

Comparative Mechanisms of Photosynthetic Carbon Acquisition in *Hizikia fusiforme* Under Submersed and Emersed Conditions

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Abstract: The economic seaweed *Hizikia fusiforme* (Harv.) Okamura (Sargassaceae, Phaeophyta) usually experiences periodical exposures to air at low tide. Photosynthetic carbon acquisition mechanisms were comparatively studied under submersed and emerged conditions in order to establish a general understanding of its photosynthetic characteristics associated with tidal cycles. When submersed in seawater, *H. fusiforme* was capable of acquiring HCO_3^- as a source of inorganic carbon (Ci) to drive photosynthesis, while emerged and exposed to air, it used atmospheric CO_2 for photosynthesis. The pH changes surrounding the *H. fusiforme* fronds had less influence on the photosynthetic rates under emerged condition than under submersed condition. When the pH was as high as 10.0, emerged *H. fusiforme* could photosynthesize efficiently, but the submersed alga exhibited very poor photosynthesis. Extracellular carbonic anhydrase (CA) played an important role in the photosynthetic acquisitions of exogenous Ci in water as well as in air. Both the concentrations of dissolved inorganic carbon in general seawater and CO_2 in air were demonstrated to limit the photosynthesis of *H. fusiforme*, which was sensitive to O_2 . It appeared that the exogenous carbon acquisition system, being dependent of external CA activity, operates in a way not enough to raise intracellular CO_2 level to prevent photorespiration. The inability of *H. fusiforme* to achieve its maximum photosynthetic rate at the current ambient Ci levels under both submersed and emerged conditions suggested that the yield of aquaculture for this economic species would respond profitably to future increases in CO_2 concentration in the sea and air.

Key words: *Hizikia fusiforme*; photosynthesis; inorganic carbon; carbonic anhydrase; submersion; emersion; tide cycle

The intertidal seaweeds spend alternatively part of their time in atmosphere and part in seawater throughout the day with the fluctuation of tidal level. They therefore undergo two very distinct environmental conditions for photosynthesis and growth. It is of general interest to study the physiology of intertidal seaweeds when considering how to deal with the high frequency cycles of the aquatic and aerial conditions (Raven, 1999; Zou and Gao, 2002a). When the tide is high, the intertidal seaweeds are submersed in seawater and exposed to two potential sources of exogenous carbon for photosynthesis: dissolved CO_2 and bicarbonate (HCO_3^-). In air-equilibrium natural seawater, at normal pH 8.2 and 20 °C, the bulk of total dissolved inorganic carbon (DIC) is HCO_3^- (ca. 2.0 mmol/L), and CO_2 (only 12 $\mu\text{mol/L}$) is less than 1 % of the total DIC. It is reported that a large number of seaweeds have developed mechanisms that permit the acquisition of HCO_3^- pool in seawater during photosynthesis (Raven, 1997; Larsson and Axelsson, 1999; Zou and Gao, 2001). Paradoxically,

intertidal seaweeds, which are exposed to atmospheric CO_2 periodically during emersion at low tide, seem to acquire HCO_3^- in seawater more efficiently than those growing in the subtidal zone (Maberly, 1990; Mercado *et al.*, 1998). An immediate change in the “ CO_2 ” supply for intertidal seaweeds will take place when they get out of the water at low tide, although a seawater film usually retains the algal thalli surface due to their viscosity and hydrophilia. The exact effect of this change in CO_2 -supply on the photosynthetic rates of intertidal seaweeds is waiting for being fully established. It appears that CO_2 will become limiting for photosynthesis more often for the seaweeds under emerged condition than under submersed condition (Gao *et al.*, 1999; Raven, 1999; Zou and Gao, 2002b; Zou and Gao, 2004).

