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Enhanced growth of the red alga *Porphyra yezoensis* Ueda in high CO_2 concentrations

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Abstract

Leafy thalli of the red alga *Porphyra yezoensis* Ueda, initiated from conchospores released from free-living conchocelis, were cultured using aeration with high CO_2. It was found that the higher the CO_2 concentration, the faster the growth of the thalli. Aeration with elevated CO_2 lowered pH in dark, but raised pH remarkably in light with the thalli, because the photosynthetic conversion of HCO_3^- to OH^- and CO_2 proceeded much faster than the dissociation of hydrated CO_2 releasing H^+. Photosynthesis of the alga was found to be enhanced in the seawater of elevated dissolved inorganic carbon (DIC, $CO_2 + HCO_3^- + CO_3^-$). It is concluded that the increased pH in the light resulted in the increase of DIC in the culture media, thus enhancing photosynthesis and growth. The relevance of the results to removal of atmospheric CO_2 by marine algae is discussed.

Introduction

With industrial activities, the increasing CO_2 concentration in the atmosphere is considered to trigger the 'greenhouse effect'. Studies are needed both to investigate the ecological impact of increasing CO_2 and to make effective use of this CO_2. There have been hardly any reports on the effects of elevated CO_2 concentration on marine macroalgae. However, the brown alga *Fucus serratus* has recently been reported to survive in 5% CO_2 for three weeks, with its photosynthetic physiology being affected (Johnston & Raven, 1990).

The red alga *Porphyra yezoensis* Ueda has been cultivated in Japan, and several studies have been made on its ecology and physiology (Oohusa, 1980; Merrill *et al.*, 1983; Tajiri & Aruga, 1984; Kato & Aruga, 1984; Gao & Aruga, 1987). However, nothing is known about its responses to elevated CO_2 concentration. This paper presents findings on the impact of elevated CO_2 concentrations on the growth of this alga.

Material and method

From the stock of free-living conchocelis stage of *Porphyra yezoensis* (ZGRW, Miura 1990) maintained in the laboratory a cluster of conchocelis

filaments was collected and cultured in an incubator under 10:14 LD cycle at 15 °C (light period: 0800–1800 at 300 μmol photon $m^{-2}s^{-1}$). Synthetic fibres (vinylon monofilaments) were used for the conchospores to attach. Zero day was assigned to the age of the leafy thalli on the day of attachment. The thalli kept in batch culture were detached from the synthetic fibre at about 20 days old. Then 150 individual thalli (5 mm in length) were separated into three groups, one for the culture aerated with air (350 ppm CO_2), the other two for the cultures aerated with air containing 1000 and 1600 ppm CO_2, respectively.

The flasks with culture medium and thalli were placed in the CO_2 incubators (Shimadzu BEC-II-250) of 250 l capacity, in which CO_2 concentration was adjusted by mixing 99.9% CO_2 from a CO_2 cylinder with air transported from outdoor by an air compressor (Fig. 1). CO_2 concentrations of air in the incubators were monitored automatically every 10 min by infrared gas analysis (IRGA, Shimadzu URA-107, including a continuous measuring unit, Shimadzu IRA-107). Aeration was carried out by using a mini-pump (Shibata MP-2N) at a flow rate of 0.3–0.5 l min^{-1}. For culture medium, seawater collected from the sea off Miyazu facing the Sea of Japan (0.03 mg l^{-1} inorganic-N, 0.006 mg l^{-1} inorganic-P) was filtered (Whatman GF/C) and enriched with PES medium (Provasoli, 1966) after being autoclaved. Culture medium was renewed every other day. Illumination was provided by fluorescent lamps (National, 36 W). Photosynthesis was measured at 15 °C and 600 μmol photon $m^{-2}s^{-1}$ in a flow-water photosynthesis measurement system (Gao, 1989) jointed with a permeable membrane mass spectrometer (Ametek M200), and its rate was compared in the seawater with 2 mM and 10 mM DIC. The photosynthetic rate was determined from increased O_2 in the closed system. Each comparison was completed with the same 10–15 thalli on the same day within 20 min in order to avoid effects caused by diurnal photosynthetic variation (Oohusa, 1980; Gao & Umezaki, 1989; Gao, 1990). Seawater with 10 mM DIC was prepared by adding $NaHCO_3$ into the usual seawater of 2 mM DIC, and the pH was adjusted to that of the usual seawater (pH 8.2) with NaOH and HCl.

