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Kinetic Studies on the Combined Effects of Lanthanum and Cerium on the Growth of *Microcystis aeruginosa* and their Accumulation by *M. aeruginosa*

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The rare earth elements (REEs) are abundant in China, and the REEs have been extensively used in industry and agriculture production for many years in China(Sun et al.1996; Yang et al. 1998). As a result, more and more dissoluble REEs are moving into aquatic environment through surface runoff. They will affect the growth of water-living creatures especially algae (Yang et al. 1999). Presently the algal blooms have already come into being in many fresh-water lakes in China, such as Taihu lake, Chaohu lake, Donghu lake and Dianchi lake and so on (Jin et al. 1990). It is very important to study the effects of REEs on the growth of algae in order to investigate the mechanism of the break out of algal blooms and to protect the aquatic environment and ecosystem. Some studies on the effects of REEs on the growth of algae have been reported. The effects of single and combined REEs on the growth of *Chlorella* and the effects of Lanthanum (La) and La-EDTA on the growth of *Chlorella* have also been researched (Hu et al. 1996 ; Wang et al. 1996). Those studied results showed that the growth of algae certainly was affected by REEs present in its growing environment. But most of these studies are on *Chlorophyta*, fewer on *Cyanobacteria*. In the recent years the *Cyanobacteria* blooms break out in most fresh-water lakes in China (Jin et al.1990). The *Microcystis aeruginosa* (*M. aeruginosa*)belongs to a kind of the *Cyanobacteria* distributes widely in nature and it is dominant algae in the algal blooms in many fresh-water lakes in China. It is necessary to study the effects of REEs on the growth of the *M. aeruginosa*. In this paper, we studied the kinetics of the combined effects of lanthanum(La) and cerium(Ce) on the growth of the *M. aeruginosa*. In fact, the algae will actively or passively accumulate REEs in its living environment. The accumulation kinetics of them by the *M. aeruginosa* were also studied in this paper. The results showed that the growth of the *M. aeruginosa* was accelerated in the combined system, and the second order kinetics adsorption model can explain the kinetics of sorption of lanthanum(La) and cerium(Ce) by the *M. aeruginosa.*

MATERIALS AND METHODS

A culture of freshwater blue green algae, *Microcystics aerugionosa* Kütz, was

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obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China. The algal were grown in HGZ medium at a temperature of 28℃±1℃, and a light intensity of 2000 lux on a 12 hr light-dark cycle, the solutions were adjusted to pH 8 and shaken manually four times a day. The concentration of the algal cells were measured by spectrophotometer at 663 nm. The relationship between the algal cell density (y) and its absorbance at 663nm (A) was a linear correlation ($y = 0.284 + 31.5A$, $r = 0.9992$, $P< 0.0001$). *M. aeruginosa* cell density were measured by spectrophotometer every 24 hr after inoculation.

In the experiment, the culture solutions were made-up by mixing the stock solutions of La^{3+} and Ce^{4+} with HGZ medium individually. The culture solutions contained La^{3+}+Ce^{4+}at initial concentrations of 0+0 (control) , 0.5 mg/L+0.05 mg/L, 0.5 mg/L+0.1 mg/L, 0.5 mg/L+0.2 mg/L, 1 mg/L+0.05 mg/L, 1 mg/L+0.1 mg/L, 1 mg/L+0.2 mg/L, 2 mg/L+ 0.05 mg/L, 2 mg/L+0.1 mg/L, 2 mg/L+0.2 mg/L were make-up in series. All of the samples were designed as parallel samples and were adjusted to pH 8. Each sample was sterilized, thereafter, proper amount of purified algae was inoculated into it and incubated under conditions of 28℃±1℃ and a light intensity of 2000 lux on a 12 hr light-dark cycle, and shaken manually fourth a day. The absorbance of each sample was measured periodically at 650 nm. In the combined system of La^{3+} and Ce^{4+}, proper amount of algal solutions (containing La^{3+}+Ce^{4+} at initial concentrations of 1 mg/L+0.05 mg/L, 1 mg/L+ 0.1 mg/L, 1 mg/L+0.2 mg/L, 0.5 mg/L+0.05 mg/L, 2 mg/L+0.05 mg/L) was drawn to centrifuge as soon as the absorbance of the solutions was measured. Abandoned the supernatant fluid and washed the surface of algae cells with redistilled water, then centrifuged again. Washed and centrifuged repeatedly for about 3 to 4 times to insure that there is no La^{3+} or Ce^{4+} on the surface of algae cells. The cell pellets were dried at 110 ℃ to a constant weight. After measuring the dry weight, the cells were digested with HNO_3 and $HClO_4$ at about 400℃. The digest was measured for the amount of La^{3+} and Ce^{4+} by ICP-AES (liberty Ax, Varian).

