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Adsorption of rare earth elements onto bacterial cell walls and its implication for REE sorption onto natural microbial mats

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Abstract

Adsorption of rare earth elements (REE) onto the cell walls of *Bacillus subtilis* (a gram-positive bacterium) and *Escherichia coli* (a gram-negative bacterium) was studied between pH 2.5 and 4.5 and at various bacterial concentrations. The distribution coefficients of REE between the bacterial cell surface and water showed a pattern with a prominent enrichment of heavy REE (HREE), including a maximum around Sm and Eu. There was also an enrichment around Pr accompanied by a decline for Nd, which was attributed to the tetrad effect. The occurrence of M-type tetrad effect suggests that REE form inner sphere complexes during their adsorption onto bacteria.

The enrichment of the distribution coefficients in the HREE region was more enhanced at higher bacterial concentrations, which could not be explained by one type of binding sites on the bacterial surface. Instead, the data are consistent with two ligand types for the sorption of REE. The pattern of bacterial distribution coefficients can be explained by the stability constants of REE with carboxylate and phosphate groups, suggesting that they are most likely responsible for the adsorption of REE on the bacterial cell surface.

Microbial mats and thermal waters at the Nakafusa hot spring (Nagano Prefecture, Japan) were also examined to evaluate whether the REE patterns of natural samples could be used as indicators of the presence of bacteria. The apparent distribution coefficients of REE displayed a pattern similar to that obtained in the laboratory experiments using pure bacterial strains. The results suggest that the REE pattern of chemical sedimentary rocks may be used to identify the bacterial contribution to the deposition of the rocks in the geological record.

Keywords: Rare earth elements; Bacteria; Cell walls; Adsorption; Microbial mat

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1. Introduction

Rare earth elements (REE; including Y in this paper) commonly behave coherently in nature, but a variety of processes can cause fractionation (e.g., Henderson, 1984; Taylor and McLennan, 1988). The fractionation pattern of REE, commonly expressed as normalized values on a logarithmic scale, provides information related to the origin of the samples and to the process leading to their formation. Furthermore, the study of REE is relevant to the dispersion of elements from radioactive waste sites because actinides, especially Am (III) and Cm (III), have similar geochemical properties as REE (e.g., Brookins, 1984).

The concentrations of REE in natural waters are influenced by their adsorption onto inorganic and organic substances and soluble complex formation with a variety of ligands (e.g., Erel and Stopler, 1993; Sholkovitz et al., 1994; Ingri et al., 2000; Tanizaki et al., 1992; Takahashi et al., 1997). It is likely that the biota in natural systems can also affect the behavior of REE. For instance, bacteria, which have a very high surface area per unit weight due to their small size, are known to efficiently adsorb various dissolved metal ions (Beveridge and Murray, 1980; Beveridge and Doyle, 1989; Fein et al., 1997; Daughney and Fein, 1998; Martinez et al., 2002; Châtellier and Fortin, 2004). Some studies have looked at the sorption of selected REE onto bacterial cells (Ferris and Beveridge, 1984; Mullen et al., 1989; Texier et al., 2000; Ozaki et al., 2002; Merroun et al., 2003; Markai et al., 2003), but they did not include the data for all REE. Brantley et al. (2001) reported uptake of all REE by a soil bacterium, but the distribution of REE between the bacterial and aqueous phases was not reported due to the low concentrations of dissolved REE at equilibrium. To our knowledge, there are still few adsorption data of all REE onto pure bacterial cells and thus no clear understanding of the potential role of bacteria on REE fractionation processes.

This paper reports for the first time the patterns of REE adsorbed onto *Bacillus subtilis* (*B. subtilis*), a gram-positive bacterium, and *Escherichia coli* (*E.coli*), a gram-negative bacterium. These two bacteria are commonly found in the environment and their cell wall properties are well characterized (e.g., Beveridge and Doyle, 1989; Fortin et al., 1997; Daughney and Fein, 1998; Daughney et al., 2001; Kelly et al., 2002; Markai et al., 2003; Boyanov et al., 2003). The binding functional groups on the bacterial cell walls were evaluated by comparing the distribution coefficients for bacteria and the stability constants of REE complexes with a variety of organic ligands based on the linear free energy relationship of complex formation between solute complex and corresponding surface complex (Stumm, 1992). Such an approach has been adopted by Byrne and Kim (1990) to evaluate the influence of REE complexation with organic ligands at the surface of particulate matter in marine systems. The nature of the adsorption sites onto bacterial cell walls is still a relatively open question for any cations including REE. Although spectroscopic analyses such as laser-induced fluorescence (Ozaki et al., 2002; Markai et al., 2003) and EXAFS (Kelly et al., 2002; Boyanov et al., 2003) have been performed, there are still some discrepancies between the conceptual models proposed by the various studies (Boyanov et al., 2003; Châtellier and Fortin, 2004). It is possible that the REE patterns can give new insight into the nature of the adsorption sites.

The laboratory data for the REE sorption are compared to those from natural microbial mat samples from the Nakafusa hot spring in the Nagano Prefecture, Japan. These mats are ideal for the examination of the role of bacteria on REE fractionation because the hot spring does not contain Fe- and Mn-oxyhydroxides, which adsorb large quantities of REE (Koeppenkastrop and De Carlo, 1992; Kawabe et al., 1999a,b).

2. Samples and experimental methods

2.1. Bacterial suspensions

The bacterial suspensions were prepared as described in Châtellier et al. (2001) and Daughney and Fein (1998). *B. subtilis* cells were cultivated in Tryptic soy broth (Difco, 30 g/L) with yeast extract (Difco, 5.0 g/L). Pre-culture tubes of 3 mL were first inoculated from a Petri dish and incubated at 37 °C for 24 h. Then, 4 Erlenmeyers containing 1 L of sterile culture medium were inoculated with the pre-cultures and grown for 7.5 h until exponential growth phase

was reached (Daughney and Fein, 1998). *E. coli* cells were grown in Tryptic soy broth (Difco, 30 g/L). A 50 mL pre-culture was incubated for 24 h, and sub-samples (10 mL) of this pre-culture were used to inoculate 4 Erlenmeyers containing 1 L of the culture medium. The bacteria were cultivated for 2.5 h and harvested in exponential phase. The bacteria were separated from the growth media by centrifugation at 6000 rpm for 15 min, washed 5 times in 1.0 mM NaCl, left to rest overnight at 4 °C, and washed again twice in 1.0 mM NaCl before being used for the sorption experiments. The dry weight of the bacteria was determined by dehydrating the suspension (15 mL) in an oven at 60 °C. The optical density (at 600 nm) of the stock suspension diluted 100 times was also determined in order to estimate its concentration before use.

