Letter

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Environ. Sci. Technol. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acs.estlett.6b00064 • Publication Date (Web): 23 Mar 2016

Rare-earth Separation Using Bacteria

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Keywords: *Roseobacter*; rare-earth elements; lanthanides; recycling; separation.

ABSTRACT

The rare-earth elements are critical to many green energy technologies but are difficult to separate from one another because of their chemical similarity. We demonstrate an alternative, biogenic method based on lanthanide adsorption to the bacteria *Roseobacter* sp. AzwK-3b, immobilized on an assay filter, followed by subsequent desorption as a function of pH. The elution desorption data suggests that the basicity of the individual lanthanides is important in determining their desorption behavior. It is found that by pre-protonating the bacteria it is possible to concentrate a solution of equal concentrations of each lanthanide to nearly 50% of the three heaviest lanthanides, Tm, Lu and Yb in just two passes. This surpasses existing industrial practice. The findings suggest that there is an opportunity to harness the diversity of bacterial surface chemistry to separate and recover technologically important rare-earth metals in an environmentally benign manner.

INTRODUCTION

It is widely recognized that the rare-earth lanthanide elements (La through Lu) are crucial constituents in advanced materials for many existing and future energy technologies [1]. The rare-earths, notably Dy, Nd and Sm, are used, for instance, in high-energy density permanent magnets in electric motors and generators such as those in electric vehicles and wind turbines [2,3]. Eu and Tb are used in phosphors for solid state lighting [4], and La and Ce, for instance, are used as anode materials in nickel metal hydride batteries. Unfortunately, because the lanthanides are chemically similar, being trivalent, and having similar ionic radii, they are difficult to separate from one another by physical or chemical means. The dependence of many green energy technologies on the lanthanides, coupled with the challenges associated with their extraction and recovery, led the U.S. Department of Energy to classify six of the lanthanides as either critical or near-critical elements [5]. This criticality as well as the search for more environmentally benign processing motivates the need for new methods of lanthanide separation and recovery, including in recycling. In this work, an alternative approach based on microbial biosorption and desorption as a function of pH is described.

The standard industrial method of separating lanthanides, after ore processing [6] to produce an aqueous mixture of the lanthanides, usually as chlorides, uses solvent extraction. In this process the solution is combined with an immiscible organic liquid such as EHEHPA (2-ethylhexyl phosphonic acid mono-2-ethylhexyl ester) [6]. The lanthanide ions partition between the organic and aqueous phases based on their basicity. In turn, these differences produce different solubilities in the two liquid phases. Then, the aqueous and organic liquids are isolated and the lanthanides are recovered from each. To increase the concentration of the recovered elements, the enriched solutions are continuously fed through numerous solvent extraction stages until the desired purity is reached [6].

Up to now, biosorption of metals to bacteria has primarily been of interest for the remediation of toxic elements, such as As, Pb and Cd, from waste water as well as to limit the release of metals from mine drainage streams. These environmentally important applications have motivated extensive studies of biosorption of toxic elements and more common metals, such as Cu, Zn, and Ni, as well as the underlying binding mechanisms [7–9]. Recently, however, a limited but convincing literature has shown that a number of the individual lanthanides can biosorb to bacterial surfaces [10–16].

In this work, we show that not only do all the lanthanides biosorb from a mixed lanthanide solution but they can then be separated under semi-continuous flow conditions with decreasing pH washes. By systematically varying the wash pH after biosorption, different lanthanides from a mixed lanthanide solution can be separated by preferentially desorbing them from the bacterial surface. We illustrate this using *Roseobacter* sp. AzwK-3b, a gram-negative marine bacterial strain whose genus has been shown to be a strong metal absorber [17].

MATERIALS AND METHODS

Media and reagents. A mixed lanthanide solution (Accutrace, New Haven, CT), a calibration standard for ICP-MS, was used as the base solution for all the work reported here. It contains 10 μg/mL of each lanthanide (except Pm), as well as Sc, Y, and Th, all dissolved in 2% nitric acid. For all the assays, this solution was first diluted with deionized water and neutralized to pH 6.0 to a concentration of 2 μg/mL.