Hizikia fusiforme, belonging to Sargassaceae (Phaeophyta), is distributed uniquely in the west-northern parts of the coast of the Pacific. It has traditionally been used as a food delicacy in China, Japan and Korea (Zhang *et al.*, 2002). Suzuki *et al.* (1996) showed that *Hizikia*

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contained higher soluble dietary fiber than other seaweeds. The extract from this seaweed has an immunomodulating activity on human, which might be useful for clinical application to treat diseases (Shan *et al.*, 1999; Katayama *et al.*, 2002). Additionally, *H. fusiforme* is an important raw material for alginates production. It now becomes one of the potential important species for seaweed cultivation, owing to its high commercial value and market demand (Zhang *et al.*, 2002). A large number of studies have been carried out on its life history (Park *et al.*, 1995; Ruan and Xu, 2001) and cultivation technique (Hwang *et al.*, 1997; Li, 2001). However, the photosynthetic characteristics of *H. fusiforme* have been less studied (Zou *et al.*, 2003). *H. fusiforme* is distributed at lower parts of the intertidal zone, frequently spending a part of tidal cycles in the emerged state. The aim of this study is to compare its photosynthetic strategies for exogenous inorganic carbon acquisition under submersed and emerged conditions, in order to establish a general knowledge about its physiological behavior associated with tidal cycles.

1 Materials and Methods

1.1 Algal materials

Hizikia fusiforme (Harv.) Okamura was collected from lower intertidal rocks along the coast of Nanao, Shantou, China when the tide went out. Samples sealed in a plastic bag with some seawater were transported to the laboratory in an insulated cooler (ca. 5 °C) within 4 h. The material was maintained in a glass aquarium tank containing filtered natural seawater (salinity ca. 33 ‰) under 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (PAR, 400-700 nm) illuminated by fluorescent tubes for 14 h out of each 24 h and at room temperature (18-22 °C). The seawater was aerated vigorously and was renewed daily. Experiments were conducted within a period of 5-d laboratory maintenance for each collection, during which the algal material showed stable photosynthetic activity. After this period, the remains were abandoned and fresh material was collected again.

1.2 Effects of pH and inhibitor on photosynthetic rates

Buffered natural seawater of varied pH values with or without the addition of acetazolamide (AZ, 100 $\mu\text{mol/L}$ of final concentration) were prepared. Different pH values were obtained by adding a known amount of biological buffers (Sigma) to give final concentrations of 20 mmol/L. TRIS was used for buffering pH 8.2 (a pH value representative of that in natural seawater) and 9.0, and CAPS for pH 10.0. AZ stock solutions prepared with 40 mmol/L NaOH, were added into the buffers to the final concentrations of 100 $\mu\text{mol/L}$. AZ is known as a relatively membrane-impermeable

inhibitor of extracellular carbonic anhydrase (CA) activity (Axelsson *et al.*, 1999; Moroney *et al.*, 2001).

Photosynthetic rates of submersed plants were measured as oxygen evolution using a Biological Oxygen Monitor (YSI Model 5300, USA) at 20 °C and at saturating photon flux density of 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The oxygen electrode was held in a temperature-controlled chamber. The fronds of *H. fusiforme* were cut into small segments (0.5-0.7 cm length) with a shape razor blade and incubated in seawater under 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 20 °C for at least 2 h before the measurements. This pre-treatment aimed to minimize the possible effect of cutting damage (wound respiration). Segments of *H. fusiforme* of about 0.3 g FW were incubated in the reaction chamber with 10 mL of buffered seawater that was magnetically stirred, and the linear O₂ evolution versus time was recorded.

The emerged photosynthetic rates were determined as CO₂ uptake with an infrared gas analyzer (LCA-4, Analytical Development Company Ltd., UK) in an open circuit under the same light/temperature conditions as for the submersed samples. Before introducing into the photosynthetic leaf chamber, the samples were respectively immersed in above seawater buffers for 30 min, aiming to adjust the pH value within the surface water film surrounding the fronds when the algal samples were emerged. The buffered seawaters used were in equilibrium with atmosphere in terms of CO₂. Thus, in our photosynthesis-determining system, the difference of CO₂ concentration between the inlet and outlet of the assimilation chamber was due to CO₂ uptake by the algal photosynthesis. The rate of CO₂ uptake (P_n) ($\mu\text{mol CO}_2\cdot\text{g}^{-1}\text{FW}\cdot\text{h}^{-1}$) was calculated as follows: $P_n = DC \times F \times 60 \times 273 / ((273+T) \times 22.4 \times FW)$, where DC is the difference in CO₂ concentration ($\mu\text{L/L}$) between the inlet and outlet air; F , the gas flow rate (L/min); T , temperature (°C); FW , fresh weight (g).