2.2. REE sorption experiments on bacteria and inorganic compounds

All experiments were carried out in Teflon containers. An REE standard solution (Spex CertiPrep., Edison, New Jersey) containing all REE elements, except Pm, at a concentration of 10.0 mg/L each was added to the bacterial suspensions of varying concentrations. The initial concentration of each element in the suspension was fixed at 100 μg/L. The concentration of bacterial cells ranged from 0.067 to 1.4 g (dry weight)/L. The pH was adjusted at values between 2.5 and 4.5 by addition of HCl or NaOH, in order to keep REE as free cations. It is known that REE form complexes with hydroxide or carbonate at pH greater than approximately 5.5 (Baes and Mesmer, 1986; Liu and Byrne, 1998). NaCl was also added in the samples at a concentration of 10 mM to fix the ionic strength. Most experiments were conducted at room temperature, i.e., at 297 K, but two experiments were also conducted at 277 ± 0.1 K and at 310 ± 0.1 K in order to examine the effect of temperature on sorption. The samples were agitated for 90 min, which was found to be sufficient to reach equilibrium (see Section 3.1). At the end of the agitation period, the aqueous phase was separated from the bacteria by filtration using hydrophilic PTFE filter (0.20 μm; Advantec, Tokyo). The amount of REE adsorbed onto the wall of Teflon containers and the filter was examined for the bacteria-free system. It was found that the amount of REE sorbed on the containers and filters in the experiments at pH below 6 was less than 3% of the amount of REE initially added to the system, which is similar to the precision, ±2%, of ICP-MS measurements.

The filtrate was acidified with conc. HNO_3 (TAMAPURE AA-100, Tama Chemicals Co., Ltd, Tokyo) to a final concentration of 2% for the determination of the concentration of REE using an inductively coupled plasma-mass spectrometer (ICP-MS; Agilent 4500 or VG PQ-3). Indium and Bi were also added at a concentration of 10 μg/L to serve as internal standards. The precision of the ICP-MS measurement was better than 2%. The distribution coefficient (K_d) of REE between the bacterial and the aqueous phases is defined as

$$K_d(\mathrm{L/g}) = \frac{[\mathrm{REE}]_{init} - [\mathrm{REE}]_{fil}}{c[\mathrm{REE}]_{fil}} \quad (1)$$

where $[REE]_{fil}$ is the concentration of REE in the filtrate, while $[REE]_{init}$ is the initial concentration of dissolved REE (=100 μg/L). The c (g/L) is the ratio of bacteria (dry weight) and water, which ranged from 0.067 g/L to 1.4 g/L. The blank concentration of each REE was estimated by analysis of the filtrate of a bacterial suspension prepared without any addition of the REE standard solution, and was found to be less than 5 ng/L for every REE except for La (5–10 ng/L) and Ce (10–15 ng/L).

For the sorption experiments on model compounds, methylcarboxylate cellulose (Sigma, St. Louis; fibrous form, 0.6 meq/g) and Bio-Rex 70 (Bio-Rad, Hercules, California; polyacrylate resin, 3.5 meq/g) were selected as representative polycarboxylates, whereas corn starch and amylose (amylose B, MW: ca. 16,000, Nacalai Tesque, Inc., Kyoto) were used as polysaccharide-rich compounds. All the compounds are insoluble in water. Polysaccharides have similar properties as the extracellular polymeric substances around some microorganisms (Welch and Vandevivere, 1994; Welch et al., 1999). Sephadex gel (G-100; Sigma), which consists of synthetic polymers of cyclodextrin bridged by glycerol-ether bonds, was also used as an analogue of bacterial extracellular polysaccharides. Finally, elemental sulfur (Wako Chem., Tokyo) was used for the experiment because it is present in

the microbial mat (sulfur turf) of the Nakafusa hot spring water. Each sample (100 mg, dry weight) was rinsed with Milli-Q water (10 mL) more than 5 times without drying prior to use. The REE leached by 2% HNO_3 from the materials were negligible, showing that the impurities of REE originally contained in the reagents can be neglected. The sorbents (100 mg) were mixed with 100 μg/L REE solution (10.0 mL) followed by pH adjustment using NaOH or HCl solution. The concentrations of REE in the filtrate were determined by ICP-MS as described above.

2.3. REE concentrations in natural microbial mat

The REE abundance of natural microbial mats and thermal water was also investigated in samples from the Nakafusa hot spring in the Nagano Prefecture, Japan (36°23′15”N, 137°45′00”E). Geochemical and microbiological characteristics of the hot spring are given in Sugiura et al. (2001), Nakagawa and Fukui (2002), and Kato et al. (2004). The effluent water flows down along the concrete wall of a dam. The temperature of the water ranges from 50 to 70 °C, the electric conductivity from 30 to 50 mS/m, the pH from 8 to 8.5, the Eh from −140 to 50 mV, and [S^{2-}] from 2 to 3 mg/L (Kato et al., 2004). Feather-like microbial mats of various colors (green, orange, white, and brown) grow in small hot-water streams flowing through cracks in the dam. The mat consists mainly of large sickle-shaped bacteria, extracellular bacterial polymers, and elemental sulfur particles. They contain cyanobacteria, phototrophic bacteria, sulfate-reducing bacteria, etc. Two microbial mat samples were collected: a sulfur turf, i.e., a white/yellowish mat rich in sulfate-reducing bacteria (Sample 1), and a brown mat rich in phototrophic bacteria (Sample 2). The concentrations of C, H, N and S of the samples were determined using a CHNS analyzer (Perkin Elmer 2400 II). Samples 1 and 2 contain 84.2 wt.% S and 4.07 wt.% C, and 72.2 wt.% S and 8.06 wt.% C, respectively (Table 1).

The mat samples were digested at 85 °C for 24 h in a mixture of H_2O_2 solution (30%, 5 mL) and HNO_3 solution (0.020 M, 3 mL). The residue in the leachate after the filtration (hydrophilic PFTE, 0.45 μm; Advantec) was essentially composed of S (>99.9 wt.%), which confirmed that the organic material had been completely digested. Aluminum and Fe concentrations in the leachate were determined by graphite-furnace atomic absorption spectroscopy (GF-AAS; Shimazu AA6650) with an auto sampling system (Shimadzu ASC-6100). The REE concentrations were measured in the same leachate using an ICP-MS (VG PQ-3). Thermal water samples were also collected upstream of the dam (78.6 °C, pH 8.34, Eh-170.2 mV). The samples were filtered with a PTFE filter (0.20 μm), acidified to 2% HNO_3, and the REE concentrations were determined with an ICP-MS after concentrating the REE using an ion-exchange resin as described in Takahashi et al. (2002).