Roseobacter sp. AzwK-3b is a bacterial strain from Elkhorn Slough, a coastal estuary close to Monterey Bay, CA [18]. It was grown in artificial seawater (ASW) first sterilized by autoclaving at 120°C for 15 min. A single stock of *Roseobacter* sp. AzwK-3b was created by inoculating 1 L of sterile ASW with *Roseobacter* sp. AzwK-3b and allowing it to incubate at 37°C for approximately 2 months. The biomass was kept refrigerated, and was sterilely removed from this stock as wet biomass for all the experiments. When dried, the mass of bacteria was found to be 0.05 mg/mL of media.

Continuous flow filtration assay. The assay was developed to quantify lanthanide biosorption as well as exposing the bacteria and biosorbed lanthanides to various pH washes. 2mL of the bacterial media (~0.1 mg) were immobilized on a 25 mm diameter hydrophilic, polypropylene filter (Pall, Port Washington, NY, GHP Acrodisc) and a syringe pump was used to flow solutions past the bacteria. The filter was selected because its average pore size (0.2 μm) was smaller than the diameter of the bacteria (0.8 μm). As described in the Supporting Information, a constant, optimized flow rate of 2.5 mL/min was used for all the assays and it was demonstrated that no biosorption occurred on the filter absent the bacteria. The biosorption step consisted of passing 1 mL of the mixed lanthanide solution through the filter. This was followed by a 5 mL deionized water wash (pH 7) to remove any lanthanides not bound to the bacteria. For the desorption, a series of 5 mL nitric acid solutions, from pH 6 to pH 1.5, in intervals of pH 0.5, was successively pumped past the bacteria on the filter.

ICP-MS. The masses of the lanthanides absorbed and desorbed were determined by ICP-MS of their concentrations in 5 mL aliquots.

Pre-protonation. The bacterial surface was pre-protonated in the same apparatus using 5 mL solutions of pH 2.5 nitric acid.

Lanthanide separation. The same flow method was used but with additional passes (stages) over fresh bacteria pre-protonated with different pH washes as described in the flow diagram in the Supporting Information, Fig S2.

RESULTS AND DISCUSSION

The biosorption of the individual lanthanides to the *Roseobacter* sp. AzwK-3b bacteria from equi-concentration lanthanide solutions at pH 7 is shown in Figure 1. The bacteria strongly absorbed each lanthanide with a slight statistical preference for the middle lanthanides. The total

biosorption was found to vary from one batch of bacteria to another. We attributed this to variations in the effective bacterial surface area exposed to the fluid flow in the assays resulting from variations in the local density of the bacteria immobilized on the filter. Despite this, the relative values within the lanthanide series of each biosorption run were consistent.

After adsorption, the bacteria were washed at successively decreasing pH. The mass of each lanthanide desorbed is shown in Figure 2. The data indicates that a larger fraction of the lighter lanthanides desorbed with the highest pH washes, while the reverse is true for the lowest pH washes. Moreover, comparison of the masses desorbed indicates that the heaviest lanthanides, in particular Tm, Yb and Lu, were preferentially desorbed at the lowest pH's. Comparison of the data for the heaviest, and smallest lanthanide, Lu, with the lightest and largest lanthanide ion, La, indicates that twenty-five times greater mass of Lu desorbed at the lowest pH than La. The variation was quantified by a desorption ratio, R_{AB}, the ratio of the desorbed masses of two different lanthanides, A and B, at the same pH. The equivalent separation factor, α_{AB}, used in other branches of separation chemistry [19], is the ratio $(R_{AB})_1/(R_{AB})_2$ where the subscripts refer to the pH at which the desorption masses are compared. For illustration, the separation factors between four pairs of neighboring lanthanides are compared in Table S1 in Supporting Information.

The biosorption and pH desorption results are consistent with lanthanide ions binding to, and desorbing from, sites on the bacterial surface according to their acid dissociation constants (pK_a's). [Strictly speaking, the pK_a of a surface site is the pH at which 50% of the lanthanides desorb from a surface site and are replaced by protons]. Although the number of distinct surface binding sites on the *Roseobacter* sp. AzwK-3b is unknown, the desorption elutions suggest that there are, possibly, three types of sites to which lanthanide ions can absorb. These broad maxima, which occur at approximate pK_a's of 5.5-6.0, in the range 3.0-4.5 and about 2.0 are quite reproducible from run to run using *Roseobacter* sp. AzwK-3b so it is conjectured that these correspond to the presence of possibly three distinctive types of binding sites on the bacterial surface. The results in figure 2 indicate that surface sites having higher pK_a's tend to bind the lighter, more basic lanthanides, and those having lower pK_a's tend to bind the heavier, more acidic lanthanides. The underlying reasons for the correlation between the observed lanthanide desorption with pK_a and the basicity of the lanthanide ion is not known. The simplest explanation is that it is related to the well-established, systematic decrease in basicity with

increasing atomic mass across lanthanide series and the associated decreasing ionic size across the series, the so-called lanthanide contraction [20,21].

