known that the enzyme, Rubisco, which catalyses the key reaction of carbon assimilation, uses inorganic carbon as carbon dioxide, most of the inorganic carbon dissolved in the sea is in the form of the bicarbonate ion due to the high pH value and high salinity in seawater[1]. However, evidence has shown that the intertidal macroalgae can transport bicarbonate ions into their cells to drive photosynthetic carbon fixation[2,3]. The primary pathway of bicarbonate use in marine macroalgae is the extracellular dehydration of HCO_3^- by carbonic anhydrase (CA) to form CO_2, which then is taken up into the algal cells[2]. The direct uptake of HCO_3^- has also been demonstrated in green macroalgae *Ulva lactuca*[4] and *Enteromorpha intestinalis*[5], in which HCO_3^- uptake could be facilitated by a mechanism with similar properties to the anion-exchange protein of red blood cells (AE1).

The intertidal macroalgae have to be exposed to air when the tide goes out. The photosynthetic adaptations to emersion-related stress and the comparative photosynthetic rates between in air and in water have been previously well examined[6,7]. Some of the upper intertidal species even get enhanced photosynthetic rates in air compared to in water[8]. It appears that the carbon fixation during emersion may represent a significant contribution to the total carbon fixation of the macroalgae. Nevertheless, the external inorganic carbon resource for photosynthesis and its acquisition mechanism by intertidal macroalgae are poorly understood under emersed state compared to submersed state[9]. In terrestrial higher plants, CO_2 flows from the atmosphere to intracellular air spaces through the stomatal pore, and diffuses across the wall, plasmalemma, cytosol and the chloroplast envelope, till to the stroma. Stomatal opening controls the partial pressure of CO_2 in the intracellular air spaces[10]. However, macroalgae possess no anatomical features such as stomata or waxy cuticules. Therefore, the acquisition mechanism of external carbon for photosynthesis in emersed macroalgae must differ from that in land plants, albeit both of them photosynthesize in air. There always exists a surface seawater film surrounding the algal thalli when they get exposed to air due to the viscosity and hydrophilia of the thalli surface. Additionally, this water film may be maintained

by high humidity, sea spray, shading rocks and extensive shingle-overlapping and piling-up. Is it possible that the intertidal macroalgae utilize the dissolved inorganic carbon in the water film surrounding the thalli under emersed state in a similar way to that under submersed state? If so, then to what an extent does the external carbon resource for photosynthesis by emersed macroalgae depend on the water film surrounding the algal thalli?

Porphyra haitanensis (Bangiales, Rhodophyta) is commonly found in the upper intertidal zone along the coast of Zhejiang, Fujian, and Guangdong in China. It is an economically important seaweed used for food, and is the principal species cultivated on a commercial scale in southern China. Photosynthetic inorganic carbon utilization is one of the fundamental biological problems concerning the aquaculture of *P. haitanensis*. The mechanism of photosynthetic bicarbonate utilization in this species when submersed in seawater had previously been investigated[11]. *P. haitanensis* is usually cultivated by the pole-system, experiencing periodic emersion when the tide goes out. The aim of this study is to describe the exogenous inorganic carbon resource for photosynthesis and its acquisition mechanism in this alga when exposed to air at low tide.

1   Materials and methods

1.1   Plant materials

Thalli of *Porphyra haitanensis* T. J. Chang et B. F. Zheng (about 3 4 weeks after seeding) were collected in November to December 2000, from Nanao Island, Shantou, China, where it was cultivated by the pole-system in a bay with a shallow sandy bottom. The unwounded and healthy thalli were selected and sealed in plastic bags with some seawater, and were transported to the laboratory in a cooler (4) within 4 hours, and were maintained in filtered seawater in glass aquaria at room temperature (18 22) and about $100 \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (light-dark cycle 12 h 12 h). The seawater was aerated and renewed by half of the amount every day. Samples were used for experiments within 4 days of maintenance, a period during which stable photosynthetic activity was recognized. After this period, the remains were discarded and fresh algal materials were re-collected.