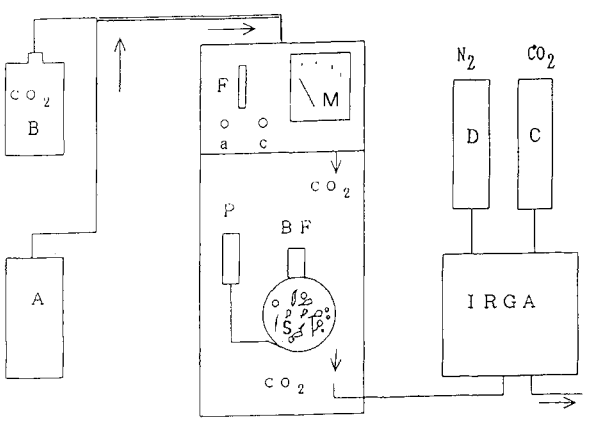
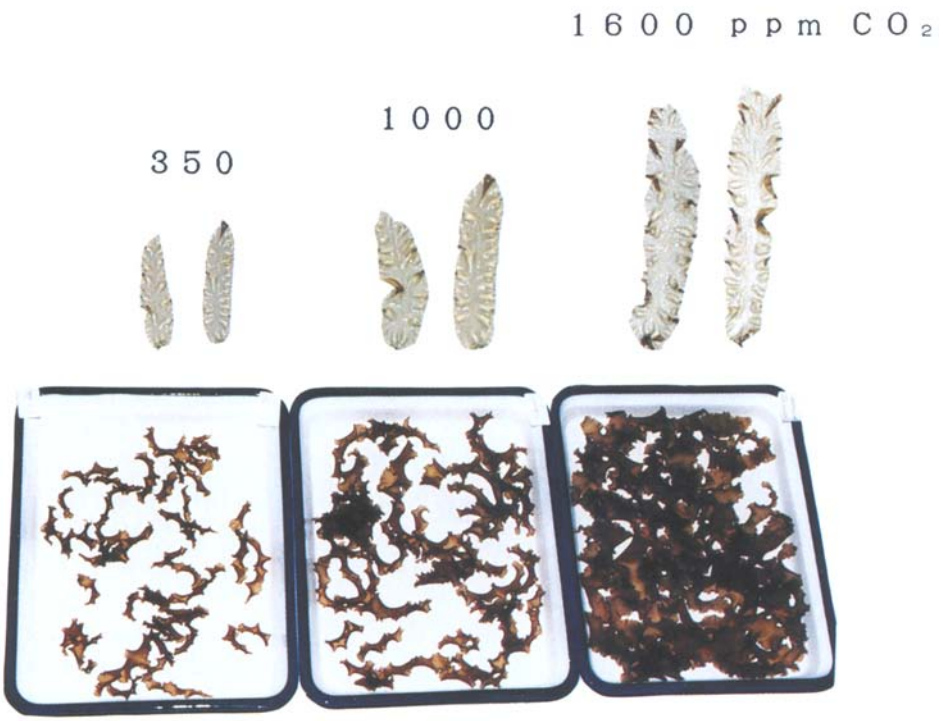


Fig. 1. Illustration for indoor culture of *Porphyra yezoensis* under elevated CO_2. A, air compressor; B, CO_2 cylinder; F, flow meter; M, CO_2 monitor; a, CO_2 inlet needle valve; c, air inlet needle valve; P, mini-pump; BF, culture flask; S, *P. yezoensis* thallus; C, CO_2 cylinder for calibration; D, N_2 cylinder for calibration; IRGA, infrared gas analyzer.

