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Effects of elevated CO_2 on sensitivity of six species of algae and interspecific competition of three species of algae

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Abstract: Effects of elevated CO_2 (5000 μl/L) on sensitivity comparison of six species of algae and interspecific competition of three species of algae were investigated. The results showed that, the cell densities of six species of algae grown in elevated CO_2 significantly increased compared to those in ambient CO_2 (360 μl/L), and with the time prolonged, the increasing extent increased. Therefore, elevated CO_2 can promote the growth of six species of algae. However, there were differences in sensitivity between six species of algae. Based on the effects of elevated CO_2 on biomass, the sensitive order (from high to low) was *Platymanas* sp., *Platymanas subcordiformis, Nitzschia closterium, Isochrysis galbana* Parke 8701, *Dunaliella salina, Chlorella* sp., on the condition of solitary cultivation. Compared to ambient CO_2, elevated CO_2 promoted the growth of three species of algae, *Platymanas subcordiformis, Nitzschia closterium* and *Isochrysis galbana* Parke 8701 under the condition of mixed cultivation. The sensitivity of the three species to elevated CO_2 in mixed cultivation changed a lot compared to the condition of solitary cultivation. When grown in elevated CO_2 under the condition of mixed cultivation, the sensitive order from high to low were *Nitzschia clostertium, Platymonas subcordiformis* and *Isochrysis galbana* Parke 8701. However, under the condition of solitary cultivation, the sensitive order in elevated CO_2 was *Isochrysis galbana* Parke 8701, *Nitzschia clostertium, Platymonas subcordiformis*, from sensitive to less sensitive. On the day 21, the dominant algae, the sub-dominant algae and inferior algae grown in elevated CO_2 did not change. However, the population increasing dynamic and composition proportion of three algal species have significantly changed.

Keywords: elevated CO_2; microalgae; sensitivity; interspecific competition

Introduction

The atmospheric CO_2 concentration increases at a rate of 1.5 μmol/(mol·a) and will double compared to pre-industrial levels around the year 2050 (Watson *et al.*, 1990). Its effects and mechanism by elevated CO_2 on global climate, ecological environment, biological diversity and agriculture production have become the hot point in the related research. There were many reports on the effects of elevated CO_2 on algae at the physical and biochemical levels. Studies showed that high concentration CO_2 could promote the growth of microalgae (Xia and Gao, 2001; Hu and Gao, 2001) and increase photosynthesis(Xia and Gao, 2002). Hein and Sand-Jensen (1997) verified that CO_2 enrichment could increase the primary production of marine phytoplankton through field investigation on the relationship between marine phytoplankton population and CO_2 concentration changes. Riebesell *et al.* (1993) speculated that CO_2 enrichment would promote the growth of marine microalgae through indoor experiment. Effects of high concentration CO_2 (5%) on photosynthetic characteristics and carbon fixation mechanism of algae have been widely studied (Badger and Gallagher, 1987). These studies elucidated the physical and biochemical changes of microalgae in high concentration CO_2 (5%) , for example, the *Chlamydomonas reinhardtii* cell grown in 5% CO_2, the affinity for CO_2 reduced, the CO_2 compensation points increased (Moroney and Tolbert, 1985), and the carbonic anhydrase (CA) activity decreased(Patel and Merrett, 1986). In addition, it has been verified that many freshwater unicell microalgae and bluegreen algae exist CO_2 concentrated mechani sm (CCM), the formation of CCM needs low concentration CO_2 and illumination (Berry *et al.*, 1976), while high concentration CO_2 (1% or much higher) inhibited the synthesis CCM and CA enzyme (Rave, 1991).

Studies of sensitivity comparison of CO_2 enrichment on higher plants have been reported. Because of the differences in plant photosynthetic ecological types (C_3, C_4, CAM), the ecological effects of concentration on different types of plant crops are significantly different. Since C_3 crops were more sensitive to CO_2 concentration enrichment than C_4 crops, therefore, the contribution of CO_2 enhancement on C_3 crops was much bigger than C_4 crops (Wang *et al.*, 1998). However, studies on sensitivity comparison of CO_2 enhancement on marine microalgae are rarely reported.

