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Removal of rare earth elements by algal flagellate *Euglena gracilis*

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Abstract

Removal of rare earth elements (REEs) from an acid solution by an algal flagellate, *Euglena gracilis*, was studied. Sixteen kinds of REEs were spiked to the solution at a final concentration of 10 $\mu g\,L^{-1}$ for each element. *E. gracilis* cells grown in the solution under 12 h light–dark cycles efficiently removed REEs during the 21-day experimental period. Significant removal was observed from days 14 to 21 of incubation. On the last day, concentrations of REEs were less than 0.7 $\mu g\,L^{-1}$ except for Sc. The concentration of Sc was $2.8 \pm 0.4\,\mu g\,L^{-1}$, suggesting that Sc removal was relatively difficult compared to the other REEs. Among REEs, the same level of removal was observed for light REEs (La–Eu) but not for heavy REEs (Ga–Lu). Heavy REEs removal tended to decrease with an increase in atomic number. The removal of REEs by *E. gracilis* were affected by lighting conditions because *E. gracilis* cells grown in the dark had removed just a small amount of REEs, less than 10% of total, through day 21 of incubation.

Keywords: Euglena; Removal; Rare earth elements; Light conditions

1. Introduction

Rare earth elements (REEs) are widely applied especially in superconductors and specialized alloys utilized by high-tech industries. Wastewater treatment sludge from manufacturing plants can be contaminated with REEs [1]. Moreover, release of REEs to the environment has been observed through land application of wastewater sludge. Although the small quantities of rare earth elements beneficially increase the quantity and quality of crop yields [2], too large quantities of REEs are toxic to plants, and they also could be toxic to animals and humans through food chain concentration. For health safety of the public, it is necessary to eliminate excess amounts of REEs from the environment.

We studied removal of REEs from an acid solution by algal flagellate *Euglena gracilis* Z. Generally, Euglenophyta including *E. gracilis* live in various aquatic environments such as freshwater, brackish water, and seawater. In addition, *E. gracilis* can grow under a broad range of pH. Lin et al. [3] showed that *E. gracilis* 277 had great potential for transportation of Nd^{3+} from a surrounding solution to the cell compartments. However, removal of other REEs by the alga and suitable conditions for removal have not been clarified yet. Since *Euglena* exhibit both plant and animal characteristics, determination of light condition effects on REE removal is necessary. In the present study, removal of sixteen REEs by *E. gracilis* and the importance of light on their removal are described.

2. Experimental

E. gracilis Klebs, strain Z, an euglenoid flagellate, was used throughout the experiment. The cells were pre-cultured in a nutrient-rich medium (NRM: 1.0 g tryptone, 0.1 g yeast extract, 1.0 g dextrose, 0.1 μg Vitamin B12 in 100 mL distilled water; pH, 3.5) for 7 days at 25 °C. The cells were

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harvested by centrifugation, washed three times with NRM and then inoculated into 30 mL of fresh NRM containing sixteen REEs (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu). These REEs were provided from a mixed standard solution for ICP-MS (XSTC-1, Spex Inc., New Jersey, USA). Each REE had a final concentration of 10 μg L^{-1}. The *E. gracilis* culture was incubated at 25 °C under 12 h light–dark cycles or in the dark (shield the light).

To determine the removal of REEs from the culture solution by *E. gracilis* cells, the soluble and insoluble fractions of the culture were separated with a 0.2 μm pore-size filter. The REE content of the insoluble fraction was defined as REEs removed by the cells. The concentration of REEs in the insoluble fraction was estimated by subtracting the concentrations of REEs in the soluble fraction from their initial concentrations. The REE content of the filtrate (soluble fraction) was determined by ICP-MS (Agilent 7500a, Yokogawa Analytical Systems Inc. Tokyo, Japan) after dilution of the sample with deionized water (>18 MΩ).

Total number of cells and the pH of the culture solution were measured on day 0, 7, 14, and 21 of incubation. Enumeration was done under a light microscope by counting at least 100 cells. Values of pH were measured with a compact pH meter B-212 (Horiba, Kyoto, Japan).

