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Effects of lanthanum(III) and EDTA on the growth and competition of *Microcystis aeruginosa* and *Scenedesmus quadricauda*

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Abstract

Rare earth elements (REEs) are widely used to increase crop production in China. However, little attention has been paid to their impacts on aquatic ecology. Batch cultivation was used here to study the effects of lanthanum (La) and EDTA on the growth and competition of the cyanobacterium *Microcystis aeruginosa* and the green alga *Scenedesmus quadricauda*. When EDTA was present at a very low concentration ($0.269\,\mu\text{mol}\,\text{L}^{-1}$), low lanthanum concentrations ($\leqslant 7.2\,\mu\text{mol}\,\text{L}^{-1}$) had little stimulative effect on the growth of *M. aeruginosa* and *S. quadricauda*, whereas a high lanthanum concentration ($72\,\mu\text{mol}\,\text{L}^{-1}$) had significant inhibitory effect on both of them. The results of cultivation experiments suggested that the inhibitory effect on *M. aeruginosa* was higher than that on *S. quadricauda* and *S. quadricauda* could become dominant in mixed cultures. When lanthanum was not added to the culture medium, high EDTA concentrations ($>13.4\,\mu\text{mol}\,\text{L}^{-1}$) had a great inhibitory effect on the growth of *M. aeruginosa* but little effect on the growth of *S. quadricauda*, which could become dominant in the mixed cultures.

Lanthanum and EDTA had complex effects on the growth and competition of *M. aeruginosa* and *S. quadricauda.* EDTA did not change the stimulation of low lanthanum concentrations on both, but at intermediated concentrations (2.69–$13.4\,\mu\text{mol}\,\text{L}^{-1}$) it could greatly alleviate lanthanum inhibition on *M. aeruginosa*; thus, *M. aeruginosa* would dominate *S. quadricauda* in these mixed cultures. Lanthanum at low concentration ($7.2\,\mu\text{mol}\,\text{L}^{-1}$) could also alleviate the inhibition of high EDTA on *M. aeruginosa*, but did not alter the outcome of the competition.

Keywords: Lanthanum; Rare earth elements; *Microcystis*; *Scenedesmus*; Competition

Introduction

Rare earth elements (REEs) have been widely used for decades as fertilizer to increase the crop production in China (Hu et al. 2004; Qiu et al. 2004; Wang et al. 2006). As a result, more and more REEs are accumulated into aquatic environments. Studies have found that low REEs concentrations can stimulate land plant growth, accelerate their uptake of nutrients, and improve their photosynthesis and tolerance (Xie et al. 2002; Wang et al. 2003; Hu et al. 2004; Ge et al. 2006). Since REEs have substantial effects on the growth of land plants, their impacts on aquatic ecology become a concern (Sun et al. 1997; Yang et al. 1999). The response of

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phytoplankton, which is the primary producer, to REEs has attracted much more attention than other aquatic cultures.

Studies have found that low concentrations of REEs can stimulate phytoplankton growth to different extent. Some researches suggested that low concentrations of lanthanum had a great stimulation on the growth of the cyanobacterium *Microcystis aeruginosa* Kütz (Qian et al. 2003), the green algae *Selenastrum capricornutum* Printz (Yin et al. 1998), the dinoflagellate *Alexandrium tamarense* (Lebour) Balech (Xing et al. 2002), etc. However, other studies reported that the stimulation of low concentration lanthanum on phytoplankton such as *M. aeruginosa* (Zhou et al. 2003), *Chlamydomonas reinhardtii* Dang. (Shi and Zhao 1987) and *Chlorella vulgaris* Beij. (Wang et al. 1996) was minor. Moreover, some studies did not find any stimulation of REEs on algae such as *Chlorella ellipsoidea* Gren. (Hu et al. 1996).

A number of experiments have suggested that high concentrations of lanthanum inhibit the growth of phytoplankton. Lin et al. (2003) indicated that lanthanum inhibited the *Microcystis* growth in batch culture experiments when its concentration was higher than 104 μmol L^{-1}; Song and Hu (2000) suggested that lanthanum inhibited algal growth when its concentration was higher than 72 μmol L^{-1}; Shi and Zhao (1987) reported that lanthanum began to inhibit the growth of *C. reinharditi* when the concentration reached 144 μmol L^{-1}.