Dukes (2002) proposed that elevation of ambient CO_2 concentration could change population composition of plant community. When CO_2 concentration was elevated, the responses of different types and stains in plant community were different and further cause the changes of composition proportion and components. For example, in the community of mixed grown C_3

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and C_4 plants, the CO_2 compensation points of C_3 plants were relatively higher, the photosynthetic rate increased and the growth quickened when CO_2 concentration was elevated; the CO_2 compensation points of C_4 plants were relatively lower; the promotion of CO_2 concentration enhancement on photosynthetic rate was less and sometime it existed minus increase. Therefore, C_3 plants generally have the competitive predominance, and C_4 plants may disappear and become extinct, so that the composition proportion and components of community changed. The studies of relationship between aquatic plants (especially algae) and CO_2 enrichment were at least lag 10 years than those of terrestrial plants(Bowes, 1993). During the last decade, there are still few reports on the studies of CO_2 enrichment on sensitivity and population competition of microalgae. In the previous study of the relationship between elevated CO_2 and algae, the used CO_2 concentration was twice or several times of the ambient CO_2 level (Gao *et al*., 1991, 1993), as well as 1% CO_2(10000 μl/L)(Mercado *et al*., 1999) and 5% CO_2 (50000 μl/L) (Andria *et al*., 1999) were also used. The effects of different CO_2 concentrations on algal growth were different. Here, we investigated the sensitivity comparison with six species of microalgae in elevated CO_2 (5000 μl/L) and effects of CO_2 enrichment on interspecific competition among the three algal species in mixed cultivation.

1 Materials and methods

1.1 Algal species and culture conditions

Six algal species, *Chlorella* sp., *Dunaliella salina*, *Isochrysis galbana* Parke 8701, *Nitzschia closterium*, *Platymanas subcordiformis*, *Platymanas* sp. (provided by Marine Microalgae Research Center, Ocean University of China) were cultured in Erlenmeyer flasks with f/2 medium (Guillard and Rhyter, 1962) (the medium of *Dunaliella salina* should be added by 8—10 gNaCl/L sea water). The initial pH of the culture was 8.0 ±0.1. Cultures were grown at (20±1)℃ under a 14:10 dark/light cycle of 50 μmol photon/(m^2·s) illumination.

1.2 CO_2 concentrations

CO_2 concentrations of 360 μl/L (ambient CO_2) and 5000 μl/L (elevated CO_2) were maintained throughout the experiment by using a CO_2 injection system (provided by Qingdao Heli Industral Gas Center, Qingdao, China) and the gas flow rate was controlled at 300 ml/min by using air flow meter (LZB-3, Qingdao Hua Yi Meter Factory, Qingdao, China). There was a definite distance between the bubbling glass tube and the algal suspension surface.

1.3 Measurement of cell density

Algae were fixed by Lugol's iodine solution. The cell density was measured every 24 h by counting the culture in a haemacytometer. All experiments were carried on triplicate.

1.4 Measurement of specific growth rate

The specific growth rate(μ) was calculated by the following Equation:

$$\mu = (\ln N_2 - \ln N_1)/(t_2 - t_1)$$

where N_2 and N_1 are the numbers of cells at t_2 and t_1(d), respectively.

1.5 Statistic analysis

The significant differences between the growth of ambient CO_2 and that of elevated CO_2 of the algal species were analyzed by the software of SPSS 13.0. It was considered significant when $P<0.05$, extremely significant when $P<0.01$, not significant when $P>0.05$.

2 Results

2.1 Sensitivity comparison of CO_2 enrichment on six algal species

Under the condition of ambient CO_2 (360 μl/L) and elevated CO_2 (5000 μl/L), the growth of algal species cultured for 5 d (Fig.1) shows that the cell density increased as time increasing. In addition, by SPSS analysis, the cell densities of the six algal species grown in elevated CO_2 significantly increased than that grown on ambient CO_2 ($P<0.05$) and as time prolonged, the increasing scale was larger. Therefore, the CO_2 enrichment can promote the growth of these algal species. The regressive equations of the six algal species grown at ambient and elevated CO_2 are shown in Table 1.