1.3 Inorganic carbon-dependent photosynthetic rates

DIC-free seawater was prepared by removing inorganic carbon (Ci) from the natural seawater by lowering pH to less than 4.0 with 0.5 mol/L HCl and sparging with pure N₂ gas for 2 h at least. A known amount of TRIS (Sigma) was added to give a final concentration of 20 mmol/L, and the pH was then adjusted to 8.2 with freshly prepared 0.5 mol/L NaOH and 0.5 mol/L HCl. All manipulations were carried out under N₂. Segments of *H. fusiforme* of about 0.3 g FW were incubated in the reaction chamber with 10 mL of buffered DIC-free seawater. The algae were left to photosynthesize to deplete the possible Ci present in the medium and in the algal cells till no further O₂ evolved, which took about 20 min. Aliquots of NaHCO₃ stock solution were then

injected into the chamber in order to create the appropriate final concentrations of Ci in the reaction medium. O_2 evolution was recorded after addition of NaHCO_3 . Additionally, the Ci -dependent O_2 evolution (i.e. P-C response curve) was carried out with the presence of AZ (100 $\mu\text{mol/L}$).

The P-C response curve was also determined in air (under emersed condition). Samples were pretreated in buffered seawater (Tris 20 mmol/L , pH 8.2) with or without the addition of AZ (100 $\mu\text{mol/L}$ of final concentration) for 30 min. Photosynthetic CO_2 uptake was then determined at different CO_2 concentrations (over the range of 2.6–62.4 $\mu\text{mol/L}$). CO_2 in the ambient air was removed to varied degrees by pumping it through a soda lime column to obtain lower concentrations of CO_2 . Concentrations of CO_2 higher than ambient air were obtained by injecting pure CO_2 before pumping ambient air into an air bag (1 m^3). The air bags were used to maintain constant CO_2 supply.

1.4 Oxygen sensitivity

Photosynthetic O_2 evolution rate of submersed samples was respectively measured at two levels of O_2 , i.e. less than 30% of air-equilibrated level of O_2 (low O_2) which was achieved by bubbling N_2 gas into the reaction chamber, and 100% of air-equilibration concentration of O_2 (ambient O_2). Similarly, the emersed photosynthetic CO_2 uptake was respectively examined under normal atmosphere (ambient O_2 , 21% of O_2 concentration) and under atmosphere with low O_2 concentration (< 6%) which was obtained by pumping air through a $\text{Na}_2\text{S}_2\text{O}_3$ solution. The O_2 concentration in air and in water were examined by the Infrared Gas Analyzer (CGT-7000, Shimadzu Corporation, Japan) and the Biological Oxygen Monitor (YSI Model 5300, USA) respectively.

1.5 Calculations of the photosynthetic parameters and theoretical photosynthetic rates

Ci -saturated maximal rate of photosynthesis (V_{max}) and half-saturation constant ($K_{0.5}$, the inorganic carbon concentration required to give half of V_{max}) were estimated by fitting the P-C curve to the Michaelis-Menten equation. The maximum rates of CO_2 supply derived from spontaneous (uncatalysed) dehydration of HCO_3^- in seawater were calculated according to Matsuda *et al.* (2001) as the theoretical rates of CO_2 supply for photosynthesis. The presumption was adopted that the algal samples consumed CO_2 at a rate causing the CO_2 concentration in seawater surrounding the algae to approach zero. This gave a theoretical maximal rate of uncatalysed conversion of CO_2 from HCO_3^- in seawater. The flux of CO_2 across the surface seawater film under emersed condition was not considered. The volume of the surface water film surrounding the algal