Table 1
REE abundances in microbial mats (decomposed by H_2O_2 and HNO_3) and hot spring water in the Nakabusa hot spring

	Microbial mat (mg/kg)		Hot spring water (ng/kg)
	Sample 1 (sulfate reducing bacteria)	Sample 2 (phototorophic bacteria)	
Y	4.34	2.60	5.79
La	8.58	5.35	6.08
Ce	19.5	14.1	18.4
Pr	2.17	1.37	1.48
Nd	7.51	4.98	5.45
Sm	1.38	0.953	1.16
Eu	0.108	0.0786	0.111
Gd	1.23	0.810	1.07
Tb	0.148	0.0983	0.159
Dy	0.735	0.492	0.936
Ho	0.135	0.0905	0.190
Er	0.401	0.272	0.544
Tm	0.0593	0.0414	0.0775
Yb	0.397	0.273	0.469
Lu	0.0640	0.0438	0.0656
C	4.07 wt.%	8.06 wt.%	
H	1.01 wt.%	0.43 wt.%	
N	0.91 wt.%	1.71 wt.%	
S	84.17 wt.%	72.20 wt.%	

Total concentrations of C, H, N, and S of the microbial mats were also included.

3. Results

3.1. Kinetic effects of the sorption on bacteria

The distribution coefficients (K_d) of REE after different agitation periods are shown in Fig. 1 for *B. subtilis* at a concentration of 0.39 g/L and at pH 4.0

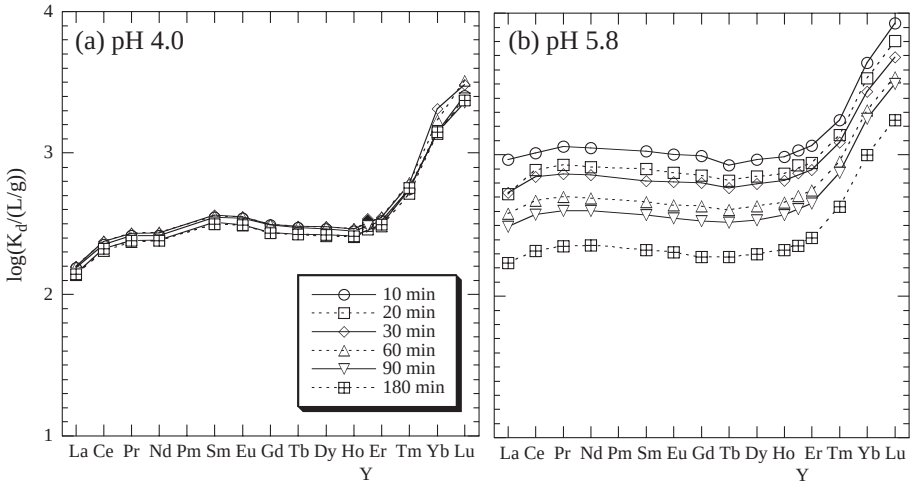

Fig. 1. Time dependence of distribution coefficients of REE, K_d, between *Bacillus subtilis* and aqueous solutions. The value for Y was plotted between Ho and Er, since the ionic radius of Y is close to that of Ho. The elapsed time is measured after the addition of the REE at (a) pH 4.0 and (b) pH 5.8. The concentration of *B. subtilis* was 0.39 g/L and the initial concentration of each REE was 100 μg/L.

and 5.8. The almost identical REE patterns obtained for the 10- to 180-min agitation periods at pH 4.0 (Fig. 1a) indicate that the sorption equilibrium was reached in less than 10 min. Similar results were also obtained at pH 4.5 (data not shown). At pH 5.8, however, the K_d values decreased with increasing agitation periods, although the patterns were similar (Fig. 1b), suggesting that REE that were originally adsorbed on the bacterial cells were released back into solution. This was most likely due to the release of soluble organic molecules from the bacterial cells, which can form complexes with REE. As a result, all additional experiments were carried out at pH values below 4.

3.2. REE distribution patterns of the bacterial species

The REE distribution patterns for the adsorption of the REE onto bacterial cells are shown at various bacterial concentrations and pH for *B. subtilis* in Fig. 2 and for *E. coli,* in Fig. 3. The K_d values increased with increasing bacterial concentration at a given pH and increasing pH at a given bacterial concentration (Figs. 2 and 3). The relationship between K_d and pH has been observed for other metal cations on various bacterial species (e.g., Daughney and Fein, 1998; Markai et al., 2003) and has been attributed to the lesser competition of cations for the binding sites at higher pH (Châtellier and Fortin, 2004).

All REE patterns were also characterized by a strong enrichment in the heavy REE (HREE) region for Tm, Yb and Lu, and by a moderate enrichment around the middle REE (MREE) region (Figs. 1–3). The pattern is distinctly different from the patterns of REE adsorbed on various minerals, including Fe (hydr)oxide, Mn (hydr)oxide, and clay minerals (Koeppenkastrop and De Carlo, 1992; Kawabe et al., 1999a,b; Takahashi et al., 2000; Coppin et al., 2002). Hence, the distinctive pattern for the adsorption of REE onto both gram-positive bacteria (*B. subtilis*) and gram-negative bacteria (*E. Coli*) suggests that the pattern may provide a signature of the bacterial activity in natural samples, as explained in Section 4.3. Similar enrichment of HREE from Er to Lu was observed for a soil bacterium (*Arthrobacter* sp.) (Brantley et al., 2001), but the cause for the enrichment was not convincing, because (i) the experiment was not a simple adsorption experiment and (ii) the data for light REE (LREE) were not shown.

Another notable feature of our experimental results is the tetrad effect, i.e., convex curves appearing in four regions in the REE pattern: La–Ce–Pr–Nd, (Pm)–Sm–Eu–Gd, Gd–Tb–Dy–Ho, and Er–Tm–Yb–Lu (Peppard et al., 1969; Jørgensen, 1970; Nugent, 1970; Masuda and Ikeuchi, 1979; Kawabe, 1992; Bau, 1996). However, the existence of the tetrad effect in nature has often been debated (McLennan, 1994).