1.2   Preparation of the seawater with different pH and with presence of carbonic anhydrase (CA) inhibitors

The filtered (GF/C filter, Whatman) sterile natural seawater was used to prepare pH-buffered seawater. A known amount of biological buffers (from Sigma) was added to give a concentration of 25 mmol/L and the pH was adjusted to the desired value with freshly prepared 0.1 mol/L NaOH and 0.1 mol/L HCl. The pH was measured with a pH meter (420A, Orion) fitted with a glass/calomel combination electrode. Different pH values were obtained with different buffers. The pH of 6.0, 8.2 (a pH representative of that in natural seawater) and 10.0 were achieved with 2-(N-morpholino) ethane-sulfonic acid (MES), 2-amino-2 (hydroxymethyl)-1, 3-Propanediol (TRIS) and 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), respectively. The carbonic anhydrase (CA) inhibitors, acetazolamide (AZ) and 6-ethoxyzolamide (EZ) (both from Sigma) were also used. It is generally believed that AZ cannot penetrate the algal cells and thus only acts on extracellular CA[12]. The EZ can penetrate into the cells and therefore inhibits both extracellular and intracellular CA[13]. Both AZ and EZ were dissolved in 40 mmol/L NaOH at a concentration of 50 mmol/L for preparing stock solutions. The final concentration of AZ or EZ adding into pH-buffered seawater was $100 \mu \text{mol}/L$.

1.3   Measurement of photosynthetic carbon dioxide uptake and the effects of pH and CA inhibitors

Photosynthetic rates of CO_2 uptake by thalli of emersed *P. haitanensis* were measured by infrared gas analysis using a leaf chamber analyzer (LCA-4, Analytical Development Company, UK) in an open gas line system. The measurements were performed at $600 \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (PAR, an irradiance which could fully saturate the photosynthesis of *P. haitanensis*) and temperature of 20 . Light was provided with a halogen lamp (150 W, Hikari-J) from above. Temperature was controlled by maintaining the leaf chamber in a temperature-controlled cabinet. The rate of CO_2 uptake (P_n) [μmol CO_2 g (d. wt)$^{-1}$ h^{-1}] was calculated as follows:

$$P_n = C \times F \times 60 \times 273/[(273 + T) \times 22.4 \times DW],$$

where C is the difference in CO_2 concentration (μL/L) between the inlet and outlet air; F, the gas flow rate (L/min); T, temperature ( ); DW, dry

weight （g , 80   , 24 h）.

In order to examine the effects of the pH value of the surface water film of thalli and CA inhibitors , the algal examples were rinsed in different pH-buffered seawater with or without CA inhibitors for 30 min prior to introduction into the chamber for determining CO_2 uptake. The seawater with different pH values was in equilibrium with atmosphere. Thus , in our CO_2 uptake determining system , the CO_2 changes between the inlet and outlet air resulted from photosynthetic CO_2 uptake by the algal samples.

1. 4   The effects of atmospheric CO_2 concentration on CO_2 uptake

The algal samples with different pH in the surface water film were examined at varied atmospheric CO_2 supply levels (in a range of 60   1440μL/L) by altering the CO_2 concentration of the inlet air within the analyzer. CO_2 in the ambient air was removed to different extents by pumping it through a soda lime column through operating the analyzer to obtain lower concentrations of CO_2 （60 , 120 , 180μL/L）. The concentration of CO_2 higher than ambient atmospheric CO_2 level （720 , 1080 , and 1440μL/L）were obtained by injecting pure CO_2 before pumping outdoor air into air bags （$0.5\,m^3$）. The air bags were used for all measurements to store the air with higher concentration of CO_2 and to maintain constant supply of each CO_2 concentration.

1. 5   Calculation of the theoretical maximum rate of CO_2 production within the surface water film of thalli

The theoretical maximum rate of CO_2 production derived from spontaneous （uncatalysed）dehydration of HCO_3^- in the surface water film of algal thalli for a given salinity , temperature and pH was calculated according to Matsuda et al. [14] The assumption was made that the alga consumed CO_2 at a rate causing the CO_2 concentration to approach zero. This gave a theoretical maximal rate of conversion of CO_2 from HCO_3^- within the water film. The flux of CO_2 across the surface water film was not taken into account. The unit of calculated values was turned into the same one as the measured rates of CO_2 uptake by algal samples. The rate of spontaneous conversion of CO_2 from HCO_3^- （d[CO_2]/dt）can be described by the following equations :