3. Results

3.1. Removal concentrations of REEs

Concentrations of REEs in the solution of the *E. gracilis* culture under the light–dark condition decreased time-dependently (Fig. 1). A significant decrease of REEs was observed after 14 days of incubation. The REE concentrations were less than 0.7 μg L^{-1} except for Sc at the end of the experimental period. The concentration of Sc was 2.8 μg L^{-1} at day 21 of incubation, suggesting that removal of Sc was more difficult for *E. gracilis* than the other REEs. During the experimental period, REEs in a culture medium without *E. gracilis* cells (positive control) remained soluble (data not shown). Thus, *E. gracilis* cells apparently removed REEs from the acid culture solution under the light–dark condition.

Compared to the light–dark condition, only a small removal of REEs was observed under the dark culture condition (Fig. 2). The REEs concentrations remained at more than 8.8 μg L^{-1} even after 21 days of incubation. Possibly, the ability of *E. gracilis* for removing REEs was controlled by lighting conditions.

3.2. Removal patterns of REEs

As seen in Fig. 1, there was a certain removal pattern for REEs: removal amounts of light REEs (La–Eu) were similar on days 14 and 21 of incubation, but those of heavy REEs (Ga–Lu) decreased with an increasing in atomic number. Similar removal patterns for both light and heavy REEs

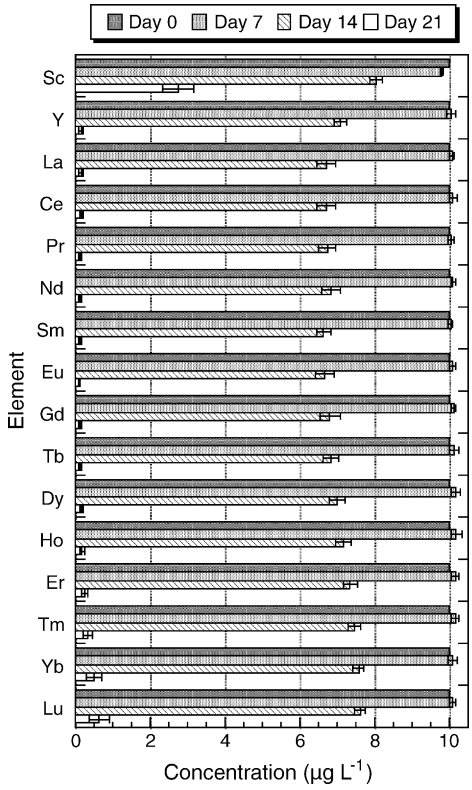

Fig. 1. Concentrations of REEs in the culture solution of *E. gracilis* grown under 12 h light–dark cycles. Initial concentrations of each REEs were 10 μg L^{-1}.

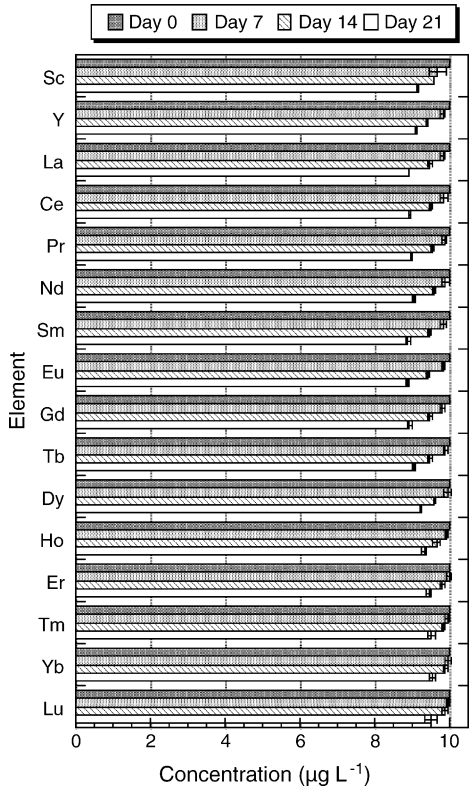

Fig. 2. Concentrations of REEs in the culture solution of *E. gracilis* grown in the dark. Initial concentrations of each REEs were 10 μg L^{-1}.