Compared to the effect of total metal concentration, the metal speciation is of greater importance on the bioavailability (Stumm and Morgan 1996). Orgainc ligands, mainly aquatic humic substances (AHSs), exist universally in natural water. They have a great influence on metal speciation, and thus on the phytoplankton growth. Many studies have investigated the influence of organic ligands on several metals and their availability to phytoplankton. For example, the complexation of iron with strong chelators will result in iron limitation and algal growth inhibition (Li et al. 1996; Imai et al. 1999; Gress et al. 2004).

REEs are transition metals, and trivalent REEs have high tendency to form complexes in natural water (Diatloff et al. 1993; Johannesson and Lyons 1994). Sun et al. (1997) demonstrated that REEs complexed with strong chelating ligands are less available to *C. vulgaris*. However, studies on the influence of REEs alone with organic ligands on algal growth are quite limited.

Cyanobacterial blooms often cause serious problems in natural freshwater systems, such as unpleasant odors, water deoxygenation and clogging of water treatment systems. However, green algal blooms are not so bothering. Up to now, there are few researches on the complex effects of REEs and organic ligands on the growth competition of cyanobacteria and green algae. EDTA is a typically non-specific complexing ligands like AHSs. The objective of this study is to investigate the effects of lanthanum(III) and EDTA on the growth competition of cyanobacterium *M. aeruginosa* and green algae *S. quadricauda*.

Materials and methods

Algae

M. aeruginosa Kütz (FACHB-912) was supplied by Freshwater Algae Culture Collection, Institute Hydrobiology (FACHB), Chinese Academy of Sciences. It was originally isolated from Lake Taihu. *Scenedesmus quadricauda* (Turp.) Brèb. were isolated from Lake Taihu in 2003. They were maintained at 28 °C under a 12 h light:12 h dark cycle in M_{11} medium, which consists of 1180 μmol $NaNO_3$, 57.5 μmol K_2HPO_4, 305 μmol $MgSO_4 \cdot 7H_2O$, 272 μmol $CaCl_2 \cdot 2H_2O$, 189 μmol Na_2CO_3, 18 μmol Fe-citrate and 2.69 μmol $Na_2EDTA \cdot 2H_2O$ in 1 L deionized water. Both cultures were not axenic, but the bacterial biomass was low (<2%) during cultivation. Lake Taihu is located in the east of China between 30°55′42″–31°33′50″N, and 119°53′45″–126°36′15″E, with a area about 2338 km^2 and a mean depth of 1.9 m. Now it suffered serious eutrophication.

Lanthanum stock solution

A stock solution of 7200 μmol L^{-1} La(III) was prepared by dissolving $La(NO_3)_3 \cdot 6H_2O$ into deionized water. Its pH was adjusted to 2 by adding 0.5 μmol L^{-1} HCl. The stock solution was kept at 4 °C in refrigerator before use.

Growth experiments

M. aeruginosa and *S. quadricauda* were grown in M_{11} medium with some modification on Fe-citrate and EDTA concentration. The concentration of Fe-citrate was decreased to 3 μmol L^{-1} to reduce the influence of citrate on the speciation of lanthanum. The effects of lanthanum at 0, 0.72, 7.2 and 72 μmol L^{-1} (0, 0.1, 1 and 10 mg L^{-1}) were investigated, and the concentrations of EDTA varied from 0.269 to 26.9 μmol L^{-1}. The initial pH was adjusted to 8.0 with 0.5 mol L^{-1} HCl or NaOH.

Cultivations were performed in 500 mL conical flasks (200 mL culture medium) in triplicate at 28 °C, 25 μmol photons m^{-2} s^{-1} under a 12 h light:12 h dark cycle. The inoculation density was 1×10^4 cells mL^{-1}. Flasks were shaken by hand and then their places were changed randomly two times a day. Cell density was counted with a haemacytometer (TATAI, Minatos) under a microscope, and about 30–300 cells were counted once. Counting was carried out three times for each sample.