$$d[CO_2]/dt = K_1 \times [DIC]/A$$

$$+ (K_3 \times [DIC] \times [H^+])/K_{H_2CO_3}/A ,$$

and $\qquad A = 1 + [H^+]/K_1 + K_2/[H^+]$

where [DIC] is the concentration of dissolved inorganic carbon in seawater. K_1 and K_3 are the rate constants of reactions HCO_3^- $CO_2 + OH^-$ and H_2CO_3 $CO_2 + H_2O$, respectively. $K_{H_2CO_3}$ and K_2 respectively are the dissociation constants of the reactions $H^+ + HCO_3^-$ H_2CO_3 and $H^+ + CO_3^{2-}$ HCO_3^- . The values of K_1 , K_3 , and $K_{H_2CO_3}$ and K_2 are according to Johnson[15] and Stumm and Morgan[16].

The volume of the surface water film surrounding the thalli was roughly estimated using difference between the weight of the thalli before and after blotting off surface water. The value obtained from this procedure was 1.1 ± 0.3 mL per gram fresh weight of alga sample. The dissolved inorganic carbon concentration of the water film used for the calculation was set to be the same as the natural seawater （2.2 mmol/L）, but the concentration of dissolved inorganic carbon at pH 6.0 was adopted according to Beer and Koch[17].

1. 6   Statistics

The data were expressed by the mean values ± standard deviation （ n 3）. Statistical significance of means was tested with t-test or ANOVA at $p <$ 0.05.

2   Results

2. 1   The effects of pH and CA inhibitors on CO_2 uptake

The pH values of the surface water film had significant and positive effects on the photosynthetic CO_2 uptake of the thalli of *P. haitanensis* under emersed state （Fig. 1）. CO_2 uptake rates at pH 8.2 and 10.0 were greater by 1.0 time and 1.4 times compared to that at pH 6.0 , respectively. The extracellular CA inhibitor , AZ , showed an insignificant inhibitory effect on the CO_2 uptake at pH 6.0. However AZ dramatically depressed the CO_2 uptake at pH 8.2 and pH 10.0 compared to control. The total CA inhibitor , EZ , considerably depressed the CO_2 uptake at all pH values assayed. It was shown that EZ produced a greater inhibition than AZ.

The theoretical maximum value of CO_2 production derived from the uncatalyzed dehydration of

HCO_3^- in the water film was calculated at different pH values, to test whether or not the inorganic carbon contained in the water film was enough to account for the measured CO_2 fluxes. The theoretical maximum rates of CO_2 production were 12. 55, 15. 79, and 0. 63 μmol/g/h (the unit was turned into the same one as CO_2 uptake by algal sample) at pH 6. 0, 8. 2 and 10. 0, respectively. Table 1 compared the theoretical values and measured values. It is shown that the measured CO_2 flux exceeds substantially that supported by the theoretical calculated values at all treatments. The ratios of measured to the theoretical rates increase by two orders of magnitude with increasing pH from 6. 0 or 8. 2 to 10. 0. Additionally, the ratios in the presence of CA inhibitors, AZ or EZ, are lower than the control, especially in the case of EZ.
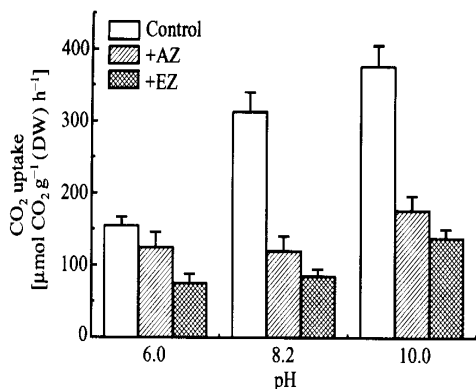
case is the rate saturated by the present day air concentration of CO_2 (360μL/L), suggesting the CO_2-limitation of CO_2 uptake by emersed *P. haitanensis*. At pH 6. 0, the rate of CO_2 uptake essentially increases linearly with increasing atmospheric CO_2 concentrations in the range tested (60　1440μL/L). At pH 8. 2 and 10. 0, there is a linear relationship between CO_2 uptake rates and the atmospheric CO_2 concentrations below the present CO_2 level, and thereafter, CO_2 uptake rate increased in a way of asymptote and tends to be saturated at 4-time of present CO_2 level (1440μL/L). CO_2 uptake rates at present atmospheric CO_2 level are 40. 3 %, 66. 6 % and 73. 9 % of that at 4-time of present day CO_2 level at pH 6. 0, 8. 2 and 10. 0, respectively, implying that the degree of limitation of CO_2 uptake of emersed *P. haitanensis* is more severe at lower pH.
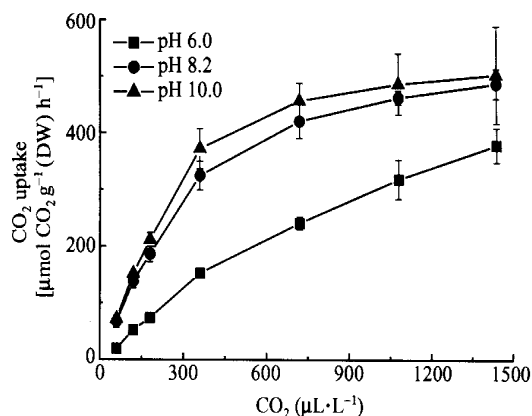