RESULTS AND DISCUSSION

The kinetics curves of the growth of the *Microcystis aerugionosa* at different initial concentrations of La^{3+} and Ce^{4+} in the system are shown in Figure1. The curves like curves of exponential function. Using the kinetic equation (1) to fit the curves of the growth of the algal cells.

$$N_t = N_0 e^{kt} \quad (1)$$

Where, N_t is the concentration of the algal cells at time t ($\times 10^6$ cell / mL) ; k is the growth rate constant of the *M. aerugionosa* (/d) ; N_0 is the extrapolate initial concentration of the algal cells ($\times 10^6$ cell / mL) ; t is time (d) . The equation (1) can be expressed as

$$\ln N_t = \ln N_0 + kt \quad (2)$$

The experimental and calculation values are shown in Table 1. The results showed that the growth rate constants (k) of the *M. aerugionosa* in the different combined systems of $La^{3+}$and $Ce^{4+}$are all greater than that of the control system, and the value of k in the combined system of La^{3+}(0.5 mg/L)and Ce^{4+}(0.05 mg/L) is the greatest among the systems. From

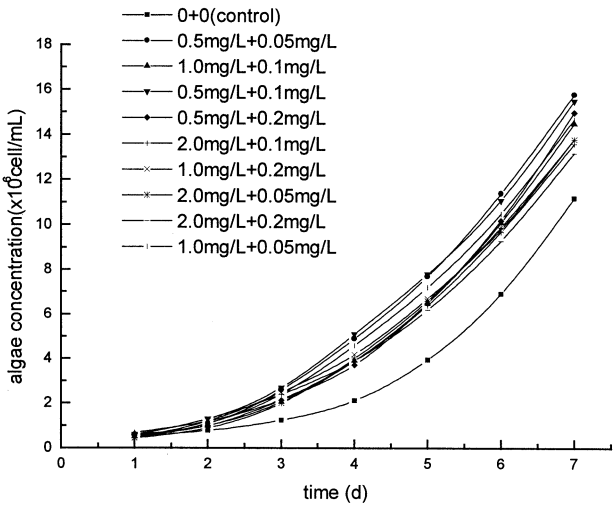

Figure 1. Kinetics curves of the *M. aerugionosa* growth under combined effects of La^{3+} and Ce^{4+}.

Table 1. Kinetic parameters of the *M.aerugionosa* growth under combined effects of La^{3+} and Ce^{4+} at 28℃.

La^{3+} (mg/L)	0	0.5	0.5	0.5	1	1	1	2	2	2
+	+	+	+	+	+	+	+	+	+	+
Ce^{4+} (mg/L)	0	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2
N_0 ($\times 10^6$cell/mL)	0.524	0.563	0.566	0.540	0.545	0.507	1.547	0.583	0.512	0.576
r^2	0.998	0.986	0.993	0.985	0.996	0.996	0.983	0.988	0.990	0.982
k(/d)	12.55	13.02	13.00	12.97	12.86	12.84	12.82	12.76	12.73	12.71

system of La^{3+}(0.5 mg/L)and Ce^{4+}(0.05 mg/L) is the greatest among the systems. From these results we can conclude that the growth of the *M. aerugionosa* was accelerated in the combined system.