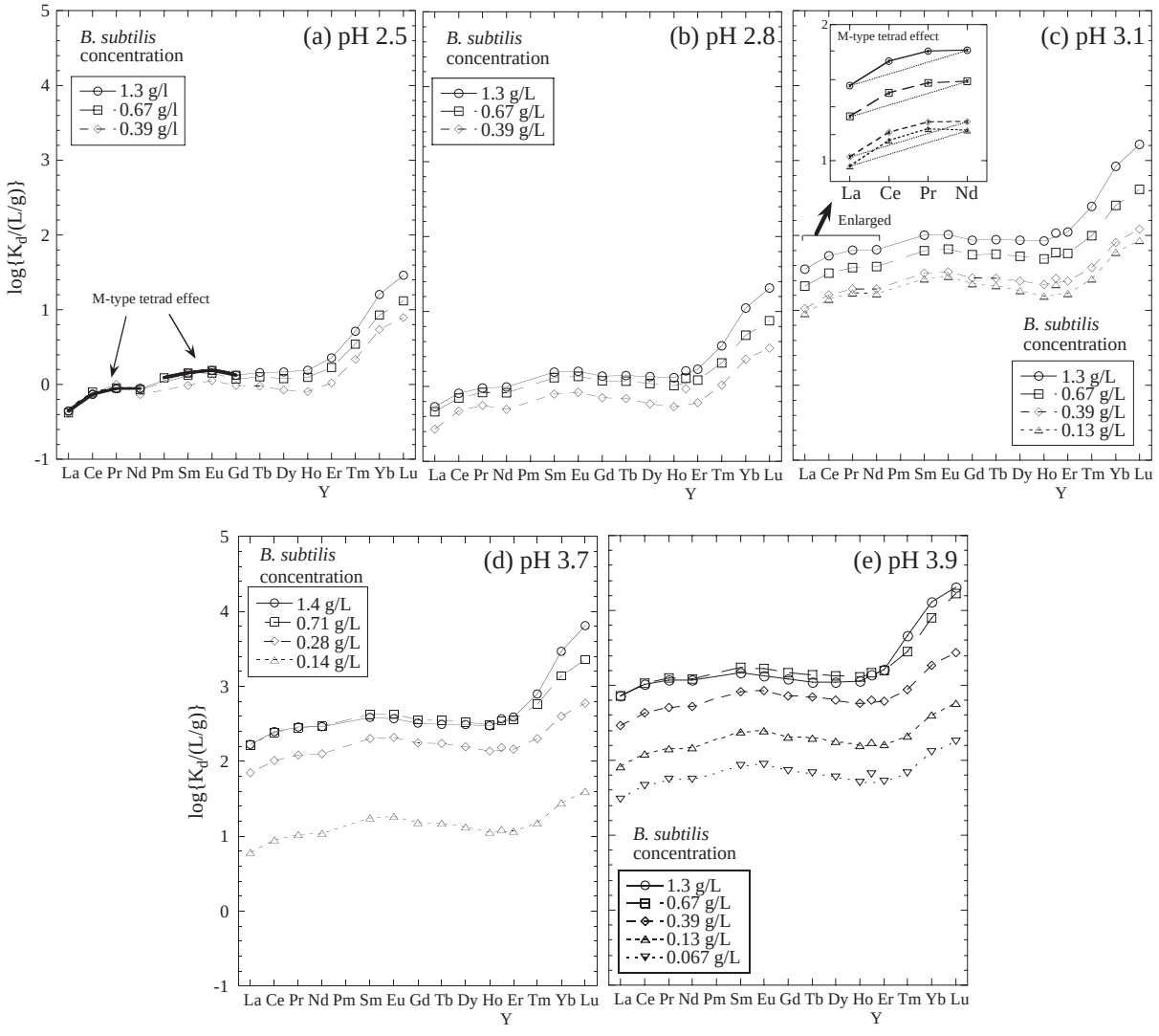

Fig. 2. Distribution coefficients, K_d, of REE for *Bacillus subtilis* at various pH values between 2.5 and 3.9. The concentration of *B. subtilis* is indicated in each diagram and the introduced concentration of each REE was 100 μg/L.

In our sorption experiments, the M-type tetrad effect was apparent for all bacterial REE patterns (Figs. 1–3). The effect was particularly obvious for the first two tetrads (La–Ce–Pr–Nd, (Pm)–Sm–Eu–Gd) (Fig. 2a). In Fig. 2c, the La–Ce–Pr–Nd part is enlarged, where the error values are less than the size of the symbols. It is obvious that our experimental data were above the interpolated lines, showing that the fractionation by the tetrad effect occurred as a result of the sorption onto bacteria.

3.3. REE abundances in natural microbial mats

The REE concentrations of two samples of microbial mat and thermal water from the Nakafusa hot spring are presented in Table 1, along with the content of C, H, N, and S of the microbial mat. The apparent distribution coefficients of REE between the mat and the water are shown in Fig. 4a. The two samples yielded similar results with respect to the enrichment of Tm, Yb, and Lu and the patterns were similar to

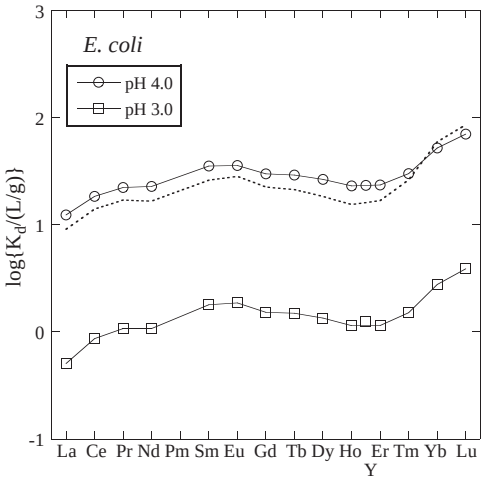

Fig. 3. Distribution coefficients, K_d, of REE for *Escherichia coli* at pH 3.0 and 4.0. The concentration of *E. coli* was 0.20 g/L and the introduced concentration of each REE was 100 μg/L. For comparison, the distribution coefficients for *Bacillus subtilis* (concentration of *B. subtlis*: 0.13 g/L; pH 3.1) are shown as a dotted curve.

those obtained from the laboratory experiments with pure bacterial strains.

3.4. REE distribution patterns of inorganic compounds

Model compounds, i.e., methylcarboxyl cellulose and polymethacrylate (Bio-Rex 70), which are rich in carboxylates, were also used for REE sorption experiments (Fig. 5). The patterns are similar to those of the stability constants of REE complexes with simple carboxylates, such as acetate and propionate (Fig. 6a), and the bacterial REE patterns (Figs. 1–3). This suggests that carboxylates sites on the bacterial surfaces are likely be significant for the adsorption of REE.

3.5. Temperature effect on REE sorption

The temperature dependence of K_d in the presence of bacterial cultures was examined at pH 3.8, where the solution was divided into three batches and kept at 277 K, 297 K, and 310 K for 90 min (Fig. 7a). The difference in the pH values due to the temperature change was less than 0.07 pH unit and the K_d values increased with increasing temperature. In addition, the enrichment of HREE was more profound at higher temperature.

4. Discussion

4.1. Type of binding sites involved in REE sorption

The stability constants of metal complexes bound to surface binding sites are correlated to the corresponding complex formation in solution (Stumm, 1992). The linear free energy relation can therefore