Fig. 1.　The effects of pH and CA inhibitors on photosynthetic CO_2 uptake rate of emersed *Porphyra haitanensis*.

Table 1.　The ratios of observed to theoretical calculated rates of photosynthesis of emersed *Porphyra haitanensis* at different pH and with the presence of CA inhibitors

pH	Control	+ AZ	+ EZ
6. 0	12. 3 ± 1. 0	10. 0 ± 1. 7	6. 1 ± 1. 0
8. 2	19. 8 ± 1. 8	7. 6 ± 1. 2	5. 4 ± 0. 6
10. 0	598. 1 ± 48. 1	281. 1 ± 31. 8	221. 4 ± 18. 1

2. 2　The effects of atmospheric CO_2 concentration on CO_2 uptake

Fig. 2 shows that the atmospheric CO_2 concentration affects significantly photosynthetic CO_2 uptake rate of emersed *P. haitanensis* at different pH. In no

Fig. 2.　The responses of photosynthetic CO_2 uptake rates of emersed *Porphyra haitanensis* to atmospheric CO_2 concentration at different pH values.

Table 2 shows the ratio of measured to calculated CO_2 uptake rate at varied atmospheric CO_2 concentrations. The values of all these ratios are higher than 1, suggesting that the CO_2 supply from the dissolved inorganic carbon within the surface water film of the thalli is not high enough to account for the rates of CO_2 uptake by emersed *P. haitanensis*. The change patterns of the values of ratios with external atmospheric CO_2 supply level are similar to those of CO_2 uptake by *P. haitanensis* shown in Fig. 2.

Table 2.　The ratios of observed to theoretical calculated rates of photosynthesis of emersed *Porphyra haitanensis* at different CO_2 concentrations and pH values

pH	CO_2 concentration (μL/L)						
	60	120	180	360	720	1080	1440
6. 0	1. 5 ± 0. 6	4. 2 ± 0. 2	5. 9 ± 0. 4	12. 1 ± 0. 7	19. 2 ± 0. 9	25. 4 ± 2. 8	30. 2 ± 2. 4
8. 2	4. 3 ± 0. 7	8. 8 ± 0. 8	11. 8 ± 0. 9	20. 6 ± 1. 6	26. 7 ± 1. 9	29. 3 ± 0. 8	30. 9 ± 1. 7
10. 0	114. 1 ± 7. 4	241. 6 ± 4. 7	336. 6 ± 19. 7	592. 6 ± 56. 6	727. 3 ± 50. 1	775. 0 ± 86. 7	801. 5 ± 136. 8