The mechanism of the effects of La^{3+} and Ce^{4+} on the growth of *Microcystis* may be explained that the La^{3+} or Ce^{4+} could incorporate with phosphatide or transporting protein on algal cells membrane to improve the stability of cell membrane, and to prevent the nutrients in algal cells from osmosizing out and improve capacity for algae against adversity (Yuan et al. 1995). The mechanism also may be expressed that slight amount of La^{3+} or Ce^{4+} could improve or strengthen the structure, function and activity of photosynthesizing organs in algal cells, stimulate the metabolism and nutrient uptake of algal cells, and accelerate the growth of algae. Larger amount of La^{3+} and Ce^{4+} in algal cells can inhibit the activity of adenylate-cyclase, RNA-synthetase, collagenase and glutamate-synthetase, and inhibit the growth of algae by manners of competitively incorporating activity centers, displacing Ca^{2+} in activity centers and competing with or displacing Mg^{2+} in the algal cells (Yang et al.

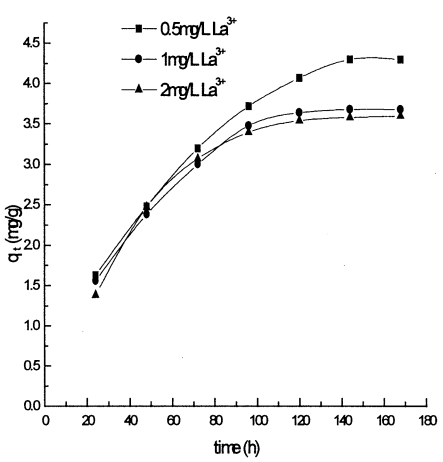

Figure 2. Kinetic accumulation curves by the *M. aerugionosa* at 1 mg / L of La^{3+} with the presence of Ce^{4+} at different initial concentrations in the combined system.

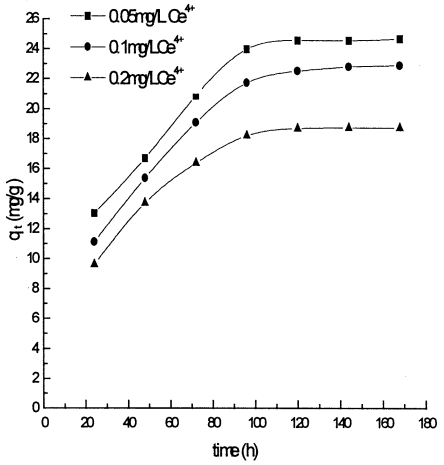

Figure 3. Kinetic accumulation curves by the *M. aerugionosa* at 0.05 mg / L of Ce^{4+} with the presence of La^{3+} at different initial concentrations in the combined system.

Table 2. Second order adsorption kinetic constants of La^{3+} and Ce^{4+} in the combined system at 28℃.

REEs	REEs combination	Initial concentration (mg/L)	Expected q_e (mg /g)	Observed q_e (mg /g)	% Deviation	$k_{2,ad}$ (g/mg/h)	r^2
La^{3+}	$La^{3+}+Ce^{4+}$	1+0.05	25.1	24.7	1.59	2.34×10^{-3}	0.994
La^{3+}	$La^{3+}+Ce^{4+}$	1+01	23.23	22.93	1.29	4.13×10^{-3}	0.997
La^{3+}	$La^{3+}+Ce^{4+}$	1+0.2	19.15	18.76	2.04	3.92×10^{-3}	0.997
Ce^{4+}	$La^{3+}+Ce^{4+}$	0.5+0.05	4.47	4.3	3.8	0.56	0.997
Ce^{4+}	$La^{3+}+Ce^{4+}$	1+0.05	3.75	3.68	1.87	0.81	0.994
Ce^{4+}	$La^{3+}+Ce^{4+}$	2+0.05	3.68	3.6	2.17	0.86	0.999

2000).