Effect of Pre-Protonation. Evidence for the solution pH controlling individual lanthanide desorption was sought using pre-protonation experiments in which the bacteria were first washed with a highly acidic solution (pH 2.5) and then exposed to the mixed lanthanides. It would be expected that upon pre-protonation, protons preferentially absorb to all the surface sites having a pK_a higher than the pre-protonation pH. Then, on exposure to the lanthanide solution, there would correspondingly be lower absorption of the lanthanides to those sites pre-protonated by washing at pH 2.5. Specifically, sites having lower pK_a's would not be protonated and consequently would bind the heaviest lanthanides just as they do without pre-protonation. The experimental findings are shown in Figure 3, on the same scale as the data in Figure 2. As anticipated, substantially less of each lanthanide desorbed with pH washes above the pre-protonation pH, whereas similar values of the lanthanide masses were recovered using pH washes below the pre-protonation pH.

Lanthanide Separation. The observed variation in pH at which different lanthanides preferentially desorb provides the basis for the possible use of bacteria in separating and recovering individual lanthanides from solution. While the separation factors achievable in a single elution assay are significant, it is likely that multiple biosorption-desorption steps would be required to attain a desired level of enrichment just as in the current solvent extraction process. To demonstrate the efficacy of such a multiple step process in purifying the heaviest lanthanides, the continuous flow assay was repeated by passing the lanthanide solution over fresh, pre-protonated bacteria (see methods and the flow diagram in Supporting Information for details). The results are presented in Figure 4 (a) showing a progressive enrichment of the three heaviest lanthanides which, after the second pass, the solution contained 18 wt % Yb and 30 wt % Lu. While the value of the pre-protonation pH was specifically selected to preferentially separate the heaviest lanthanides, it was found, but not shown in this publication, that the pre-protonation pH could be adjusted, to recover and cycle different washes through the assay in order to recover other groups of lanthanides, such as the middle lanthanides.

To illustrate the potential of the bacterial separation approach, the quantitative findings in Figure 4 (a) can be compared to the standard industrial method, using solvent extraction, of separating the lanthanides (see Figure 4(b)). The comparison is based on calculating the enrichment after two stages of the industrial solvent extraction method using the separation factors cited for the process [6]. The calculations are in the Supporting Information. The two-pass biosorption-desorption enrichment process using *Roseobacter* sp. AzwK-3b achieves comparable, if not superior, purities to the industrial process.

Although the results suggest that preferential binding of lanthanide ions depends on the pH, more detailed studies, for instance by EXAFS, are clearly needed to identify the functional groups responsible for the binding to specific lanthanides. However, it is also possible surface molecules, such as polysaccharides and lipids, as well as functional groups, such as carboxyl groups, can also bind to some of the lanthanides. Takahashi *et al.* (2005), for example, has reported the preferential adsorption of the middle rare earths to carboxyl groups from molecules such as acetate and propionate. Similarly, there are biosorption studies [10,12–14,22,23] showing that the heaviest lanthanides preferentially bind to phosphate groups, which have a pK_a (~2.0) [24], consistent with our findings that the last lanthanides, Yb and Lu, to desorb are also the most acidic. However, it is likely that the lanthanide binding is more complicated and that there is not only competition between the protons and the lanthanide ions in solution for specific surface sites but also between different lanthanides. Furthermore, it is extremely unlikely that specific lanthanides will bind to specific sites, and more likely there is a distribution in binding energies as well as local steric effects involved. These questions clearly warrant more detailed structural biochemical characterization of lanthanide binding, but until then the interpretation in terms of the values of pK_a seems useful if too simplistic [25].