3 Discussion

Our previous work reported that when submersed in seawater during high tide, *Porphyra haitanensis* utilized HCO_3^- as its principal bulk external inorganic carbon source to drive photosynthetic CO_2 fixation and O_2 evolution[11]. As the concentrations of HCO_3^- in seawater were drastically reduced with the increasing pH value from 8.2 to 10.0, photosynthetic O_2 evolution at pH 10.0 was substantially lower than that at pH 8.2[11]. When the tide went out, *P. haitanensis* would be exposed to air with a surface water film surrounding the thalli. The surface water film, which had roughly $30\mu m$ thickness, possessed the same concentration of dissolved inorganic carbon and pH value as bulk seawater. The change pattern of photosynthetic performances with pH value for the thalli of *P. haitanensis* under emersed state would have been similar to that under submersed state, if the emersed *P. haitanensis* mainly used HCO_3^- of the surface water film as its external carbon resource for photosynthesis. However, this study showed the reverse results of the responses of photosynthetic performances to pH values. The photosynthetic CO_2 uptake by thalli of emersed *P. haitanensis* was not reduced at high pH. In fact, the CO_2 uptake rate by emersed alga at pH 10.0 was even a little higher than that at pH 8.2, and was significantly greater than that at pH 6.0. The reasonable explanation could only be the difference of mechanism of inorganic carbon utilization between emersed and submersed *P. haitanensis*. In contrast to the submersed *P. haitanensis*, emersed *P. haitanensis* principally utilized atmospheric CO_2 as its external carbon resource for photosynthesis, as proposed by Mercado and Niell[9]. Other results presented in this study, i.e. the theoretical maximum rates of CO_2 production derived from the spontaneous dehydration of HCO_3^- within the surface water film were much lower than the photosynthetic rates of CO_2 uptake by emersed *P. haitanensis* with or without CA inhibitors, suggested that the dissolved inorganic carbon within the surface water film could far from support the observed CO_2 uptake by emersed *P. haitanensis*, and the main external inorganic carbon for emersed photosynthesis could only come from atmosphere. These results also suggested that the measured rates of CO_2 uptake by emersed *P. haitanensis* reflect the real rates of photosynthesis, because the contributions of dissolved inorganic carbon of surface water film to photosynthesis were rather low and could be ignored.

During photosynthetic metabolism, atmospheric CO_2 must cross through the surface water film of the thalli of *P. haitanensis* before entering into the algal cells. Thus, the surface water film acts as an air-water interface. The driving force for the CO_2 flux across the water film is therefore the concentration gradient of CO_2 between atmosphere and the water film[18]. Provided that the thalli had no photosynthetic (and respiratory) metabolism, there would be no concentration gradient and thus no net CO_2 flux between air and the water film. Thus, the CO_2 flux from atmosphere to the water film resulted from the CO_2 uptake by photosynthesis from the water film. In the previous work, we showed that the photosynthesis of *P. haitanensis* was ultimately dependent on diffusive entry of CO_2, which derived from the dehydration of the bulk HCO_3^- in seawater catalyzed by extracellular CA[11]. A model describing the underlying mechanism for external inorganic carbon acquisition by *P. haitanensis* under emersed state during low tide was proposed. On the outside of the water film towards atmosphere, the gaseous CO_2 was dissolved into the water film and conversed into HCO_3^- (hydration reaction): $CO_{2(gaseous)} \quad CO_{2(aqueous)} + H_2O \quad H_2CO_3 \quad H^+ + HCO_3^-$. HCO_3^- diffusively transferred to the side of the water film towards the algal cells where the dehydration reaction occurred: $HCO_3^- + H^+ \quad CO_2 + H_2O$. The CO_2 production derived from dehydration then passed through plasma membrane and entered into the cells interior via active uptake or passive diffusion. High pH in the water film could enhance the dissolution of gaseous CO_2 and the transformation of CO_2 into HCO_3^-, and thereby accelerate the CO_2 flux across the air-water interface[18]. This is the cause why the pH value had a positive effect on the rate of CO_2 uptake by emersed *P. haitanensis*. At the same time, CA rapidly catalyzed the interversion of CO_2 and HCO_3^- [1,13]. *P. haitanensis* possessed high extracellular and intracellular CA activities[11], and their role in carbon assimilation by emersed *P. haitanensis* could be clearly recognized from the depression of CO_2 uptake by CA inhibitors, AZ and EZ. It could be postulated that the enhancement of CO_2 flux by the extraceullar CA was due to a rapid hydration of air-CO_2 to HCO_3^- in the outside of the water film and the reverse conversion (dehydration) of HCO_3^- to CO_2 inside of the water

film catalyzed by the enzyme. In case of pH 6.0, as the equilibrium reactions of inorganic carbon forms were much shifted towards the CO_2, which thus reduced the role of extracellular CA, consequently, AZ had hardly any effect on the CO_2 uptake. On the other hand, the intracellular CA facilitated the transport of inorganic carbon within cells from cytoplasm to chloroplast by accelerating the interconversion of CO_2 and HCO_3^- [13,19]. Therefore, EZ, which could penetrate into the cells and inhibit both extracellular and intracellular CA, had more pronounced inhibitory effects on CO_2 uptake than AZ.