samples was estimated as a mass difference before and after blotting off the superficial water. The value obtained from this procedure was (0.6 \pm 0.2) mL per gram fresh weight of alga sample. The theoretical rate of CO_2 supply ($d(\text{CO}_2)/dt$) was calculated by the following equations: $d(\text{CO}_2)/dt = K_1 \times (\text{DIC})/A + K_3 \times (\text{DIC}) \times [\text{H}^+]/K_{\text{H}_2\text{CO}_3}/A$, and $A = 1 + [\text{H}^+]/K_1 + K_2/[\text{H}^+]$, where $[\text{DIC}]$ is the concentration of dissolved inorganic carbon in seawater. K_1 and K_3 are the rate constants of reactions $\text{HCO}_3^- \rightarrow \text{CO}_2 + \text{OH}^-$ and $\text{H}_2\text{CO}_3 \rightarrow \text{CO}_2 + \text{H}_2\text{O}$, respectively. $K_{\text{H}_2\text{CO}_3}$ and K_2 represent respectively the dissociation constants of the reactions $\text{H}^+ + \text{HCO}_3^- \rightarrow \text{H}_2\text{CO}_3$ and $\text{H}^+ + \text{CO}_3^{2-} \rightarrow \text{HCO}_3^-$. The values of K_1 , K_3 , $K_{\text{H}_2\text{CO}_3}$ and K_2 are according to Johnson (1982) and Stumm and Morgan (1996). Photosynthetic rates based on O_2 evolution or CO_2 uptake were compared by assuming the photosynthetic quotient of 1.0.

1.6 Statistics

The data were expressed as the mean values \pm SE (n = 3). Statistical significance of the data was tested with ANOVA or *t*-test at $P < 0.05$.

2 Results

2.1 Effects of pH and AZ on the photosynthetic rates under submersed and emersed conditions

Figure 1 shows the effects of pH and AZ on photosynthetic rates of *H. fusiforme* fronds under submersed and emersed conditions.

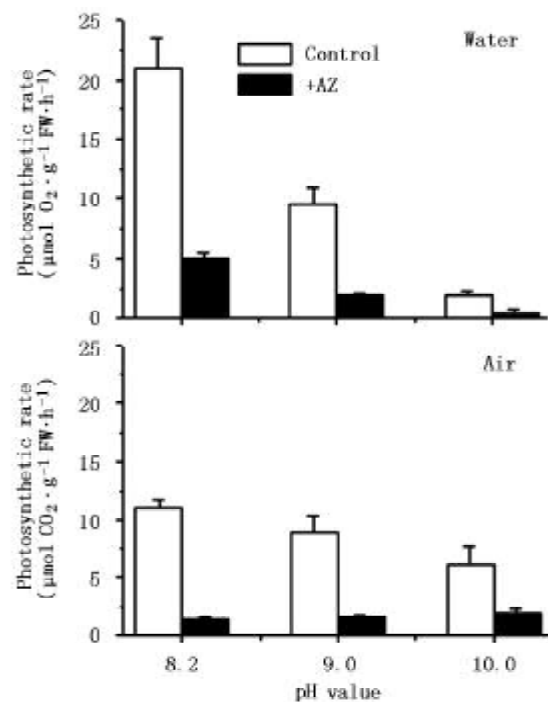


Fig. 1. Effects of pH and acetazolamide (AZ) on net photosynthetic rates (P_n) of *Hizikia fusiforme* under submersed and emersed conditions.

emerged conditions. Photosynthetic O₂ evolution of submersed plants was reduced drastically as the pH in the seawater increased from 8.2 to 10.0. O₂ evolution at pH 9.0 and 10.0 was reduced by 54.4 % and 90.5 %, respectively, compared to that at pH 8.2. By contrast, there was only a slight decrease in photosynthetic CO₂ uptake of emerged plants with the increasing pH in the surface water film surrounding *H. fusiforme* fronds. There was no significant difference ($P > 0.1$) in CO₂ uptake between pH at 8.2 and 9.0. CO₂ uptake at 10.0 was reduced by 44.5% compared to that at pH 8.2. It was shown that the photosynthetic rate was significantly ($P < 0.01$) greater at pH 8.2, but was conspicuously ($P < 0.01$) lower at pH 10.0, in submersed plants than in emerged plants. AZ remarkably ($P < 0.01$) inhibited the photosynthetic rate at all the pH values tested for both submersed plants and emerged plants. The inhibitory effect of AZ ranged from 76 % to 82 % in water, but from 87 % to 68 % in air, when the pH value raised from 8.2 to 10.0.