Although at only the laboratory scale and not optimized, our results suggest that the bacterial sorption-elution desorption process may be more benign than current commercial solvent extraction processes. We used *Roseobacter* sp. AzwK-3b as the biosorbing material but it is anticipated that lanthanide separation will be achievable using other bacteria since the surface groups implicated in this work commonly occur on the surfaces of other bacteria and are not expected to be unique to *Roseobacter* sp. AzwK-3b. Indeed, similar results but differing in the numerical values of separation factors have been obtained with three other bacteria, *Shewanella oneidensis, Sphingobacterium sp.* and *Halomonas sp*. An example is shown in Figure S3 in the

Supporting Information for lanthanide desorption from *Shewanella oneidensis,* another bacteria known to be a metal absorber. Given the rich variety of bacterial surface chemistries, it is also likely that other bacteria will exhibit significantly greater differentiation in binding different lanthanides. It is also possible that other metals can be separated from one another using similar absorption-desorption elution methods. This may also be important in separating specific heavy metals after bioremediation.

ACKNOWLEDGEMENTS

This work was initiated with a grant from the Harvard University Center for the Environment and then carried out with funding from the Office of Naval Research under Grant No. N00014-11-1-0894. We are grateful to Dr. C. Hansel for providing the bacteria used in this work, to P. Girguis for his advice, and A. Cortinas, A. Magyar and R. Diebold for their assistance. Some data reported in this paper are included in the Supporting Information. A patent application, PCT/US2014/022253, regarding this work has been submitted and is pending at the time of writing.

CONFLICT OF INTEREST

No conflict of interest declared.

SUPPORTING INFORMATION

Supporting Information Available: Additional methods, calculations and figures, as referenced in the text, are in the supporting information. This material is available free of charge via the Internet at http://pubs.acs.org.

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FIGURE CAPTIONS

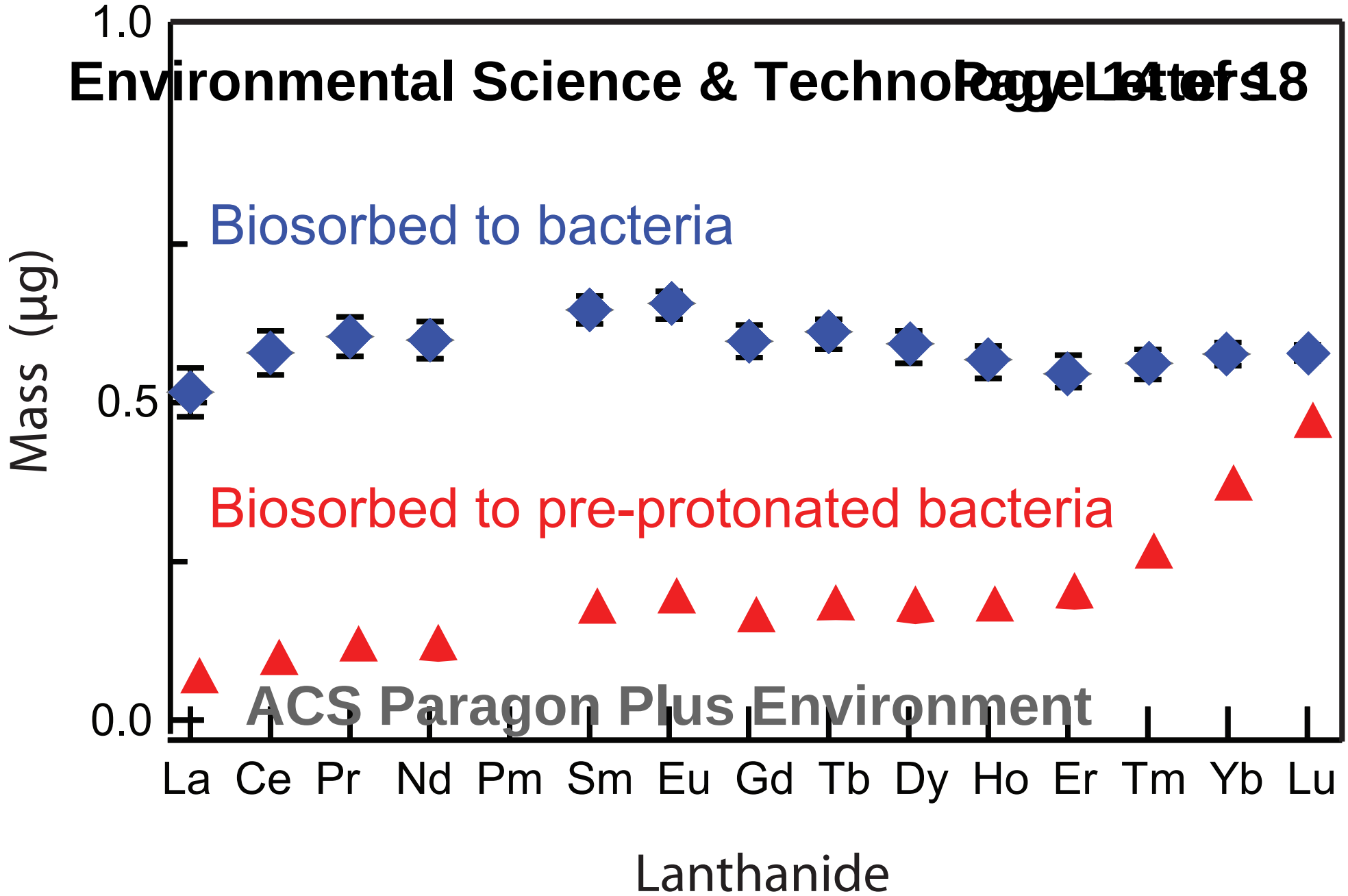
Figure 1. Mass of each lanthanide adsorbed to *Roseobacter* sp. AzwK-3b from an equi- mass 1 mL solution of all the rare-earths during the filtration assay. Biosorption was almost independent of the lanthanide atomic number although there is a slight preference for the middle lanthanides. The mass of the lanthanides adsorbed to the bacterial surface after first protonating the surface at pH 2.5 is also shown. There is reduced biosorption of the lighter rare-earths but similar biosorption of the heavier rare-earths compared to biosorption before protonation. (Repeated in triplicate. The error bars are commensurate with the symbol size)