Results

Significant differences (t-test, $P<0.01$) in both length and width of *Porphyra yezoensis* thalli were found 10 days later in the cultures aerated with different concentrations of CO_2 (Fig. 2). Growth of the thalli was fastest in 1600 ppm CO_2 culture, intermediate in 1000 ppm CO_2 culture and slowest in 350 ppm CO_2 culture, i.e. it was enhanced with an increase in CO_2 concentrations from 350 to 1600 ppm. Twenty days later, the thallus length or width in the cultures with 1000 and 1600 ppm CO_2 was about 1.4 and 1.9 times that in 350 ppm CO_2 culture. Clear differences can be seen among the biomass of 50 thalli in respective cultures, and no extraordinary morphological features were observed (photo in Fig. 2). No significant difference (t-test, $P>0.05$) in the length/width ratio of thalli (average 3.5) was found under various CO_2 concentrations throughout culture period.

Aeration with air of 1000 and 1600 ppm CO_2 reduced pH from 8.2 (350 ppm CO_2) to 7.8 and 7.2, respectively, in the culture media without algal



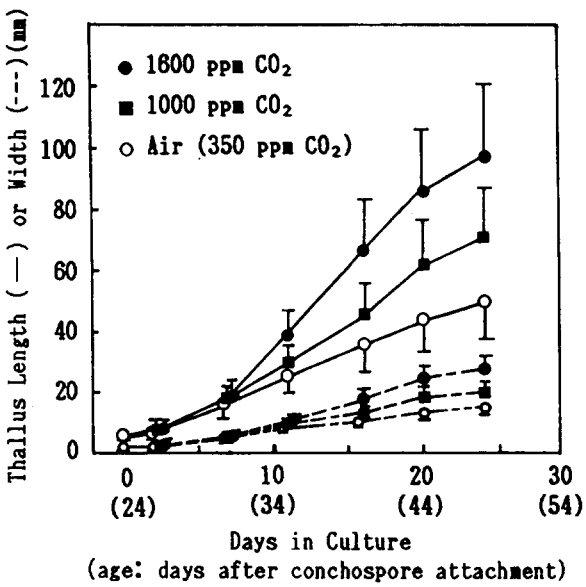


Fig. 2. Growth of *Porphyra yezoensis* thalli under various CO_2 concentrations. The mean length and width of 50 thalli are indicated with SD (n = 50). (Photo, biomass of the respective 50 thalli after 20 days in culture).

thalli or with algal thalli in dark, but not much with algal thalli in light. It was found that pH of the culture media with thalli lowered to reach the lowest value during dark period but rose to reach the highest value during the light period (Fig. 3A), and the higher the CO_2 concentration, the bigger the pH rise in light (Fig. 3B).

Figure 4 shows changes of pH with flow rates

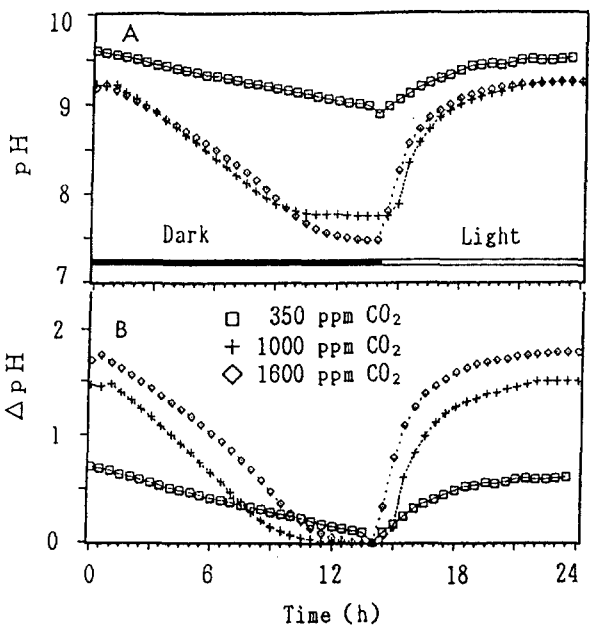


Fig. 3. Diurnal variations of pH (A) and its extends (△ pH, B) in culture media with the thalli of *Porphyra yezoensis* aerated with air of 350, 1000 and 1600 ppm CO_2. The black bar indicates dark period, and the white bar light period. Measured with 18 to 21 days culture, one day after the culture medium was renewed.