Statistical analyses

T-test for multiple pairwise comparisons was performed to determine the significance of growth curves at different conditions.

Results

Effects of EDTA on the growth and competition of *M. aeruginosa* and *S. quadricauda*

Without the addition of lanthanum, *M. aeruginosa* grew very well in single cultures when EDTA concentrations were lower than $2.69\,\mu\text{mol}\,\text{L}^{-1}$, but the growth was suppressed when EDTA concentrations were higher than $13.4\,\mu\text{mol}\,\text{L}^{-1}$ (Fig. 1A). In contrast, under the same conditions, *S. quadricauda* grew well, in which the growth curves did not show a significant difference when EDTA concentrations changing from 0.269 to $26.9\,\mu\text{mol}\,\text{L}^{-1}$ (Fig. 1B) ($P = 0.2832$, 0.1341, and 0.791, when EDTA increased from $0.269\,\mu\text{mol}\,\text{L}^{-1}$ to 2.69, 13.4, and $26.9\,\mu\text{mol}\,\text{L}^{-1}$, individually; $P > 0.05$, paired test). It suggested that high EDTA concentrations ($\geqslant 13.4\,\mu\text{mol}\,\text{L}^{-1}$) could inhibit the growth of *M. aeruginosa*, but not the growth of *S. quadricauda*.

The growth competition of *M. aeruginosa* and *S. quadricauda* in mixed cultures indicated that neither of them was affected by EDTA at concentration of 0.269 and $2.69\,\mu\text{mol}\,\text{L}^{-1}$, but only *S. quadricauda* grew well at EDTA concentrations from 13.4 to $26.9\,\mu\text{mol}\,\text{L}^{-1}$ (Fig. 2, A1–A4). It might be deduced that under high EDTA concentrations ($\geqslant 13.4\,\mu\text{mol}\,\text{L}^{-1}$) *S. quadricauda* would become the dominant species.

Effects of lanthanum on growth and competition of *M. aeruginosa* and *S. quadricauda* at different EDTA concentrations

When EDTA concentration was low ($0.269\,\mu\text{mol}\,\text{L}^{-1}$), low lanthanum concentrations ($\leqslant 7.2\,\mu\text{mol}\,\text{L}^{-1}$) had no stimulative effect on the growth of *M. aeruginosa* and *S. quadricauda* in single cultures; however, a high lanthanum concentration ($72\,\mu\text{mol}\,\text{L}^{-1}$) significantly inhibited the growth of *M. aeruginosa* and *S. quadricauda*, and the inhibitory effect on *M. aeruginosa* was greater (Fig. 3A and E).

Growth competition of *M. aeruginosa* and *S. quadricauda* in mixed cultures indicated that neither of them became dominant when lanthanum concentrations were low ($\leqslant 7.2\,\mu\text{mol}\,\text{L}^{-1}$), whereas *S. quadricauda* was the superior species in high lanthanum concentrations, $72\,\mu\text{mol}\,\text{L}^{-1}$ (Fig. 2, A1–D1).

Interaction of lanthanum and EDTA on the growth and competition of *M. aeruginosa* and *S. quadricauda*

At elevated EDTA concentrations, the effects of lanthanum on the growth of *M. aeruginosa* and *S. quadricauda* may change greatly (Fig. 3). The observed inhibition by high lanthanum concentrations on *M. aeruginosa* growth in the cultures of $0.269\,\mu\text{mol}\,\text{L}^{-1}$ EDTA was greatly alleviated when EDTA concentration was raised to $2.69–13.4\,\mu\text{mol}\,\text{L}^{-1}$. Furthermore, in cultures containing more than $13.4\,\mu\text{mol}\,\text{L}^{-1}$ EDTA,