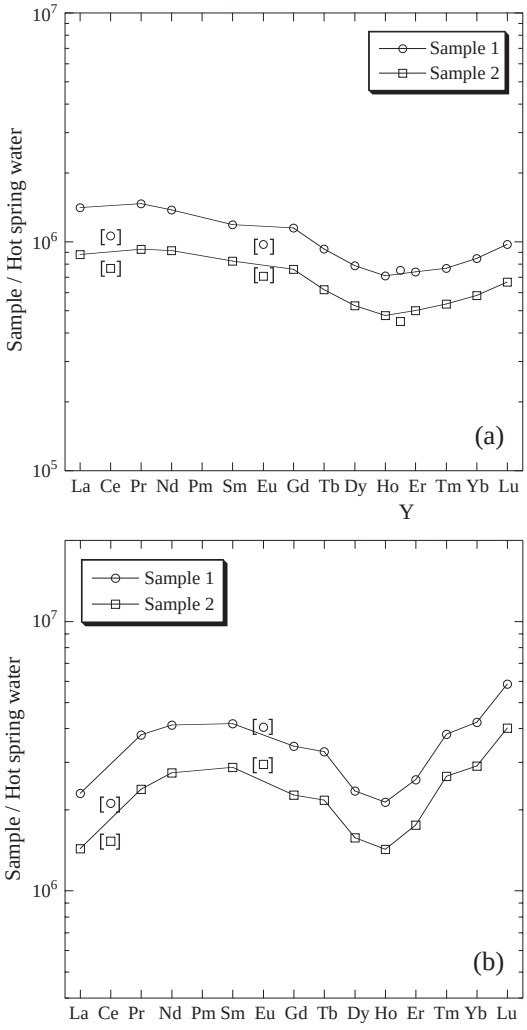

Fig. 4. The concentration patterns of REE in microbial mats in the Nakafusa hot spring where the concentrations are normalized by the (a) total concentrations or (b) calculated free concentrations (removing the REE complexed with OH) in the co-existing hot spring water. The values for Ce and Eu are shown in brackets, since these two elements commonly display anomalous behavior due to their redox-sensitive properties.

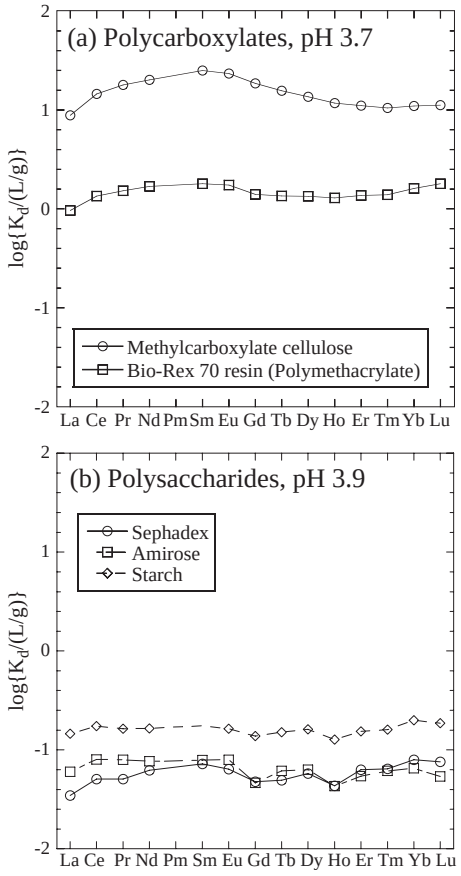

Fig. 5. Distribution coefficients of REE, log (K_d), for (a) carboxylmethyl cellulose and polymethacrylate (the Bio-Rex 70 resin); (b) Sephadex gel, amylose, and corn starch.

be applied to evaluating the binding sites of REE on bacterial cells. The complexation of H^+or $REE^{3+}$with a ligand R^L on the bacterial surface is expressed by the following equations;

$$R^L + H^+ \leftrightarrow R^L - H \tag{2}$$

$$R^L + REE_i^{3+} \leftrightarrow R^L - REE_i \tag{3}$$

$$K^L = \frac{[R^L] \cdot [H^+]}{[R^L - H]} \tag{4}$$

$$\beta_i^L = \frac{[R^L - REE_i]}{[R^L] \cdot [REE_i^{3+}]} \tag{5}$$

where (i) K^L is the proton dissociation constant of the binding site at the bacterial surface, (ii) REE_i^{3+} and β_i^L are non-complexed, free cation of ith REE and the stability constant of the complex, respectively, and (iii) symbols in brackets denote their concentrations. Assuming that only one type of sites is available for REE, the REE distribution pattern is expressed by:

$$\{K_d^i\}_{i=1,16} = \{K_d(La), K_d(Ce), \ldots, K_d(Lu)\} = \left\{ \frac{[R^L - REE_i]}{[REE_i]_{aq}} \right\}_{i=1,16} \tag{6}$$

where $[REE_i]_{aq}$ is the concentration of ith REE dissolved in the solution. Strictly speaking, soluble complexes of REE with hydroxide and carbonate need to be considered, but such complexes are not significant at low pH (pH<5.5). Therefore, $[REE_i]_{aq}$ in our experiments was equal to $[REE_i^{3+}]$, which leads to:

$$\{\log\ (K_d^i)\}_{i=1,16} = \left\{ \log\left(\frac{[R^L - REE_i]}{[REE_i^{3+}]} \right) \right\}_{i=1,16} = \{\log(\beta_i^L) + \log([R^L])\}_{i=1,16} \tag{7}$$

The $[R^L]$ was considered to be identical for all REE in each batch of experiments, as the binding sites on bacterial surface are indistinguishable to all REE. Given that $[R^L]$ is common to all REE, the distribution pattern reflects the pattern for the stability constants of surface complexes, β_i^L. The linear free energy relation suggests that the values of log β_i^L should have a linear relation to the log β_i values, stability constants for equivalent metal complexes in solution (Stumm, 1992):

$$\{\log(K_d^i)\}_{i=1,16} = \{\log(\beta_i^L [R^L])\}_{i=1,16} = \{a\ \log(\beta_i) + b\}_{i=1,16} \tag{8}$$

where a and b are constants.

The REE patterns obtained from the experiments were not precisely parallel to each other. The HREE enrichment was more enhanced in the solution with greater bacterial concentrations (Fig. 2). This is not consistent with Eq. (7), indicating that more than one type of sites were involved. If we assume that two