of air and addition of carbonic anhydrase (CA) into the culture medium with the thalli in the light. When CA was added into the culture media with a saturating aeration rate (about 1 l min^{-1}), the rise of pH was suppressed. It was also found that pH dropped abruptly to reach a constant value during the dark period when CA was added into the culture medium.

Light-saturated net photosynthesis of the alga was found to be much higher in seawater with 10 mM DIC than in usual seawater with 2 mM DIC, independent of culture background of CO_2 concentrations (Fig. 5). The photosynthetic rate in the seawater with 10 mM DIC was enhanced by 31%, 17% and 40% in the thalli cultured with 350, 1000 and 1600 ppm CO_2, respectively.

Discussion

CO_2 physically dissolved in culture media ($[CO_2]aq$) can reach an equilibrium with that in the bubbling gas (PCO_2), being hydrated to form carbonic acid, which dissociates to bicarbonate, then to carbonate as follows (Stum & Morgan, 1981):

$$cPCO_2 \rightleftharpoons [CO_2]aq \quad (1)$$

$$[CO_2]aq + H_2O \rightleftharpoons H_2CO_3 \quad (2)$$

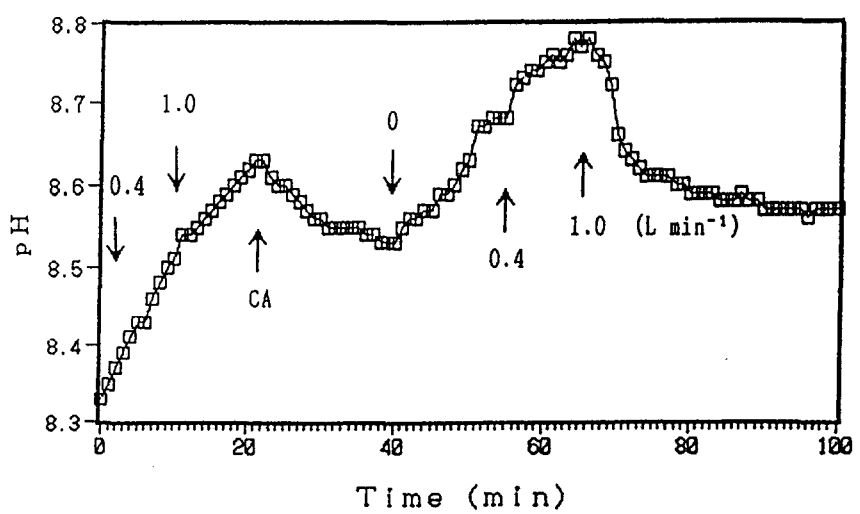


Fig. 4. Changes of pH with flow rates of air and the presence of carbonic anhydrase (CA) in the culture medium with thalli of *Porphyra yezoensis*. The arrows indicate the time for change of flow rate of air and the addition of CA.