Table 1 Regressive equations for cell numbers (y) and time (x) of the six algal species grown at elevated CO_2 and ambient CO_2

Algal species	Regressive equation		R^2	
	Ambient CO_2 (360 μl/L)	Elevated CO_2 (5000 μl/L)	Ambient CO_2 (360 μl/L)	Elevated CO_2 (5000 μl/L)
Chlorella sp.	y=433.89x+986.95	y=497.46x+954.19	0.9903	0.9921
Platymanas subcordiformis	y=14.486x+44.952	y=21.314x+45.048	0.9832	0.9884
Isochrysis galbana Parke 8701	y=74.2x−304.67	y=100.34x−296.14	0.9937	0.9955
Nitzschia closterium	y=71.17x+123.24	y=99.514x+115.71	0.9801	0.9803
Dunaliella salina	y=66.486x+162.29	y=89.543x+149.81	0.9935	0.9915
Platymanas sp.	y=27.771x+21.571	y=44.514x+17.048	0.9359	0.9591

As there were differences among the six algal species, therefore, their sensitivity of CO_2 enrichment on biomass increasing were different. The rates of regressive slope for the six algal species are shown in Table 2. The order of regressive slope rate was *Platymanas* sp., *Platymanas subcordiformis*, *Nitzschia*

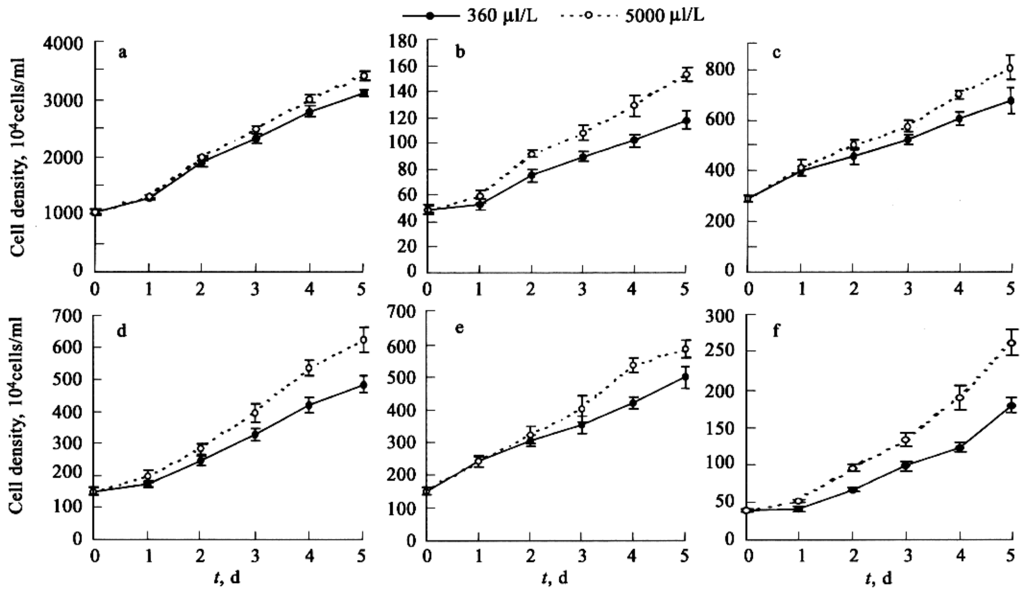

Fig.1 Effects of elevated CO_2 on the growth of *Chlorella* sp.(a), *Platymanas subcordiformis*(b), *Isochrysis galbana* Parke 8701(c), *Nitzschia closterium* (d), *Dunaliella salina*(e) and *Platymanas* sp.(f)

closterium, *Isochrysis galbana* Parke 8701, *Dunaliella salina*, and *Chlorella* sp. *Platymanas* sp. was the most sensitive to CO_2 enrichment; while *Chlorella* sp. was the least.