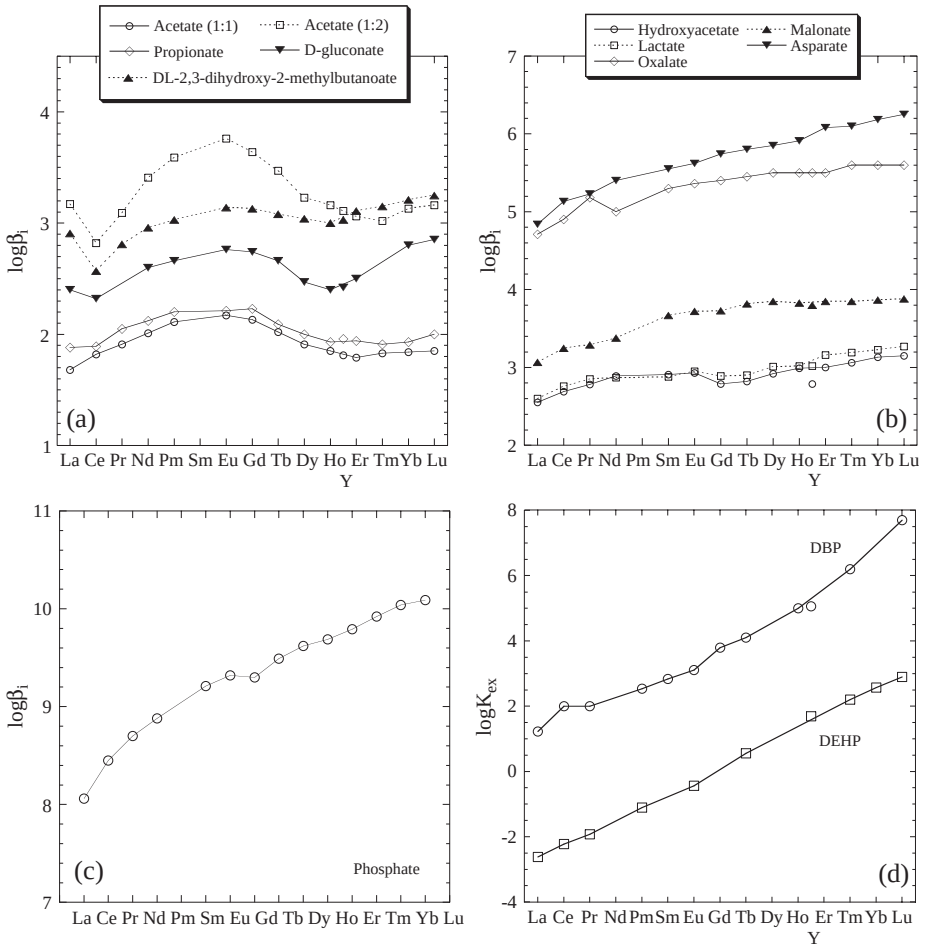

Fig. 6. Stability constants, β_i, of complexes for REE with (a) simple carboxylic acid (1:1 and 1:2 complexes) and carboxylic acid with at least two-OH groups in the ligand, (b) bidentate carboxylic acid and amino acid, and (c) phosphoric acid. (d) Extraction constants, K_{ex}, of REE for di(n-butyl) phosphoric acid (DBP) and di(2-ethyl-hexyl) phosphoric acid (DEHP).

types of ligands (L and L') were at play, Eq. (7) then becomes:

$$\begin{aligned}\left\{\log(K_{\mathrm{d}}^{i})\right\}_{i=1,16} &= \left\{\log(\beta_i^{\mathrm{L}}\cdot[\mathrm{R}^{\mathrm{L}}]+\beta_i^{\mathrm{L}'}\cdot[\mathrm{R}^{\mathrm{L}'}])\right\}_{i=1,16}\\ &= \left\{\log(\beta_i^{\mathrm{L}}+\alpha\cdot\beta_i^{\mathrm{L}'})+\log([\mathrm{R}^{\mathrm{L}}])\right\}_{i=1,16}\end{aligned} \tag{9}$$

where $\alpha=[\mathrm{R}^{\mathrm{L}'}]/[\mathrm{R}^{\mathrm{L}}]$. When the bacterial concentration is high, binding sites are not fully occupied. According to Eq. (4) and defining c^{L} and $c^{\mathrm{L}'}$ as the total concentrations of ligands L and L′, this corresponds to an asymptotic situation where $\alpha=\alpha_0=[c^{\mathrm{L}'}\cdot(1+[\mathrm{H}^+]/K^{\mathrm{L}})]/[c^{\mathrm{L}}\cdot(1+[\mathrm{H}^+]/K^{\mathrm{L}'})]$ is a constant at a given pH and the REE distribution pattern is parallel to $\left\{\log(\beta_i^{\mathrm{L}}+\alpha_0\cdot\beta_i^{\mathrm{L}'})\right\}_{i=1,16}$. When the bacterial concentration decreases, it is likely that one of the two types of ligands, for instance L, will be saturated first with the REE. As soon as a significant fraction of the ligand L is complexed with REE, the ratio α starts to increase. Hence, the REE distribution pattern changes as the concentration ratio of bacteria and REE changes.

It has been suggested that carboxylate and phosphate sites are mainly responsible for the adsorption of cations onto bacterial cells (Fein et al., 1997; Daughney and Fein, 1998; Daughney et al., 2001; Kelly et al., 2002; Markai et al., 2003; Boyanov et al.,

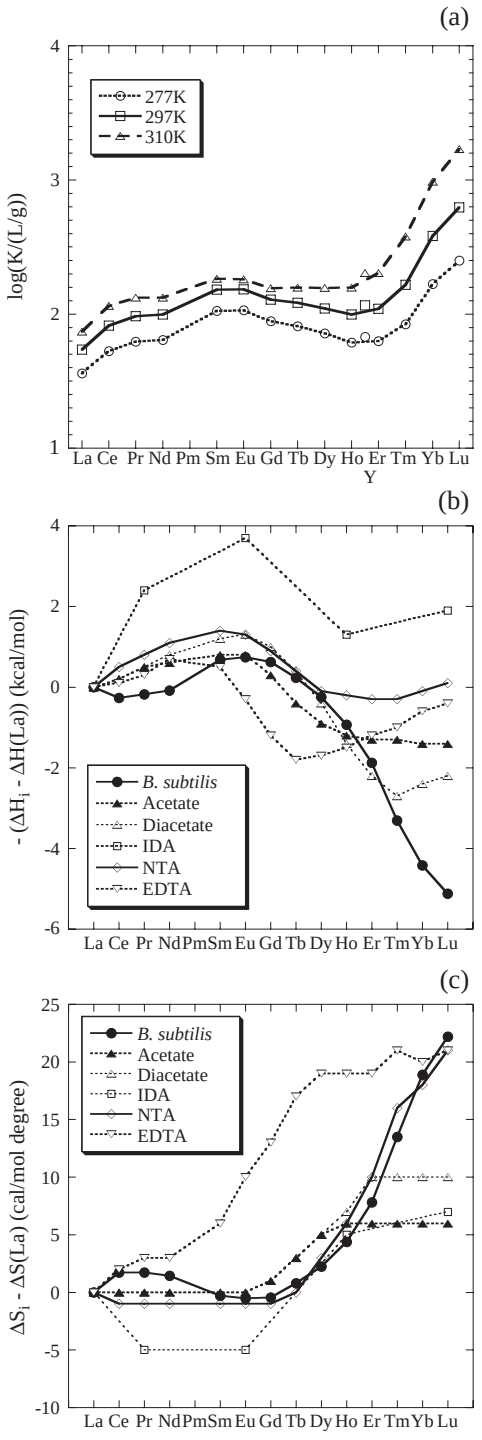

Fig. 7. (a) Temperature dependence of the distribution coefficients, K_d, of REE for *B. subtilis* at a concentration of 0.33 g/L and at pH 3.8. Variations of (b) ΔH_i values relative to ΔH (La) and (c) ΔS_i values relative to ΔS (La) for the adsorption on *B. subtilis* and complexation with various carboxylate ligands (Smith and Martell, 1987). IDA—iminodiacetic acid; NTA—nitrotriacetic acid; EDTA—ethylenediaminetetraacetic acid.