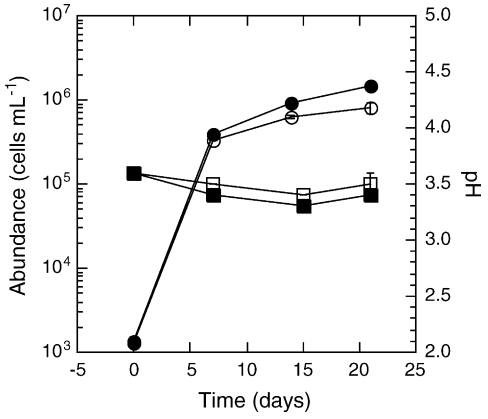

Fig. 3. Abundance of *E. gracilis* cells (circles) and pH in the solution of the *E. gracilis* culture (squares). Blackened and open marks denote results for light–dark cycles and dark conditions, respectively. Bars indicate the standard deviations of three replicates.

were also observed in the dark condition although only a small amount of the REEs was removed (Fig. 2).

3.3. Cell abundance and pH

E. gracilis cells grew exponentially for the first 7 days of incubation for both light conditions (Fig. 3) after which the abundances changed between them. The abundances of *E. gracilis* cells under the light–dark condition were about 1.5 and 1.8 times higher than those in the dark conditions at days 14 and 21, respectively. The abundance in the dark at day 21 reached the same level as that under the light–dark condition at day 14.

During the experimental periods, changes in pH value were small for the culture solution under both light conditions (Fig. 3). The values tended to decrease for both until day 14, but they returned to the initial value by day 21.

4. Discussion

In this study, *E. gracilis* showed some ability to remove REEs from acidic solutions. This ability appeared under the light–dark condition (Fig. 1), but not under the dark condition (Fig. 2). From these results, it was concluded lighting conditions would be an important factor in removal of REEs by *E. gracilis*.

Light conditions made a slight difference in *E. gracilis* abundance (Fig. 3), but the low abundance of cells in the dark condition was not able to fully explain the small removal of REEs in the dark (Fig. 2). On the last day of incubation, the number of cells grown in the dark reached the same level as grown in the light-dark culture at day 14 of incubation. Removal amounts of REEs, however, were significantly different between the two light conditions. Maximum amounts of removal for the dark (day 21) and light–dark (day 14) conditions were 1.1 $\mu g\,L^{-1}$ (Fig. 2) and 3.4 $\mu g\,L^{-1}$ of Sm (Fig. 1), respectively. Consequently, a difference in the abundance of *E. gracilis* cells under different light conditions was not an important factor influencing REE removal. Physiological activities involving photosynthetic activity could play a crucial role in REE removal. It was interesting to note that little removal (Fig. 1) was observed during the exponential growth period (days 0–7, Fig. 3) even under the light–dark condition. Possibly, the removal of REEs by *E. gracilis* cells would be a passive reaction rather than an active uptake.

The solubility of REEs depends on the pH. For instance, precipitation occurs at about pH 6.2 for lutetium and pH 8 for lanthanum during titration with soluble alkali [4]. In the present study, REEs in the initial culture medium were highly soluble because the pH was 3.6, and only small amounts of REEs were removed from the positive control for light–dark and dark conditions (data not shown). In addition, pH ranged from 3.3 to 3.6 for both light conditions of *E. gracilis* cultures and the initial pH value was never exceeded during the experimental period (Fig. 3). Removal of REEs from the acidic culture solution, therefore, was not caused by precipitation associated with an increasing pH.

A certain pattern of REE removal by *E. garacilis* was found (Figs. 1 and 2): removal amounts of heavy REEs decreased progressively with atomic number. For REEs, the atomic radius decreases with increasing atomic number. The atomic radius is responsible for elemental chemical properties such as ionic characteristics, covalent characteristics, and solubility [4]. The removal pattern of REEs by *E. garacilis* could depend on chemical and physical properties governed by the atomic radius. Differences in removal amounts between light and heavy REEs may be caused by hydration in aqueous solution because the inner sphere water coordination is 9 for light and 8 for heavy REE ions [5–7]. However, more detail studies of the REEs removal mechanism by *E. gracilis* are needed to establish any relationships between chemical properties and removal amount of REEs.

In this study, removal of REEs by *E. gracilis* from acidic solution was demonstrated. This ability of *E. gracilis* suggests a potential use in wastewater remediation. In addition, *Euglena* lives in various aquatic environments, and may be concerned with some aspects of REE behavior in these environments.

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