Table 2 Ratio of regressive slope for the six algal species grown at elevated CO_2 and ambient CO_2

Algal species	Rate of regressive slope	Ratio
Chlorella sp.	497.46 : 433.89	1.15
Platymanas subcordiformis	21.314 : 14.486	1.47
Isochrysis galbana Parke 8701	100.34 : 74.2	1.35
Nitzschia closterium	99.514 : 71.171	1.40
Dunaliella salina	89.543 : 66.486	1.35
Platymanas sp.	44.514 : 27.771	1.60

2.2 Effects of CO_2 enrichment on interspecific competition in three algal species under the condition of mixed cultivation

2.2.1 Effects of ambient CO_2(360 μl/L)

Fig.2 shows that during the population increasing course of control groups (bubbling with air), population increasing dynamic of *Isochrysis galbama* Park 8701 was weakly competitive mode. The increasing time was only limited on the former 5 d, the later 16 d it decreased continuously, the competitive ability was relatively weaker. The proportion of cell number of *Isochrysis galbana* Parke 8701 occupied total community cell number was ascended at the beginning and then descended, became dominant algae on the day 2 (the proportion was 45.5%), subsequently, the cell number began to decline slowly and on the day 21, the proportion of cell number occupied total community cell number was the least (the proportion was 14.6%), the population increasing dynamic of *Nitzschia clostertium* was strong

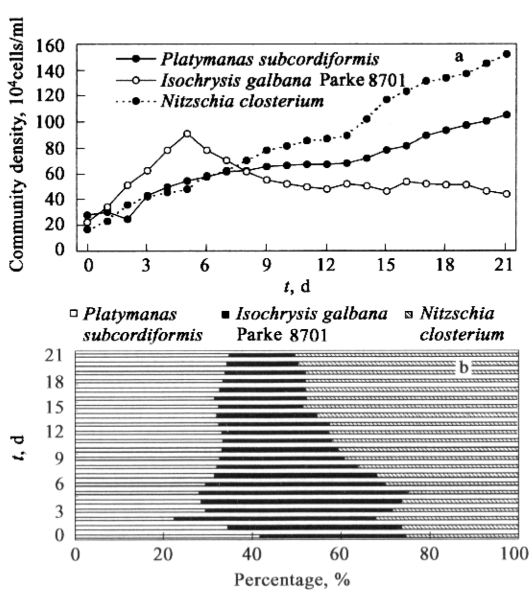

Fig.2 Population increasing dynamics(a) and quantitative percentage (b) of different algae in ambient CO_2(360 μl/L)

competitive mode. On the day 21, *Nitzschia clostertium* showed continuous increasing mode, the proportion of cell numbers of *Nitzschia clostertium* occupied total community cell number increased as time prolonged, on the day 8, cell number was occupied predominance (the occupied proportion was 36.3%), on the day 21, the cell density of *Nitzschia clostertium* was much bigger than the other two algae, *Nitzschia clostertium* was the absolutely dominant algae in algae community (the occupied proportion was 50.5%). The population increasing dynamic of *Platymonas subcordiformis* was increasing type. Namely, in 3 weeks, population was increased continuously. On the day 21, the proportion of

Platymonas subcordiformis cell number occupied total community cell number (the occupied proportion was 34.9%) was between *Nitzschia clostertium* and *Isochrysis galbana* Parke 8701, became the sub-dominant algae. Above all, in the primary of experiment, the nutrient was relatively abundant, the growth of *Isochrysis galbana* Parke 8701 was rapid, then became dominant algae in the community, but as time prolonged, nutrient maybe limited, competition occured among the population, *Nitzschia clostertium* finally obtained predominance, became the dominant algae, *Isochrysis galbana* Parke 8701 became inferior algae, while *Platymonas subcordiformis* turned from inferior algae into sub-dominant algae.