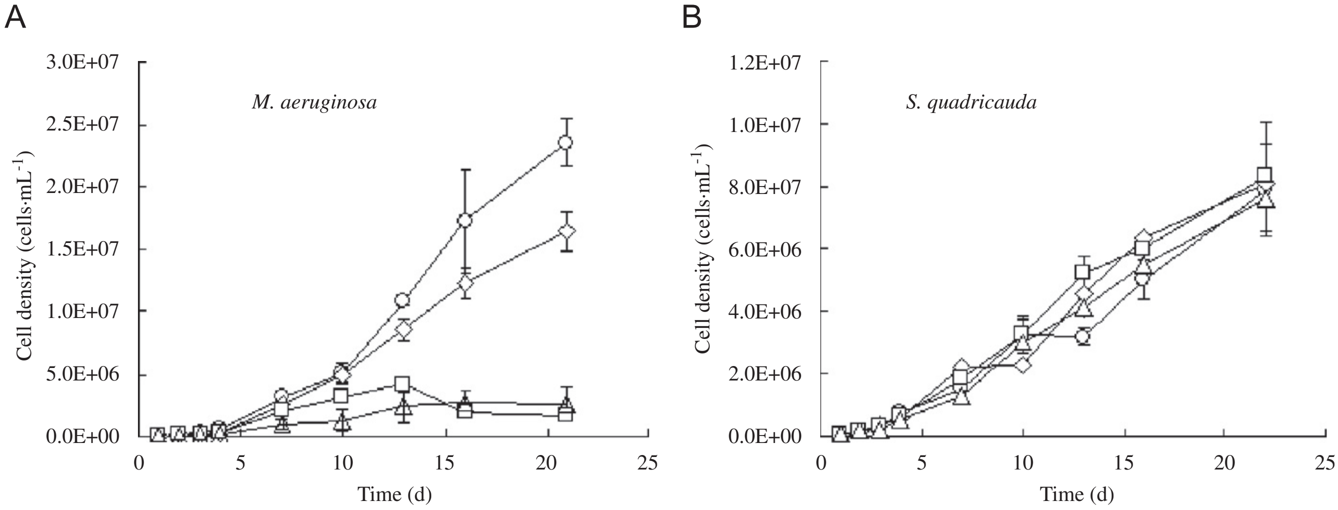

Fig. 1. Effects of EDTA on the growth of *M. aeruginosa* and *S. quadricauda* in single cultures (without lanthanum). (○) EDTA, $0.26\,\mu\text{mol}\,\text{L}^{-1}$; (◇) EDTA, $2.6\,\mu\text{mol}\,\text{L}^{-1}$; (□) EDTA, $13.4\,\mu\text{mol}\,\text{L}^{-1}$; (△) EDTA, $26.9\,\mu\text{mol}\,\text{L}^{-1}$.