2003). The stability constants (β_i in Eq. (8)) of REE–carboxylates reported by Martell and Smith (1974, 1977) show various patterns that are grouped into three. The first group consisting of simple monocarboxylates, such as acetate and propionate, displays a convex shape with a maximum around Sm and Eu and a slight enrichment in HREE (Fig. 6a). The REE patterns of the 1:1 (=REE:carboxylate) and the 1:2 complexes are similar (Fig. 6a). The second group corresponds to complexes with carboxylates including at least two OH groups in the ligand molecule (D-gluconate and DL-2,3-dihydroxy-2-methylbutanoate), which also displays a "bell-shaped" pattern as well as a distinct increase in HREE (Fig. 6a). The third group is characterized by a steady increase of logβ with increasing atomic number (Z), corresponding to complexes with bidentate carboxylate, like oxalate and malonate and amino acid like asparate (Fig. 6b). Carboxylates with only one OH group (hydroxyacetate and lactate) display similar increase of β_i with increasing Z (Fig. 6b). Stability constants of phosphate complexes estimated by Byrne and Sholkovitz (1996) show a steady increase of log β_i with increasing Z (Fig. 6c). The extraction constants (K_{ex}) of dialkylphosphoric acids, such as di(n-butyl) phosphoric acid (DBP) and Di(2-ethyl-hexyl) phosphoric acid (DEHP), also show a steady increase with increasing Z (Fig. 6d; Baes, 1962). The K_{ex} for DEHP is defined as $[\text{REE-}((\text{DEHP})_2)_3]_{org}[H^+]^3_{aq}/\{[(\text{DEHP})_2]^3_{org}[REE^{3+}]_{aq}\}$ and similar to K_{ex} for DBP. The subscripts "org" and "aq" indicate that the species are in the organic or in the aqueous phases during the extraction. The K_{ex} shows the concentration ratio of REE complexes with the extraction ligand (=$[\text{REE-}((\text{DEHP})_2)_3]_{org}$) and free REE (=$[REE^{3+}]_{aq}$) normalized by the free ligand (=$[(\text{DEHP})_2]_{org}$) at constant $[H^+]$. Therefore, K_{ex} is similar to the stability constant. The structure of the extraction ligands is $(RO)_2P(O)OH$ (R: alkyl group), where the phosphate is present as a phosphodiester linkage. This structure is in fact similar to the phosphodiester site in the teichoic

acids of bacterial cell walls (Madigan et al., 2003). Therefore, the K_{ex} values for DEHP and DBP are comparable with the binding coefficients at phosphodiester sites on bacterial cell walls. Altogether, two sites model consisting of carboxylate site with larger affinity for MREE and phosphate site with larger affinity for HREE can simulate the REE patterns of the sorption on bacteria in terms of a convex pattern in MREE, a sharp increase in HREE, and the change depending on the concentration ratio of bacteria and REE (Fig. 2). The data are thus consistent with our proposed interpretation that carboxylate and phosphate groups are the main binding sites for REE on the bacterial cell walls.

Boyanov et al. (2003) suggested that Cd^{2+} adsorbed preferentially onto phosphate groups at pH lower than 4.5, and that the contribution of carboxylates increased as pH increases. Since our solutions had pH lower than 4.5, it is possible that REE may have been preferentially sorbed to phosphate groups. This possibility is supported by the enhanced enrichment of K_d in HREE at high bacterial concentrations (Fig. 2) because HREE are preferentially bound to the phosphate group. The decrease in the enrichment of K_d in HREE at lower bacterial concentrations suggests that the contribution of carboxylates became larger due to the saturation of phosphate sites by added REE.

The pH dependence of the K_d values indicates that the phosphate groups are less important for the REE sorption at increasing pH. The values at different pH were normalized to those at pH 2.5 (Fig. 8). This indicated that the increase of the distribution coefficient with Z decreased with the increase in pH, especially between La and Ho. Considering that the logβ trend exhibited a monotonous increase with Z for the phosphate sites, it is suggested that the contribution of the phosphate sites to REE sorption on the bacterial cell walls was reduced at higher pH as suggested by Boyanov et al. (2003).

4.2. Inner sphere versus outer sphere complexation

The M-type tetrad effect, especially the first two tetrads, La–Ce–Pr–Nd and (Pm)–Sm–Eu–Gd, is not outstanding but apparent in all experimental results. This effect is attributed to different stabilization energies of REE in the ligand field due to the difference in the energy levels of the 4f electrons

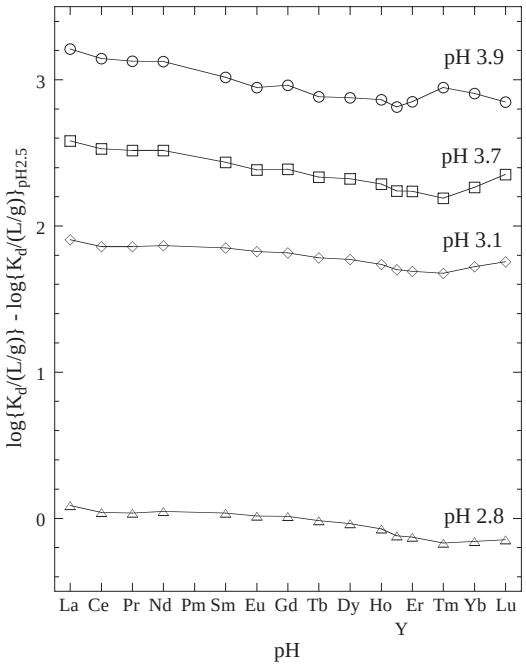

Fig. 8. The pH dependence of the distribution coefficients, K_d, of REE at various pH values ranging from pH 2.8 to 3.9 normalized to the K_d values at pH 2.5 for *B. subtilis*. The concentration of *B. subtilis* was 1.3 g/L (except at pH 3.7 where it was equal to 1.4 g/L).

among REE (Jørgensen, 1970; Nugent, 1970; Kawabe, 1992). When hydrated ion is the main REE species in the aqueous phase as in the present experiments, the tetrad effect is observed when the sorbed REE forms inner sphere complexes. Similar results were also observed for the REE sorption on the surfaces of clay, but the tetrad effect was absent when REE were sorbed as outer sphere complexes (Takahashi et al., 2000, 2004; Coppin et al., 2002). The evidence suggests that formation of inner sphere complex is responsible for the tetrad effect. Therefore, the observed tetrad effect in our experiments suggests the formation of inner sphere complexes during the adsorption of REE on bacteria. Our proposed interpretation is supported by other spectroscopic studies of the sorption of REE, such as the laser-induced fluorescent spectroscopy studies by Ozaki et al. (2002) and Markai et al. (2003) and the NMR study of Ferris and Beveridge (1984) that showed the formation of inner sphere complex of Eu(III).

The proposed interpretation is further supported by the temperature dependence of K_d at 277 K, 297 K,

and 310 K (Fig. 7). Inner sphere complexation is accompanied by the release of hydrated water molecules initially associated with the REE cations, which leads to an entropy increase. We observed that REE adsorption on the bacterial surfaces increased at higher temperature (Fig. 7a). This suggests that the complexation of the REE on the bacterial cell walls results in an increase of entropy (S). This is consistent with an inner sphere complexation, but not with outer sphere adsorption. These results are consistent with the formation of surface complexes of REE with carboxylate and phosphate sites at bacterial cell walls.