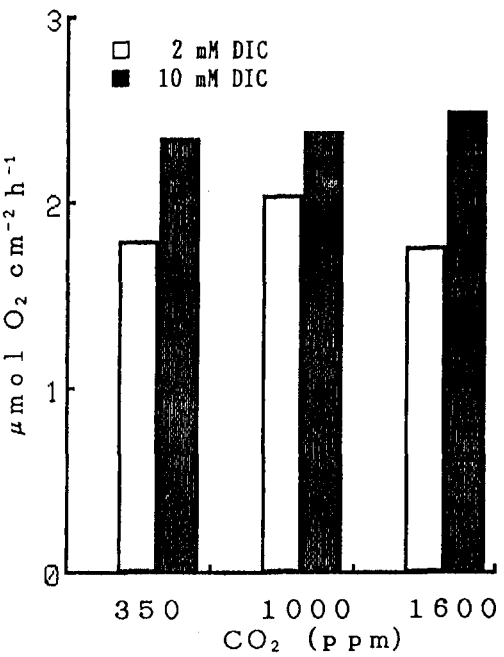


Fig. 5. Comparisons of light-saturated net photosynthetic rates of *Porphyra yezoensis* thalli cultured under various CO_2 concentrations in seawater with 2 mM and 10 mM DIC.

$$H_2CO_3 \overset{k1}{\rightleftharpoons} H^+ + HCO_3^- \quad (3)$$

$$HCO_3^- \overset{k2}{\rightleftharpoons} H^+ + CO_3^{=}, \quad (4)$$

where c and k respectively indicate the solubility of CO_2 and dissociation constant whose values depend on the temperature and chlorinity of seawater.

When CO_2 concentration in air increases, the above reactions proceed toward the right-hand side to reach a new equilibrium, which gives rise to an increase of H^+ ions in seawater. Therefore, aeration with high CO_2 can reduce pH of the culture medium. In the present study, aeration with elevated CO_2 lowered pH in the dark but raised pH in the light in the culture medium with the thalli.

It has been shown that ribulose-bisphosphate carboxylase/oxygenase (Rubisco) is the predominant initial carboxylating enzyme in seaweeds (Kremer & Kuppers, 1977; Cook & Colman, 1987; Reiskind *et al.*, 1988; Beer *et al.*, 1990), which is known to fix CO_2 not HCO_3^- (Cooper *et al.*, 1969). Nevertheless, a number of seaweeds have been reported to take up HCO_3^- (Thomas & Tregunna, 1968; Jollife & Tregunna, 1970; Sand-Jensen & Gordon, 1984; Bidwell & McLachlan, 1985; Brechignac & Andre, 1985; Cook *et al.*, 1986; Johnston & Raven, 1986; Smith & Bidwell, 1987; Axelsson & Uusitalo, 1988; Holbrook *et al.*, 1988; Lignell & Pedersen, 1989; Surif & Raven, 1989; Madsen & Maberly, 1990; Beer *et al.*, 1990). The rise of pH in the light must be attributable to photosynthetic carbon fixation in that the tremendous amount of HCO_3^- in the culture media or within the algal cells is converted to OH^- and CO_2 with catalysis of CA (Asada, 1981; Silverman, 1991), the former raising pH, and the latter being assimilated in photosynthesis:

$$HCO_3^- \overset{CA}{\rightleftharpoons} CO_2 + OH^- \quad (5)$$

$$CO_2 + H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} [H_2CO] + O_2. \quad (6)$$

However, if the production rate of OH^- ions in the reaction (5) is lower than that of H^+ ions from dissociation of hydrated CO_2 in the reactions (3) and (4), the pH rise could not be attributable to the photosynthetic utilization of HCO_3^-. Addition of CA into the culture media suppressed the rise of pH at the saturating aeration rate. This indicates that the pH change disappeared when the reactions (3) and (5) are balanced with the catalysis of CA (Fig. 4). Accordingly, pH dropped gradually during the dark period and rose quickly during the light period (Fig. 3) in the culture media without CA added, just because the reaction (3) proceeded much more slowly than the reaction (5). The faster the photosynthetic carbon fixation, the more the OH^- and the quicker the rise of pH. Because greater diurnal variations in pH were observed in the culture media aerated with high CO_2 concentration, photosynthetic carbon fixation must be much more actively advanced in such cultures.