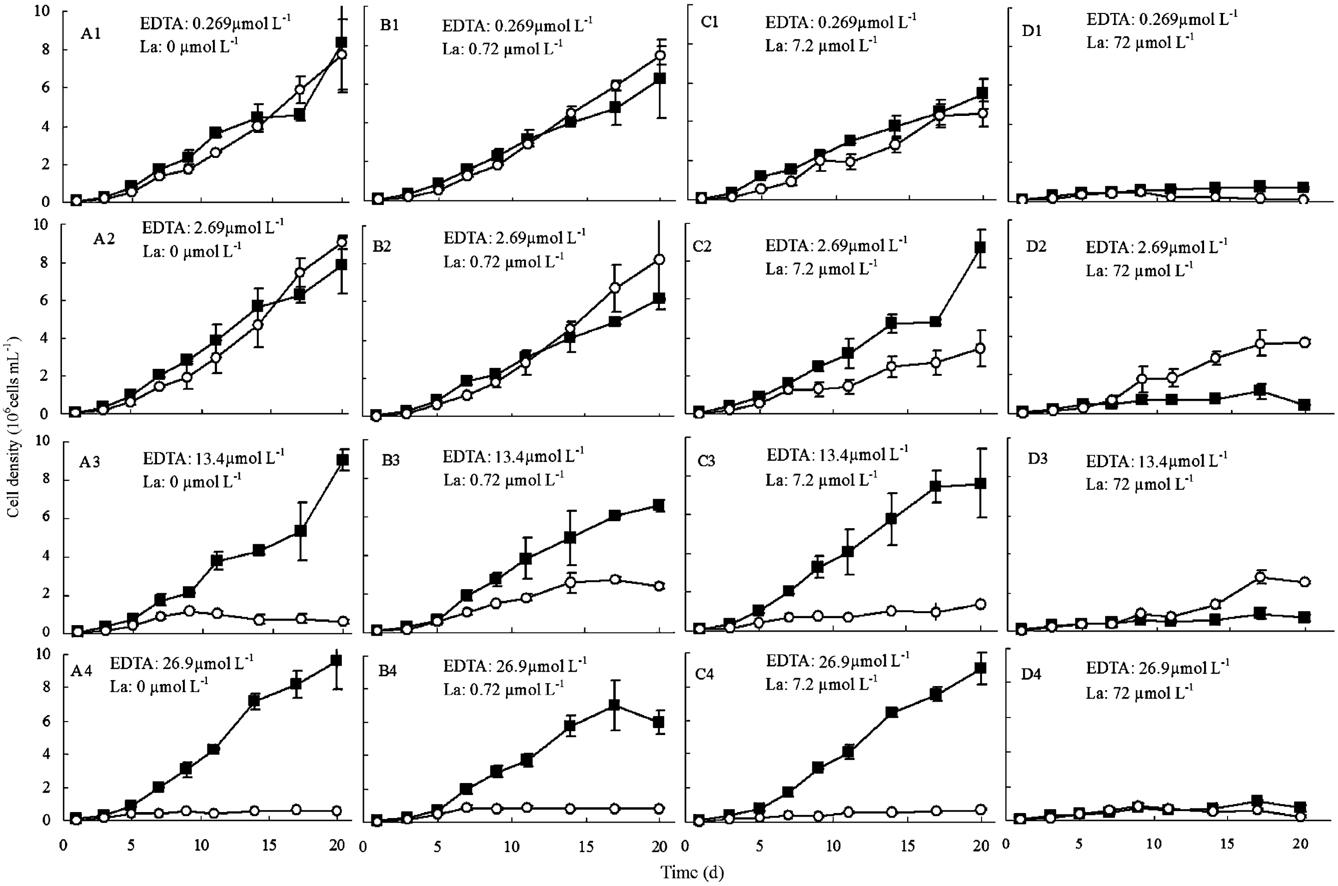

Fig. 2. Effects of lanthanum (0–72 μmol L^{-1}) on the competition of *M. aeruginosa* and *S. quadricauda* in mixed cultures at different EDTA concentrations (0.269–26.9 μmol L^{-1}). (○) *M. aeruginosa*; (■) *S. quadricauda*.

low lanthanum concentrations ($\leqslant$7.2 μmol L^{-1}) had slightly stimulative growth effect on both *M. aeruginosa* and *S. quadricauda*; their maximum biomasses (the former 20 days) were higher than those with low EDTA concentration (0.269 μmol L^{-1}).

The growths of *M. aeruginosa* and *S. quadricauda* in the mixed cultures are similar to single cultures. When both EDTA and lanthanum levels were low (Fig. 2, A1–C1, A2–C2), both algae could grow well and their maximum biomasses were comparable; since the EDTA greatly alleviated high lanthanum inhibition to *M. aeruginosa* but evidently not to *S. quadricauda*, *M. aeruginosa* could became the dominant species at high lanthanum levels (72 μmol L^{-1}) when EDTA was raised to 2.69–13.4 μmol L^{-1} (Fig. 2, D2 and D3); although low lanthanum concentration slightly alleviated the inhibitory effect of high EDTA concentration, *S. quadricauda* still outcompete *M. aeruginosa* in the experiments where lanthanum changed from 0.72 to 72 μmol L^{-1} when EDTA was at 13.4–26.9 μmol L^{-1}, (Fig. 2, A3–C3, A4–C4). When both EDTA and lanthanum were high, neither *M. aeruginosa* or *S. quadricauda* grew well.