The observed rates of CO_2 uptake by emersed *P. haitanensis* reflected the driving force for CO_2 transport across the surface water film surrounding the thalli, which derived from the concentration difference of CO_2 between atmosphere and the water film. Theoretically, the capacity of carbon assimilation by the algal cells determined the capability to deplete CO_2 in the water film. Thus, the observed rates of CO_2 uptake by emersed *P. haitanensis* were determined by both the atmospheric CO_2 concentration and the capacity of carbon assimilation by the algal cells. The present study showed that the observed CO_2 uptake increased with increasing atmospheric CO_2 concentration within the tested range (60 1440μL/L) under all pH values (pH 6.0, 8.2 and 10.0), albeit the increasing rates were reduced at pH 8.2 and 10.0 when the atmospheric CO_2 was higher than 360μL/L. This suggested that the major limiting factor for the actual rates of CO_2 uptake by emersed *P. haitanensis* was the CO_2 concentration of atmosphere within the tested range. Additionally, the pH value had negative effects on the CO_2 uptake by emersed *P. haitanensis* when measured under an identical atmospheric CO_2 concentration. However, pH value did not affect the calculated maximum rates of CO_2 uptake by emersed *P. haitanensis* using Michaelis-Menten analysis. The maximal rates of CO_2 uptake were in fact the capacity of carbon assimilation (carbon-saturated Rubisco carboxylation) of the algal cells.

Whether or not the photosynthesis of marine macroalgae is saturated by the current ambient CO_2 level is of general concern in a view of ecological impacts of atmospheric CO_2 rise mainly due to anthropogenic effects (burning of fossil fuels, deforestation). Intertidal macroalgae will be exposed to air when the tide goes out, and the CO_2 in atmosphere is

the predominant exogenous inorganic carbon source for their photosynthesis. Thus, the intertidal macroalgae might be more sensitive to the future increase in atmospheric CO_2 [2,20,21]. This study showed that the doubled atmospheric CO_2 concentration, which had been predicted to occur in the next few decades, would enhance the photosynthesis by 30% in *P. haitanensis* when emersed at low tides, and thus increase its carbon gain. In contrast, when submersed in seawater at high tides, *P. haitanensis* mainly used HCO_3^- to drive photosynthesis, the inorganic carbon composition in seawater could well saturate the photosynthesis[11]. Additionally, the dissolved inorganic carbon concentration would increase by 6% (from 2.2 to 2.34 mmol/L) when the atmospheric CO_2 doubles[16]. Consequently, increasing atmospheric CO_2 would hardly affect the photosynthetic rate of *P. haitanensis* when submersed during high tides. On the other hand, this study indicated that the dissolved inorganic carbon of the surface water film surrounding the thalli could not support the observed photosynthetic rates of emersed *P. haitanensis* at all pH value and atmospheric CO_2 tested. Moreover, the ratios of the measured to the theoretical calculated rates of CO_2 uptake increased with increasing atmospheric CO_2 concentration or pH value. This suggested that the contributions (if any) of dissolved inorganic carbon within the surface water film of thalli to photosynthetic carbon assimilation were rather low and were much reduced under higher atmospheric CO_2 concentrations or higher pH values. In case of aquaculture, *P. haitanensis* usually were planted at a large density. The thalli cover each other when the tide goes out, which would increase the diffusion barriers of atmospheric CO_2 (corresponding to the reduced atmospheric CO_2). Under this condition the dissolved inorganic carbon of surface water film surrounding the thalli could account for part of the photosynthetic carbon assimilation for emersed *P. haitanensis*. For example, the dissolved inorganic carbon of the water film might account for roughly 20% of the total carbon assimilation of emersed *P. haitanensis* when the atmospheric CO_2 concentration was 60 μL/L and the pH was 8.2. Nevertheless, the absolute value of photosynthetic carbon assimilation was rather low. Consequently, increasing atmospheric CO_2 would enhance the photosynthetic productivity of *P. haitanensis* by stimulating the photosynthesis during the period of emersion at low tides, but not the photosynthesis when submersed at high tides.

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