2.2.2 Effects of elevated CO_2 (5000 μl/L)

Fig.3 shows that when bubbled with elevated CO_2, cell densities of the three species marine microalgae all ascended greatly, on the day 21, the cell numbers of *Platymonas subcordiformis*, *Isochrysis galbana* Parke 8701, *Nitzschia clostertium*, increased by 55.2%, 93.2%, 114.6% respectively, compared to control groups(bubbling with air), and the competitive balance among population have changed. Therefore, when the three species of algae were mixed cultured, the sensitivity order of elevated CO_2 on three algal species was *Nitzschia clostertium*, *Platymonas subcordiformis*, *Isochrysis galbana* Parke 8701, *Nitzschia clostertium* was the most sensitive to CO_2 enrichment. The specific growth rate of *Nitzschia clostertium* on the day 5 (relative to the initial state(0 d)) under the condition of mixed cultivation (which was 0.409) was bigger than that of under the condition of solitary cultivation(which was 0.283). From the day 4, the cell density of *Nitzschia clostertium* ascended greatly, became dominant algae,on the day 21, the proportion of cell number occupied total community cell number was the biggest (the occupied proportion was 55.6%). Under the condition of CO_2 enrichment, biomass of *Isochrysis galbana* Parke 8701 was ascended slowly at the beginning, on the day 8, began to decline, on the day 13, began to ascend slowly again, on the day 21, the cell number was 85×10^4 cells/ml. In the control groups, the biomass of *Isochrysis galbana* Parke 8701 began to decline after the day 5, under the condition of CO_2 enrichment, the biomass of *Isochrysis galbana* Parke 8701 began to increase till to the day 8, subsequently declined slowly, but after the day 13, began to decrease. However, on the condition of solitary cultivation, the cell density of *Isochrysis galbana* Parke 8701 increased continuously with the time prolonged. The specific growth rate of *Isochrysis galbana* Parke 8701 on the day 5(relative to the initial state(0 d)) under the condition of mixed cultivation (which was 0.155) was smaller than that of under the condition of solitary cultivation (which was 0.204). Therefore, in mixed cultivation, at the experimental anaphase, nutrient maybe relatively short, *Isochrysis galbana* Parke 8701 showed weak competition, so the cell proportion began to decrease, CO_2 enrichment can make up the nutrient limitation on *Isochrysis galbana* Parke 8701, while *Platymonas subcordiformis* was the least sensitive to high CO_2, increasing dynamic was a continuous increasing mode, during the 21 d of the experiment, biomass was increased continuously and stably. On the day 21, the proportion of cell number occupied the total community cell number was 27.7%, became the sub-dominant algae.

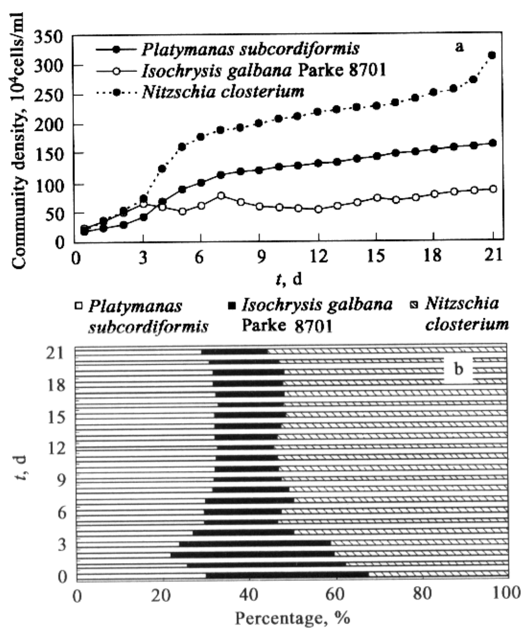

Fig.3 Population increasing dynamics(a) and quantitative percentage (b) of different algae in elevated CO_2(5000 μl/L)

3 Discussion

3.1 High concentration of CO_2 promotes the growth of microalgae

High concentration of CO_2 can promote (Gao *et al.*, 1993), inhibit (Israel *et al.*, 1999) or does not affect(Andria *et al.*, 1999) macroalgae. These different results may be due to different algal species and experimental conditions. In our experiment, CO_2 enrichment promoted the growth of the six species of marine microalgae, which has been verified in many previous studies(Liang and Yonemoto, 1999; Xia and Gao, 2001; Nobutaka *et al.*, 1992). Liang and Yonemoto (1999) found that CO_2 enrichment prolonged exponential growth phase, while Lin(1991) found that the generation time of *Spirulina subsalsa* shortened significantly when 2.5% CO_2 was added in the culture. Possibly, the CO_2 enrichment made the chloroplast more developed (Xia and Gao, 2002), and furthermore both the photosynthesis and cell division rate were increased. Therefore, the algal cell density was enhanced.