On plotting the values for q_t (the amount of La^{3+} or Ce^{4+} uptake per unit dry weight of algal cell, mg/g) versus time t (h), the kinetics curves for the accumulation of La^{3+} and Ce^{4+} by the *M. aerugionosa* were obtained and shown in the Figures 2 and 3. The data incorporated in the figures demonstrated that the adsorption was rapid in the beginning, then the process slowed down and reached saturation. In the beginning, surface adsorption may be dominant in the process. La^{3+} and Ce^{4+} incorporated with the functional groups (carboxyl, hydroxyl and amino) on the cell wall, and the process was rapidly. Thereafter, La^{3+} and Ce^{4+} having been adsorbed onto the cell surface moved slowly into the cell, and the process was slower. In the combined system, La^{3+} and Ce^{4+} competed for the sorption sites on the cell surface and the uptake of one metal decreased with the presence of the other metal, the bio-sorption

may be momnolayer sorption. The second order kinetics adsorption model (Ho et al.1996) was tried to describe the process. The equation was given as

$$t/q_t = (1/2k_{2,ad} \cdot q_e^2) + t/q_e \quad (3)$$

Where $k_{2,ad}$ is second order rate constant for sorption (g/mg/h) ; q_e is the amount of La^{3+} and Ce^{4+} adsorbed at equilibrium (mg/g) ; and q_t is the amount of La^{3+} and Ce^{4+} adsorbed at time t (mg/g) . The values for these constants are derived from regression analysis of t/q_t versus time t, and are given in the Table 2. The results showed that the second order kinetics adsorption model does provide a realistic description of the sorption process. Nevertheless, the second order kinetics adsorption model further demonstrates that the observed and expected values of q_e, the amount of La^{3+} and Ce^{4+} adsorbed at equilibrium, are similar in all cases. Thus, suggesting the applicability of this model in explaining the kinetics of sorption.

Hu et al. reported that the mechanism for algae to accumulate REEs and heavy metals was similar (Hu et al. 1984). La^{3+} and Ce^{4+} can directly penetrate through cell membrane into algal cells (Yuan et al. 1995). La^{3+} and Ce^{4+} also can move into algal cells through the anionic pathways by incorporating with Cl^{-1} and OH^{-1} in culture solution to form complex anions. Additionally, lanthanum and cerium have many properties similar to those of calcium, the La^{3+} and Ce^{4+} may enter algal cells through the specific pathways for Ca^{2+} (Yang et al. 2000).

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REFERENCES

Ho YS, Wase DAJ, Forster CF (1996) Kinetic studies of competitive heavy metal adsorption by Sphagnum moss peat. J Environ Technol 17 : 71 – 77

Hu QH, Guan LL, Ye ZJ (1996) Effect of rare earth elements on the growth of *Chlorella pyrenoides*. Environ Sci 17 : 37 – 38 (in Chinese)

Hu SC, Zhang XM, Qiu Y (1984) Preliminary observation on the toxicity of rare earth nitrate to several kinds of water-living algae. J Jangxi University 1: 22 – 24 (in Chinese)

Jin XC, Liu HL, Tu QY (1990) Eutrophication in the lakes in China. China Environmental Science Press, Beijing (in Chinese)

Sun H, Wang XR, Hua ZZ, Wu CH (1996) Bioconcentration and elimination of five light rare earth elements in carp (Cyprinus carpio L). Chemosphere 33 :1475 – 1483

Wang XR, Sun H, Xu ZA, Dai LM (1996) The effects and bioconcentration of REE La and its EDTA complex on the growth of algae. J Nanjing University 32: 460 – 465 (in Chinese)

Yang LH, Wang XR, Sun H, Zhang HS (1998) The effect of EDTA on rare earth element

bioavailability in soil ecosystem. Chemosphere 38: 2825 – 2833
Yang WD, Wang T, Lei HY, Liu JS (2000) Progress in studies on biological effects of rare earth elements. J Chinese Rare Earths 21: 62 – 70 (in Chinese)
Yang XY, Yin DQ, Sun H, Wang XR (1999) Distribution and bioavailability of rare earth elements in aquatic microcosm. Chemosphere 39 : 2443 – 2450
Yuan FZ, He MY (1995) An overview of the biochemical mechanism for the effect of rare earth elements on algal cell. J Rare Metals 19: 443 – 447 (in Chinese)