The variations of ΔH (H: enthalpy) and ΔS for the REE sorption reactions can be estimated from the REE patterns, using the following equation (Smith and Martell, 1987):

$$\log(\beta_i^{\mathrm{L}}) = -\left(\frac{\Delta H_i - T\Delta S_i}{k_{\mathrm{B}}T}\right) + C$$
$$= 0.219\cdot\left(-\frac{\Delta H_i}{T} + \Delta S_i\right) + C \qquad (10)$$

where C is an unknown constant, ΔH_i is expressed in cal/mol, and ΔS_i is expressed in cal/mol/K. Assuming that there was only one type of ligands, we used Eq. (10) to express the variations of the REE distribution coefficients as a function of the temperature in terms of the variation of ΔH_i and ΔS_i for each element. When the values of ΔH_i and ΔS_i were plotted relative to the values for La and compared with the results for various carboxylate ligands (Fig. 7b and c; Smith and Martell, 1987), it can be seen that REE patterns for *B. subtilis* are similar to those for acetate in the light and middle REE. Although we could not compare our results to those for phosphate groups due to the lack of the available data, this analysis suggests that carboxylates sites play a significant role in REE sorption on the cell surface. For HREE, it is necessary to consider a second binding site for the HREE, which presumably corresponds to phosphate groups as discussed previously.

4.3. REE pattern as a geochemical signature of past bacterial activity

The REE patterns of natural microbial mats showed an enrichment of the HREE (Fig. 4a), which is similar to the results obtained with the pure bacterial cultures. However, the natural microbial mats also displayed an enrichment of LREE (Fig. 4a), which was not observed in the laboratory. This LREE enrichment could be caused by the presence of clay or secondary iron oxyhydroxides in the samples, because they can be rich in LREE. We discount this possibility because the Al and Fe concentrations in the mat were less than 1 g/kg. In order to estimate the contribution of clays and secondary iron oxyhydroxides to the REE incorporation, we used the REE data available for shales (McLennan, 1989) and iron oxyhydroxides (Koeppenkastrop and De Carlo, 1992; Kawabe et al., 1999b). Based on the REE data, it was estimated that REE in the clay and iron oxyhydroxide particles accounted for less than 0.1% and 0.01% of the total REE in the microbial mats. Although S was the main inorganic constituent of the mats (84.2% and 72.2% in Samples 1 and 2, respectively), our laboratory experiments showed that the amounts of REE adsorbed onto elemental sulfur were insignificant; the log K_{d} values were less than -3. This indicates that S was not responsible for the REE sorption in the microbial mats.

Another possible cause for the enrichment of LREE in the microbial mats is the formation of soluble complexes of the REE in the thermal water. The distribution coefficients of natural samples correspond to the concentrations in microbial mats normalized by the total concentrations of REE in the thermal water (Fig. 4a). The solution contains free cations and dissolved complexes and the latter are likely to be significant in natural water. In alkaline thermal waters, such as the Nakafusa hot spring, hydroxide and carbonate complexes are predominant species of REE in the aqueous phase (van Middlesworth and Wood, 1997). The hydrolysis and carbonate formation are more favored with HREE than with LREE (Baes and Mesmer, 1986; Liu and Byrne, 1998), indicating that the amount of free cations are much lower than the total dissolved REE, especially for HREE. This likely resulted in an apparent reduction of the HREE enrichment in the REE patterns of the microbial mats.

Recalculation of the distribution coefficients of REE between the microbial mat samples and free cations in the solution yielded a REE pattern with an enrichment of HREE and a convex shape around

MREE (Fig. 4b). The concentrations of free REE cations were estimated by subtracting the amounts complexed with OH using the stability constants given by Baes and Mesmer (1986). The data for 25 °C were employed in the calculation because the temperature dependence is identical among REE between 25 °C and 75 °C (Wood, 1990). The corrected REE pattern in Fig. 4b simulated the sorption of free REE on microbial mats in the hydrothermal water. As a result, the REE patterns of the microbial mats were in better agreement with the patterns obtained for the bacterial cells in the laboratory, which showed a strong enrichment of the HREE and a bell-like shape around the MREE. The recalculation considering carbonate complexes was not performed due to the lack of data on carbonate concentrations in the thermal water. However, the inclusion of carbonate data should further enhance the enrichment of HREE in the microbial mat relative to free ions in the aqueous phase, which is in agreement with our interpretation.

The microbial mats contain other organic material, such as extracellular polymeric substances (EPS) consisting of polysaccharides, proteins, nucleic acids, and lipids (Flemming, 1995). REE may be bound on such organic material. We discounted this possibility for the microbial mats of the Nakafusa hot spring because our data of the sorption of REE on neutral polysaccharides (i.e., corn starch, amylose, and Sephadex gel; Fig. 5b) showed that a rather flat pattern was observed and that the magnitude of the K_d values was much lower than those obtained for *B. subtilis*. However, it is possible that EPS containing carboxylate and phosphate groups might have contributed to the sorption of REE. If such groups were present in the EPS of the biofilms, they likely led to REE patterns relatively similar to those for bacterial cells. Hence, as EPS are secreted from bacteria, this possibility does not change our conclusion that the presence of bacteria yields distinct REE pattern with an enrichment of HREE along with a convex shape around the MREE.

There is a large difference in pH between the experimental data with pure cultures and the natural microbial mat samples. The natural system has alkaline waters around pH 8, whereas the experiments were conducted below pH 6. We showed that the enrichment of HREE at low pH was caused by the presence of carboxylate and phosphate sites on the bacterial surface. These sites are most likely responsible for the REE binding also in alkaline waters, where REE patterns at higher pH will become similar to lower pH when bacteria are responsible for REE sorption. However, a study on REE abundances in natural microbial mats growing at low pH conditions is required to verify our proposed interpretation.

Finally, a recent study by Anderson and Pedersen (2003) suggested that bacteriogenic Fe (hydr)oxides can be enriched in HREE compared to inorganic materials, which is consistent with our results. Since REE are essentially immobile during diagenesis and metamorphism, the bacterial REE signature recorded during the deposition of sediments is likely to be retained in the rocks in the geological record.

5. Conclusion

This study indicates that HREE, especially, Tm, Yb, and Lu, are enriched on the cell surface of *B. subtilis* and *E. coli* compared to other REE. The REE patterns suggest that there are at least two binding sites on the bacterial cell surface, i.e., carboxylate and phosphate groups. The experimental results are consistent with the REE patterns of natural microbial mats, which also display an enrichment of HREE. Considering the relatively immobile nature of REE during the diagenesis and metamorphism, REE patterns may be used to identify the bacterial activity during the deposition of sedimentary rocks.

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