3.2 Sensitivity of microalgae to CO_2 enrichment

Because of different species of algae, the sensitivities of the six species of algae to CO_2 enrichment were different. Therefore, the increasement of cell number was different showing that the sensitivity order was *Platymanas* sp., *Platymonas subcordiformis*, *Nitzschia clostertium*, *Isochrysis galbana* Parke 8701, *Dunaliella salina*, *Chlorella* sp., from sensitive to less sensitive. Mixed cultivation changed the sensitivity of CO_2 enrichment on marine microalgae. Under the condition of mixed cultivation, the sensitivity order of elevated CO_2 on the three species of algae was *Nitzschia clostertium*, *Platymonas subcordiformis*, *Isochrysis galbana* Parke 8701, from sensitive to less sensitive. However, under the condition of solitary cultivation, the sensitivity order of elevated CO_2 on the three species of algae was *Platymonas subcordiformis*, *Nitzschia clostertium*, *Isochrysis galbana* Parke 8701, from sensitive to less sensitive. Although *Nitzschia clostertium* was less sensitive than *Platymonas subcordiformis* to high concentration of CO_2 under the condition of solitary cultivation, *Nitzschia clostertium* finally became the dominant algae, while *Platymonas subcordiformis* was sub-dominant algae since *Nitzschia clostertium* had strong competitive ability in mixed cultivation. Dukes (2002) reported that *Centaurea* responsed strongly to CO_2 enrichment under the condition of solitary cultivation, while mixed cultivation with acrawl meadow species, the increasement was not significant. Therefore, the responses of plants to CO_2 enrichment were different under the conditions of solitary cultivation and mixed cultivation. The changed sensitivity could be the cause of the sensitivity of algae to CO_2 enrichment in mixed cultivation.

3.3 Effects of CO_2 enrichment on interspecific competition

The elevated CO_2 may change population constitution of ecology system by changing atmospheric climate or even more directly be in favor of some photosynthetic approaches (Arp *et al.*, 1993) or by changing the quantity in ecology system. The elevated CO_2 will enhance net N(Hungate *et al.*, 1997) and plants can use these resources and gain the competitive predominance to CO_2 enrichment. Studies of different CO_2 concentrations on the changes of community constitutes verified that the growth rate and composition proportion of the community composed of five kinds of different plant seedlings all have significant change, after they were put into low and relative high concentration of CO_2 for 120 d (Bazzaz *et al.*, 1992).

In marine ecological system, the sensitivity differences of algae to elevated CO_2 can easily cause change of interspecific competition and community structure, and affect the whole aquatic ecological system.

On the day 21 of the experiment, all the three species of algae, the dominant algae, sub-dominant algae and inferior algae grown in high concentration of CO_2 did not change, compared to those grown in low concentration of CO_2. However, the growth rate and composition proportion of the three species of algae changed significantly. Among them, the growth rate increased, while on the day 21, the proportion of *Nitzschia closterium*, *Platymonas subcordiformis*, *Isochrysis galbana* Parke 8701 changed in turn from 50.5%, 34.9%, 14.6% to 55.6%, 27.7%, 16.7%, *Nitzschia clostertium* increased by 5.1% ($P<0.05$), *Platymonas subcordiformis* decreased by 7.2% ($P<0.05$), *Isochrysis galbana* Parke 8701 decreased by 2.1%($P>0.05$). The growth dynamics of three species of algae changed, respectively.

4 Conclusions

The results demonstrated that elevated CO_2 did not change the dominant, sub-dominant and inferior species of algae. This may be due to the CO_2 concentration or other unknown cause. In actual environment, although CO_2 concentration elevated it is still not high enough compared to the CO_2 concentration used in our experiment. Therefore, the experimental results can not completely deduce to actual environment, other environmental factors (e.g. UV-B radiation, heat shock, pathogen, toxicity and so on) should be synthetically considered and there is much still to be done.

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