Discussion

In M$_{11}$ medium, lanthanum is mainly present as La^{3+}, LaOH^{2+}, LaCO$_3^+$, LaPO$_4^0$, and LaEDTA species. When EDTA concentration is low (0.269 μmol L^{-1}), the main species of lanthanum are La^{3+}, LaOH^{2+}, LaCO$_3^+$, and LaPO$_4^0$, which are non-chelated species. In these cultures, the findings that low lanthanum concentrations had no stimulative effect on *M. aeruginosa* and *S. quadricauda* are different from those of most previous studies showing that low lanthanum concentrations have stimulative effect on algal growth (Yin et al. 1998; Xing et al. 2002; Qian et al. 2003). In cultures of higher EDTA concentrations, the observed stimulative effect of lanthanum is similar to that from previous studies.

One likely reason for observed difference is that the effect of lanthanum on phytoplankton growth is dependent on the concentration of chelating organic ligands. On one hand, trivalent lanthanum has a high affinity to form complexes with organic ligands, which will influence its availability to phytoplankton (Sun et al. 1997). Similarly, the complexation of organic ligands may also influence the activity of organic ligands.

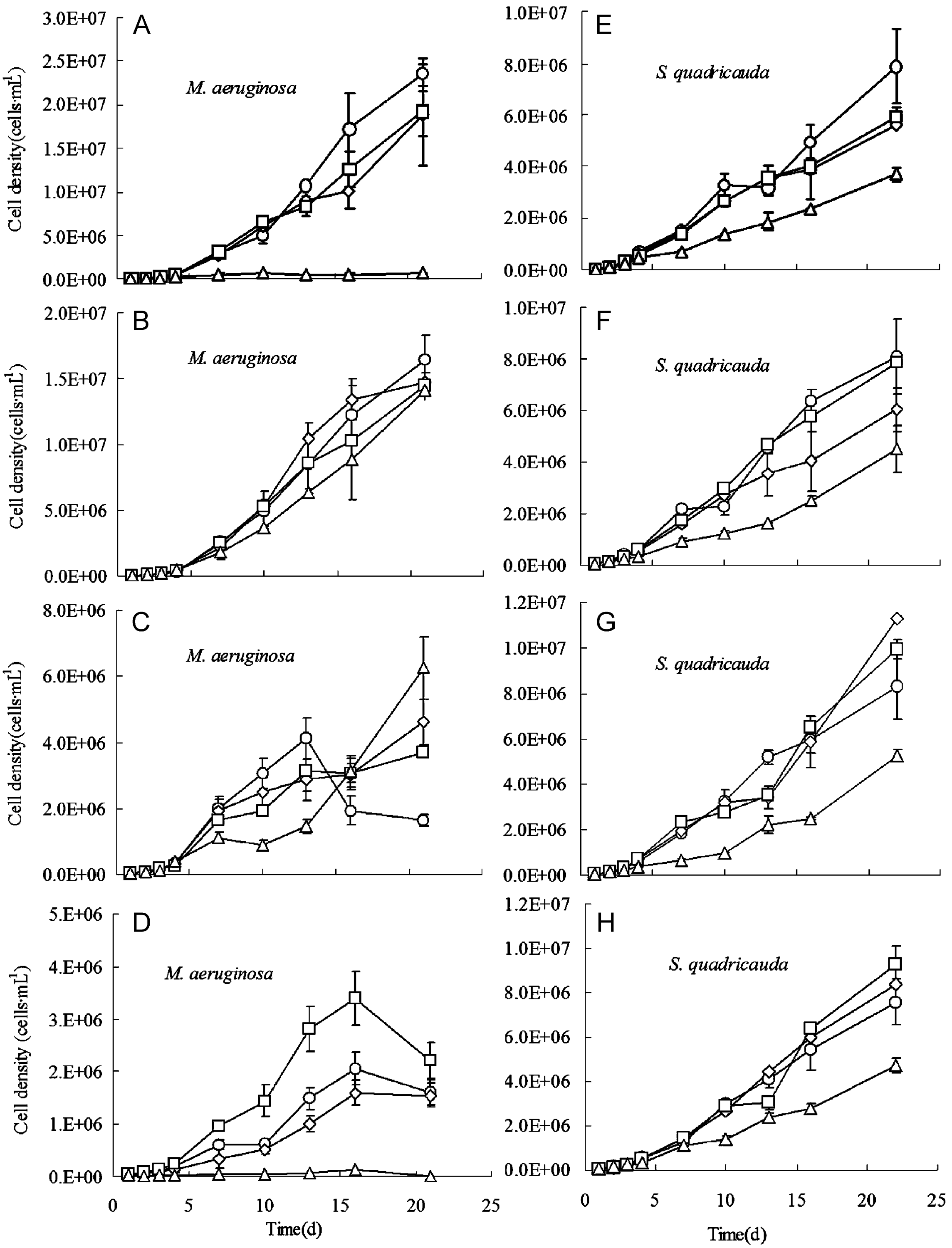

Fig. 3. Effects of lanthanum (0–72 μmol L^{-1}) on the growth of *M. aeruginosa* and *S. quadricauda* in single cultures at different EDTA concentrations (0.269–26.9 μmol L^{-1}). (○) La, 0 μmol L^{-1}; (◇) La, 0.72 μmol L^{-1}; (□) La, 7.2 μmol L^{-1}; (△) La, 72 μmol L^{-1}. (A, E) EDTA, 0.269 μmol L^{-1}; (B, F) EDTA, 2.69 μmol L^{-1}; (C, G) EDTA, 13.4 μmol L^{-1}; (D, H) EDTA, 26.9 μmol L^{-1}.

Low concentrations of EDTA can increase the solubility of many metals, and thereby, may increase their availability. For lanthanum, which has high solubility in neutral and weak alkaline solutions with a low hydrolysis, the added EDTA at 0.269–2.69 μmol L^{-1} will not likely increase the lanthanun stimulative effect on the growths of both *M. aeruginosa* and *S. quadricauda*. Sun et al. (1997) suggested that bioconcentration ability of La^{3+} with algae is higher than that of La-EDTA. It implies that La^{3+} is more available to algae than La-EDTA. Consequently, lanthanum, chelated with EDTA, would not increase its stimulative effect. At higher concentrations of EDTA, the probable reason that low lanthanum concentration (7.2 μmol L^{-1}) had stimulative effect on phytoplankton growth is that lanthanum changed the activity of EDTA.