Figure 2. The mass of individual lanthanides desorbed from *Roseobacter* sp. AzwK-3b at 0.5 pH intervals as a function of pH washes each 5 mL volume. Although the masses of the lanthanides desorbed during the two highest pH washes, pH 6 and pH 5.5, was relatively insensitive to atomic number, lower pH washes revealed marked differences with atomic number. Furthermore, the graphs indicate more light lanthanides desorbed with higher pH washes, and more heavy lanthanides desorbed with lower pH washes. Local maxima in the mass desorbed with successively lower pH suggest there may be as many as three distinct bacterial sites, corresponding to pH's of 5.5-6.0, 4.5-3.0 and 2.5, are responsible for lanthanide absorption. The error bars represent the standard deviation of at least three replicates.

Figure 3. The effect of first pre-protonating the *Roseobacter* sp. AzwK-3b with 5mL of a pH 2.5 nitric acid wash on the mass of each lanthanide desorbed during subsequent titration as a function of pH. The bacteria desorbed smaller amounts of all the lanthanides at pH washes higher than the pre-protonation wash (pH 2.5) as compared to that shown in Figure 2. Similar

masses of the lanthanides desorbed at pH washes lower than the pre-protonation pH. As shown, these were enriched in the heaviest lanthanides. Same mass scale as Figure 2. The error bars represent the standard deviation of at least three replicates.