In order to test whether or not the CO₂ supplies derived from uncatalysed spontaneous dehydration of HCO₃⁻ in seawater medium surrounding the algal fronds were enough to support the measured photosynthetic rates of O₂ evolution by emerged plants or CO₂ uptake by emerged plants, respectively, the observed rates of photosynthesis were compared with the theoretical rates (Table 1). The observed rates at all the pHs tested for the submersed plants exceeded ($P < 0.01$) those supported solely by the spontaneous CO₂ formation. This indicated that submersed *H. fusiforme* frond was capable of using external HCO₃⁻ as a source of Ci to drive photosynthetic O₂ evolution. However, the theoretical rate was fast enough to account for the observed rate of photosynthetic O₂ evolution at pH 8.2 with the presence of AZ. Under emerged condition, the ratios of observed to theoretical rates were much higher than 1.0, and those increased by two orders of magnitude with increasing pH from 8.2 to 10.0. The ratios in case of AZ were also much greater than 1.0 and dramatically increased with increasing pH, though the values of these ratios were

Table 1 Ratios of measured to theoretically calculated photosynthetic rates at different pH values for *Hizikia fusiforme* under submersed and emerged conditions

	pH 8.2	pH 9.0	pH 10.0
Water			
Control	1.5 ± 0.2	10.0 ± 1.5	55.3 ± 6.2
+AZ	0.4 ± 0.1	1.9 ± 0.2	9.8 ± 12.8
Air			
Control	36.3 ± 2.4	424.0 ± 60.6	7 699.0 ± 2 005.6
+AZ	4.9 ± 0.2	75.3 ± 8.0	2 450.0 ± 506.2

AZ, acetazolamide.

reduced compared to the controls. As the CO₂ fluxes from the atmosphere to the fronds were not taken into account in calculating the theoretical values, it could be inferred that the atmospheric CO₂ was the predominant source of Ci driving the photosynthesis of *H. fusiforme* fronds under emerged condition.

2.2 The dependence to exogenous inorganic carbon for photosynthetic rates under submersed and emerged conditions

Effects of external inorganic carbon concentrations on net photosynthetic rate with or without AZ are shown in Fig.2 for the submersed *H. fusiforme* fronds and in Fig.3 for the emerged fronds. The photosynthesis was far from saturated with ambient Ci levels under both submersed and emerged conditions. This was in accordance with the high $K_{0.5}$ values in water as well as in air (Table 2). Saturating Ci level seemed to be reached at about 8.8 mmol/L for the submersed plants. It appeared that the emerged photosynthetic rate was not to be saturated over the range of CO₂ in air (2.6-62.4 μmol/L) used in the experiments. Though the

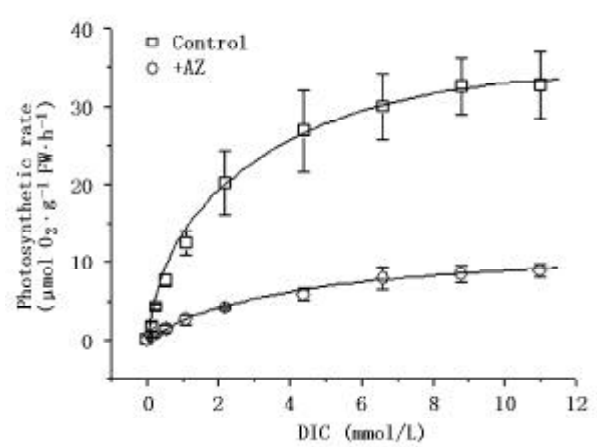


Fig.2. Photosynthetic oxygen evolution rate as a function of dissolved inorganic carbon (DIC) concentration in seawater for the submersed *Hizikia fusiforme* with or without the presence of acetazolamide (AZ).