On the other hand, photosynthesis is accom-

panied by the assimilation of NO_3^-, NH_4^+ and HPO_4^{2-} as follows (Stum & Morgan, 1981):

$$106\,CO_2 + 16\,NO_3^- + HPO_4^{2-} + 122\,H_2O + 18\,H^+ \xrightarrow{\text{light}} [C_{106}H_{263}O_{110}N_{16}P_1] + 138\,O_2$$

$$106\,CO_2 + 16\,NH_4^+ + HPO_4^{2-} + 108\,H_2O \xrightarrow{\text{light}} [C_{106}H_{263}O_{110}N_{16}P_1] + 107\,O_2 + 14\,H^+ .$$

Such processes are accompanied by the uptake of H^+ or OH^- (or release of OH^- or H^+), that is, by changes in alkalinity which is associated with charge balance. Subsequently, pH changes can result from the assimilation of ions. It was reported that the median C:N:P atomic ratio of marine macroalgae was 550:30:1 (Atkinson & Smith, 1983). Thereby, carbon must be assimilated much faster than nitrogen in the photosynthesis of marine macroalgae. Consequently, during the photosynthesis of *P. yezoensis*, NO_3^- assimilation would have a small effect on the pH rise in the culture medium with the thalli compared with the HCO_3^- utilization.

The rise of pH increases the dissolution of CO_2 into the culture medium. DIC concentrations were calculated from the pH, CO_2 solubility and the dissociation constants of equations (3) and (4) provided that the influence by the alkalinity changes resulted from the uptake of ions was negligible. The estimated DIC was 1.3–21.5 mM, 3.7–61.5 mM and 5.9–98.4 mM in the pH range of 8.0–9.0 in the culture media aerated with 350, 1000 and 1600 ppm CO_2, respectively. However, because both the solution of CO_2 and the subsequent hydration are slower processes compared to photosynthesis (Fig. 4 shows indirectly), the actual DIC in the culture medium during the light period might be considerably lower than the above estimated values. In the present study, nevertheless, the aeration of high CO_2 could bring about high DIC due to the raise pH in the culture medium.

Light-saturated net photosynthesis of the alga was enhanced in the seawater with elevated DIC (Fig. 5) i.e. photosynthesis of the alga is limited by inorganic carbon source in natural seawater as reported in some other marine macroalgae (Tseng & Sweeney, 1946; Wheeler, 1980; Holbrook *et al.*, 1988; Surif & Raven, 1989; Madsen & Maberly, 1990; Maberly, 1990). Therefore, the photosynthesis of the alga must have been accelerated in the culture media aerated with high CO_2. The accelerated photosynthesis resulted in enhanced growth of the alga.

The most important conclusion we draw from our results is that CO_2 increased up to 5 times the present level enhances the photosynthesis and growth of *Porphyra yezoensis*, and that pH and DIC in seawater are correlated to each other, i.e. increased DIC with increased CO_2 in air accelerates the photosynthesis, and the accelerated photosynthesis raises pH, which then takes turns to elevate DIC. Stum & Morgan (1981) suggested that doubling of CO_2 in the atmosphere would result, under equilibrium conditions, in a lowering of pH by 0.279 units in the mixed surface seawater of the sea. Is it possible, without understanding the above correlation, that pH of seawater would be chemically reduced with industrial emissions of CO_2 and that the lowered pH would result in a release of CO_2 into air from HCO_3^- and CO_3^{2-} in the sea?

Our findings are applicable to effective use of CO_2 with useful marine algae as well as to evaluating the effects of elevated CO_2 concentrations on marine ecology and air-sea CO_2 exchange, because our cultures were maintained in an open-system which is considered as a simulator of the globe. Photosynthetic utilization of HCO_3^- has also been reported in marine phytoplankton (Badger & Andrews, 1982; Rees, 1984; Patel & Merrett, 1986; Munoz & Merrett, 1988, 1989). We suggest that, if the majority of marine plants raise pH of seawater under elevated CO_2 concentrations, the significance of marine photosynthesis should be noticed not only in carbon fixation but also in increasing the dissolution of CO_2 into ocean.

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