In the present study, high EDTA concentrations (>13.4 μmol L^{-1}) greatly inhibited the growth of *M. aeruginosa*, but not *S. quadricauda*. EDTA had great influence on the speciation of metals other than REEs. Many studies have reported that EDTA and other organic ligands in natural water are important to the uptake of metal micronutrients, especially iron, by phytoplankton (Muggli and Harrison 1996; Gerringa et al. 2000; Matz et al. 2004). EDTA could increase the solubility of ferric oxide and thereby increase the iron availability to algae when EDTA concentration is low, but it chelates iron strongly and may decrease iron availability to some algae when EDTA concentration is high (Gerringa et al. 2000).

The effects of organic ligands on algal growth were species specific. Parparova and Yacobi (1998) added another kind of organic ligand, 8-hydroxyquinoline (8HQ) to lake water from Lake Kinneret where heavy cyanobacterial bloom occurred. They found that the photosynthesis of the cyanobacterium *M. aeruginosa* was most strongly inhibited by 8HQ additions. The result of present study that high EDTA concentrations inhibited the growth of *M. aeruginosa* but not *S. quadricauda* is similar to the results of Parparova's experiments. An explanation for this is that cyanobacteria could hardly use the iron bound with organic ligands while green algae could.

As lanthanum can chelate trivalent lanthanum with high affinity, the reason that low lanthanum concentration has stimulative effect in higher EDTA concentration is more likely that lanthanum decreased the complexiation capacity of organic ligand rather than that lanthanum is necessary to phytoplankton growth.

On the other hand, lanthanum, complexed with EDTA could alleviate its inhibition on phytoplankton growth. In the present study, intermediate EDTA concentrations of 2.69–13.4 μmol L^{-1} greatly alleviated high lanthanum inhibition on *M. aeruginosa*. But at higher EDTA concentrations (>26.9 μmol), growth inhibition reoccurred.