Table 2 The inorganic carbon-saturated maximum photosynthetic rates (V_{max}) and the apparent half-saturation constant ($K_{0.5}$) in *Hizikia fusiforme* under submersed and emerged conditions

	V_{max} ($\mu\text{mol O}_2$ or $\text{CO}_2 \cdot \text{g}^{-1}$ $\text{FW} \cdot \text{h}^{-1}$)	$K_{0.5}$ (DIC) (mmol/L)	$K_{0.5}$ (CO ₂) ($\mu\text{mol/L}$)
Water			
Control	44.7 ± 13.1	2.72 ± 0.91	14.8 ± 5.0
+AZ	11.2 ± 1.3	3.88 ± 1.76	21.2 ± 9.6
Air			
Control	49.7 ± 15.5	-	51.0 ± 14.7
+AZ	13.6 ± 5.3	-	41.2 ± 21.4

AZ, acetazolamide; DIC, dissolved inorganic carbon.

net photosynthetic rates at ambient C_i were significantly higher in submersed plants than in emerged plants, the C_i -saturated maximum photosynthetic rates (V_{max}) were similar ($P > 0.1$) in both submersed and emerged plants (Table 2). AZ strongly depressed photosynthetic activities of *H. fusiforme* fronds at all C_i levels of the measurements under submersed condition as well as emerged condition (Figs. 2, 3). However, the $K_{0.5}$ values showed no significant ($P > 0.05$) difference between with and without the presence of AZ (Table 2).

2.3 Sensitivity to O_2 concentration for photosynthetic rates under submersed and emerged conditions

Effect of O_2 concentration on photosynthetic rates under submersed and emerged conditions are shown in Fig. 4. Photosynthetic rates is significantly inhibited ($P < 0.05$) by O_2 at ambient O_2 concentration in comparison with low O_2 concentration for *H. fusiforme* fronds under both submersed

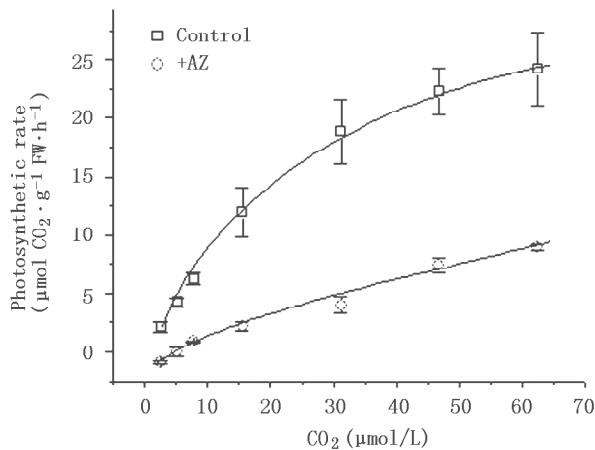


Fig. 3. Photosynthetic CO_2 uptake rate as a function of CO_2 concentration in air for the emerged *Hizikia fusiforme* with or without the presence of acetazolamide (AZ).

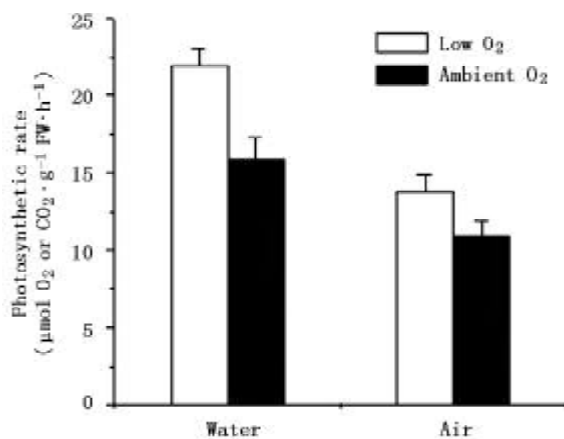


Fig. 4. Comparison of photosynthetic rates measured at low O_2 concentration and those measured at ambient O_2 concentration for *Hizikia fusiforme* under submersed and emerged conditions.

and emerged conditions, indicating a C_3 -like photosynthetic gas exchange physiology.

3 Discussion

H. fusiforme, which normally grows in low intertidal zone, will be exposed to air when the tide goes out, especially during a spring tidal cycle. However, field desiccation seldom occurs due to the continuous waves, sea spray and extensive shingle-overlapping. The morphology of coarsely-branched fronds of *H. fusiforme* further reduces the probability of desiccation. Thus, *H. fusiforme* could often maintain lengthy hydrated statue while exposed. The present work showed that *H. fusiforme* exhibited different photosynthetic activity under emerged condition compared to submersed condition, which might result from different availability of exogenous inorganic carbon for photosynthesis, and/or the mechanism of carbon acquisition between in and out of water. For example, the concentration of DIC in seawater is about 140 times greater in water than in air (2.2 mmol/L vs 15.6 µmol/L), but the diffusion rate of CO_2 in air is 10 000 times higher than in water. Oates (1985) and Romaine *et al.* (1997) guessed that the difficulty in absorbing CO_2 in its molecular form or blockage of its movement into the cells might result in the lower photosynthetic rate in air. On the other hand, the carbon-saturated maximum photosynthetic rate of *H. fusiforme* was similar between in water and in air (Table 2), implying the comparable carboxylatory capacity of Rubisco in both the environmental conditions.

Rates of theoretical CO_2 supply lower than the observed rates of photosynthetic O_2 evolution could be considered as evidence for the capacity of submersed *H. fusiforme* to acquire external HCO_3^- in seawater to drive photosynthesis, as reported in some other algae for their abilities of using HCO_3^- (Johnston *et al.*, 1992; Gao and Zou, 2001). The inhibition of photosynthetic O_2 evolution by AZ addition indicated that extracellular CA activity acted as an essential part of HCO_3^- acquisition by submersed *H. fusiforme*. It has been previously shown that extracellular CA evidently occurred in *H. fusiforme* (Zou *et al.*, 2003). In some intertidal seaweeds, there seems a poor correlation between external CA activity and the capacity of HCO_3^- acquisition, and the main cause is that those seaweeds possessed the mechanism of direct HCO_3^- uptake (Mercado *et al.*, 1998). However, the experiment of culturing *H. fusiforme* under different CO_2 concentrations was in support that the external CA activity was closely linked to the ability of acquiring HCO_3^- in seawater (Zou *et al.*, 2003). Therefore, when *Hizikia* was submersed in seawater, external CA catalyzed

the dehydration of HCO_3^- externally, and the CO_2 formed was the species of Ci that crossed the plasma membrane. However, although the rate of conversion between HCO_3^- and CO_2 catalyzed by extracellular CA activity is almost instantaneous, the resulting equilibrium CO_2 concentration is rather low at high pH (Raven, 1997; Axelsson *et al.*, 1999; Zou and Gao, 2001). As a consequence of that, the extracellular CA-mediated conversion of HCO_3^- to CO_2 is much less efficient at high pH values. This gave the physiological explanation for the results that submersed photosynthetic rate of *H. fusiforme* was conspicuously reduced with increasing pH (Fig. 1). On the other hand, the relationship between the photosynthetic rate and pH under emersed condition differed from that under submersed condition, in which the emersed photosynthetic rate did not significantly decrease as pH rose. Such discrepancy of photosynthetic performances in *H. fusiforme* between in water and in air could be ascribed to the different source of Ci for photosynthesis, as suggested by Mercado and Niell (2000). Though submersed *H. fusiforme* mainly used HCO_3^- pool in seawater for photosynthesis, emersed *H. fusiforme* acquired CO_2 in air as a principal source for photosynthesis. The CO_2 uptake rate substantially exceeded the theoretical CO_2 flux derived from the spontaneous conversion of HCO_3^- in the surface seawater film surrounding the fronds of *H. fusiforme*, supplying further evidence that the main source of Ci for emersed photosynthesis came from atmospheric CO_2 .