In natural water, aquatic humic substances (AHSs) are typically non-specific complexing ligands (Stumm and Morgan 1996), there by, their behaviors are similar to the ligand, EDTA. AHSs are composed of fulvic acid and humic acid, and 30–80% of which is dissolved organic carbon (DOC). AHSs are the largest fraction of natural organic matter in most water bodies (Thurman 1985). DOC in natural water varied in orders of magnitude, which maybe change from less than 1 mg L^{-1} to hundreds of mg L^{-1} (Steinberg et al. 2006). As molecular weight of dissolved AHSs usually less than 1000, it molar concentration may change from less than 1 μmol L^{-1} to hand hundreds μmol L^{-1}. The organic ligands concentrations covered EDTA concentration tested. Consequently, when lanthanum gets into water environments, it may interact with AHSs and play an important role in the competition of *M. aeruginosa* and *S. quadricauda*.

M. aeruginosa and *S. quadricauda* are typical cyanobacterium and green algae in shallow eutrophic lake (e.g. Lake Taihu), in which *M. aeruginosa* always form surface blooms while *S. quadricauda* do not. There are several hypotheses for the dominance of cyanobacteria in freshwater, including nutrient resource ration (N:P) competition (Smith 1983; Takamura et al. 1992), differential light requirements (Zevenboom and Mur 1980), CO_2 competition (Shapiro 1990), buoyancy regulation (Walsby 1994), high temperature tolerance (Robarts and Zohary 1987), zooplankton predation (Fulton and Pearl 1987), superior cellular nutrient storage (Pettersson et al. 1993), suppression of other algae by excretion organic compounds (Matz et al. 2004) and so on. The investigation of this study found that REE lanthanum, chelator EDTA and their interaction influence the competition of cyanobacteria and green algae. And when high concentration lanthanum was added to intermediate concentration organic ligand media, the condition favors the dominance of *M. aeruginosa*. Some lakes of China, such as Lake Taihu, REEs concentration have been accumulated in sediments. The summation concentration of REEs in the inflow river is up to 695 mg kg^{-1} (5000 μmol kg^{-1}) (Li 2006). Therefore, addition of REEs to agricultural fertilizer may affect the community structure of phytoplankton and the cyanobacterial blooms.

Conclusions

1. When lanthanum was not added, high EDTA concentrations ($\geqslant 13.4$ μmol L^{-1}) exhibited significant inhibitory effect on the growth of *M. aeruginosa*, but had no effect on the growth of *S. quadricauda*, which indicates that high EDTA concentration favors the dominance of *S. quadricauda.* It also implies that high AHSs concentration have the same effects as high EDTA concentration.
2. When EDTA was present at a low concentration (0.269 μmol L^{-1}), low lanthanum concentrations ($\leqslant 7.2$ μmol L^{-1}) had no stimulative effect on the growth of *M. aeruginosa* and *S. quadricauda*, but high lanthanum concentration (72 μmol L^{-1}) had great

growth inhibition on both of them and the inhibition on *M. aeruginosa* was greater.

3. Complexation of lanthanum with EDTA did not change the stimulative effect of low lanthanum concentrations ($\leqslant 7.2\,\mu\text{mol}\,\text{L}^{-1}$) for *M. aeruginosa* and *S. quadricauda*, but it can alleviate the inhibition effect of high EDTA concentration ($26.9\,\mu\text{mol}\,\text{L}^{-1}$) on *M. aeruginosa.*
4. Complexation of lanthanum with EDTA did not evidently change inhibitory effect of high lanthanum concentrations on *S. quadricauda*, but greatly changed the inhibitory effect on *M. aeruginosa.* EDTA at $2.69–13.4\,\mu\text{mol}\,\text{L}^{-1}$ can alleviate the inhibition of lanthanum greatly, which resulted in the dominance of *M. aeruginosa* on *S. quadricauda*. It implies that REEs fertilizer may favor cyanobacterial blooms when organic ligands in a certain concentration.

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