CO_2 in air must first be dissolved into and across through the surface water film surrounding the fronds of *H. fusiforme* before it reached the plasmolemma and was available for photosynthesis. The present results showed that AZ had a considerable inhibitory effect on photosynthetic CO_2 uptake rate of *H. fusiforme* under emersed condition, indicating that the external CA facilitated the atmospheric CO_2 acquisition. Firstly, external CA catalysed the conversion of dissolved CO_2 into HCO_3^- in the water film, which allowed a CO_2 gradient and produced a driving force facilitating the dissolving of gaseous CO_2 into water film (Portielje and Lijklema, 1995). Secondly, as CO_2 was the species of Ci that entering into cells, extracellular HCO_3^- must be dehydrated to form CO_2 before acquired by the algal cells. This process was also mediated by external CA. Therefore, external CA simultaneously catalyzed hydration and dehydration reactions in the surface water film. However, those two adverse reactions must be spatially separated from each other. It might be proposed that the role of extracellular CA in *H. fusiforme* could be regarded as a facilitating under emersion condition, whereas that as a qualitatively

essential mechanism under submersion condition, as described in some other intertidal seaweeds (Raven, 1997; Mercado and Niell, 2000; Zou and Gao, 2004).

It was noted that at high pH value (10.0), the photosynthetic rate of *H. fusiforme* under submersed condition was very low, indicating the acquisition of HCO_3^- mediated by external CA activity was not function well in seawater under such high pH. By contrast, when exposed to air, *H. fusiforme* could still photosynthesize efficiently when the pH value of the surface water film covering the fronds was as high as 10.0. Such high pH in the water film could accelerate the conversion of CO_2 into HCO_3^- and then raise the CO_2 flux across the air-water interface (Portielje and Lijklema, 1995). In case of high standing stock or low seawater motion, the pH of seawater close to *H. fusiforme* may rise due to the photosynthetic acquisition of HCO_3^- , and consequently the photosynthesis could be depressed. However, when the tide goes out, the fronds of *H. fusiforme* retain surface seawater film with high pH and they could still photosynthesize efficiently. Such emersed photosynthetic performance could confer *H. fusiforme* with ecological significance of increasing the daily carbon gain.

Marine seaweeds usually assimilate CO_2 via the C_3 biochemical pathway with ribulose-1,5-biophosphate carboxylase/oxygenase (Rubisco) as a carboxylating enzyme (Kerby and Raven, 1985; Raven, 1997). The carboxylase function of Rubisco can be competitively inhibited by O_2 , and a high intracellular O_2 : CO_2 ratio is favorable for oxygenase activity and the photorespiration pathway. However, O_2 tension had hardly effect on photosynthetic rates for many seaweeds (Kerby and Raven, 1985; Raven, 1997), i.e. they exhibited C_4 -like photosynthetic gas exchange physiology. The common explanation is that they possessed a CO_2 -concentrating mechanism (CCM) maintaining elevated CO_2 level intracellularly, which was based on the active HCO_3^- utilization system (Beer, 1994), as the well-established CCM in microalgae (Kaplan and Reinhold, 1999). The O_2 sensitivity obtained in *H. fusiforme* was consistent with a C_3 -like photosynthetic gas exchange physiology, albeit this species had the ability of HCO_3^- use. It was proposed that the external CA activity and the associated exogenous carbon acquisition mechanism in *H. fusiforme* were not enough to maintain elevated CO_2 intracellularly and to prevent photorespiration. The present results showed that the photosynthesis of *H. fusiforme* was not saturated with the current ambient Ci levels under submersed condition as well as under emersed condition, and substantially increased rates of photosynthesis could be gained by addition of DIC in seawater or CO_2 in air. It is generally believed that

the atmospheric CO₂ rises mainly due to anthropogenic effect (combustion of fossil fuels; deforestation), and consequently near-shore marine dissolved CO₂ levels may also increase (Bowes, 1993; Stumm and Morgan, 1996). Such an increase of CO₂ in air and/or in seawater would no doubt enhance the photosynthetic rate of *H. fusiforme* under both submersed and emersed conditions, which thereby would enhance the growth and production of aquaculture for this cultivated crop.

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