Figure 4. (A). Purification of the heaviest lanthanides. The mass fraction of each lanthanide initially in solution and then after the first and second passes of the same solution over freshly pre-protonated bacteria illustrate concentration enrichment of the three heaviest lanthanides, Tm, Yb and Lu. After the second pass, the solution contains 48% of the two heaviest lanthanides, Yb and Lu, exceeding the calculated enrichment performed using solvent extraction shown in panel (B). After each pass the bacteria were replaced by a new batch of bacteria and pre-protonated with a wash at pH 2.5.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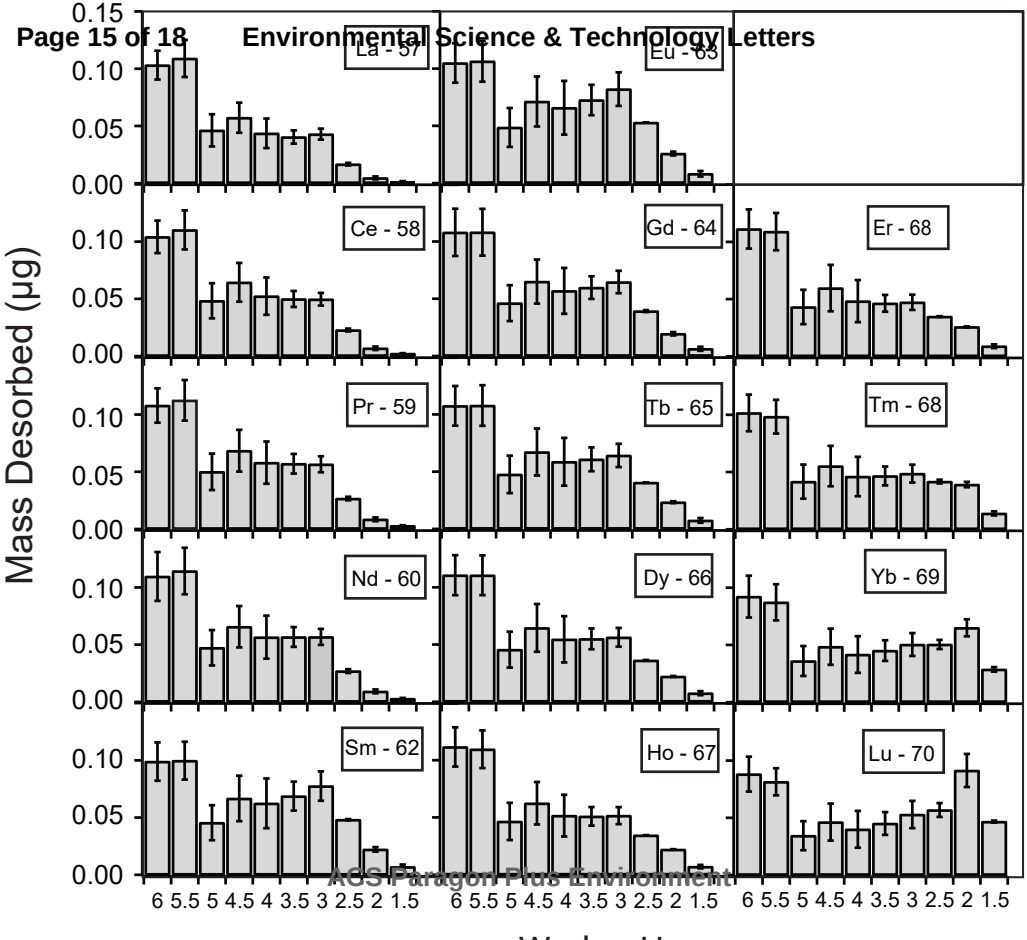
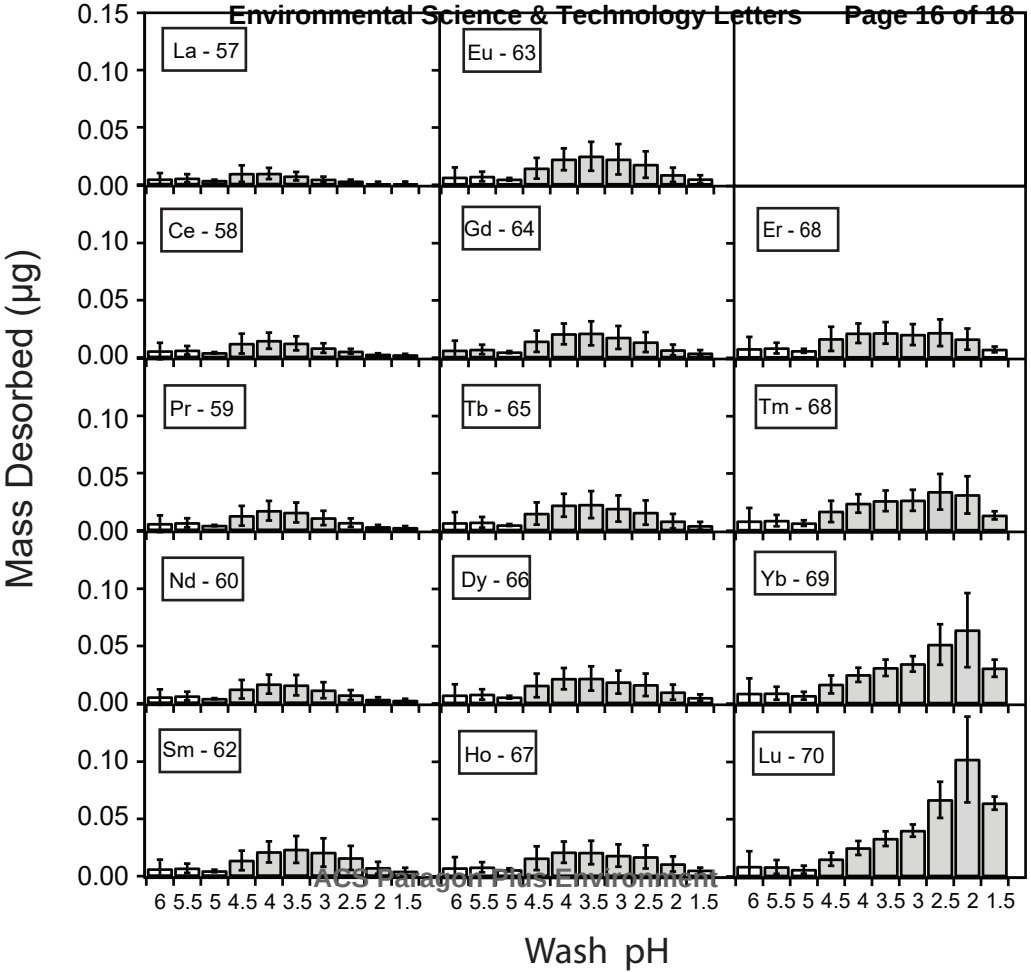
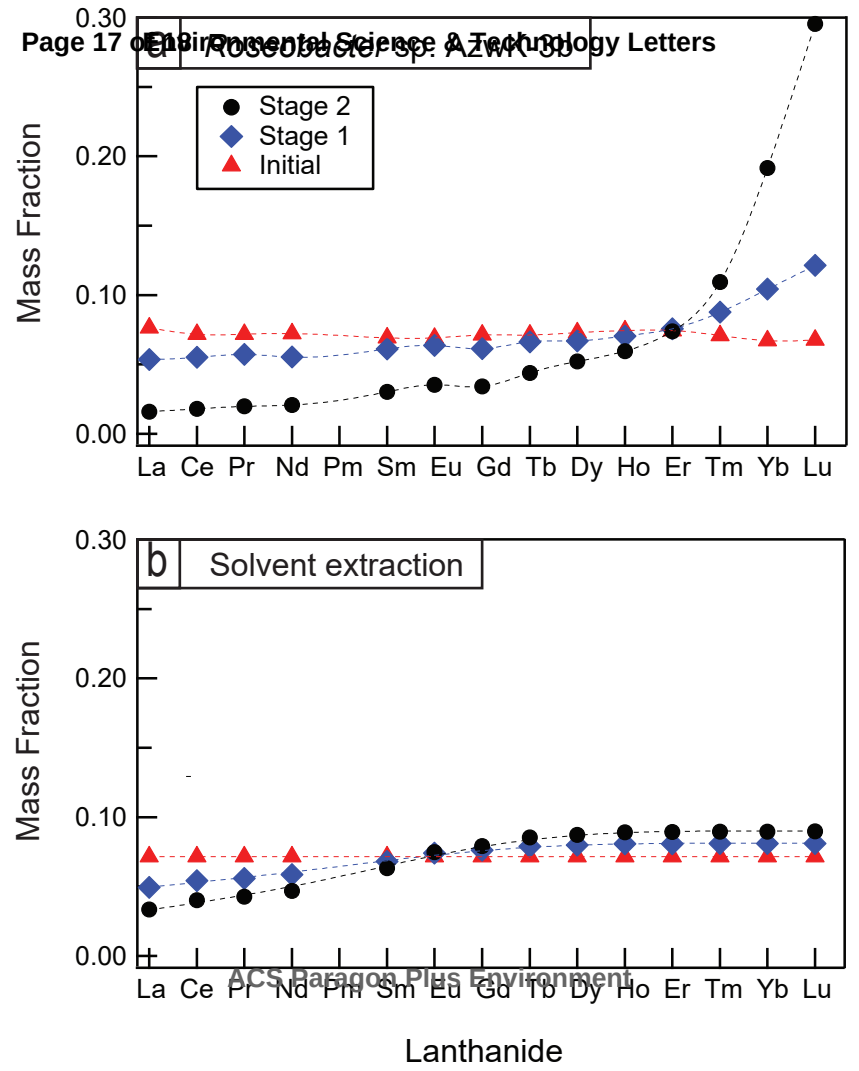
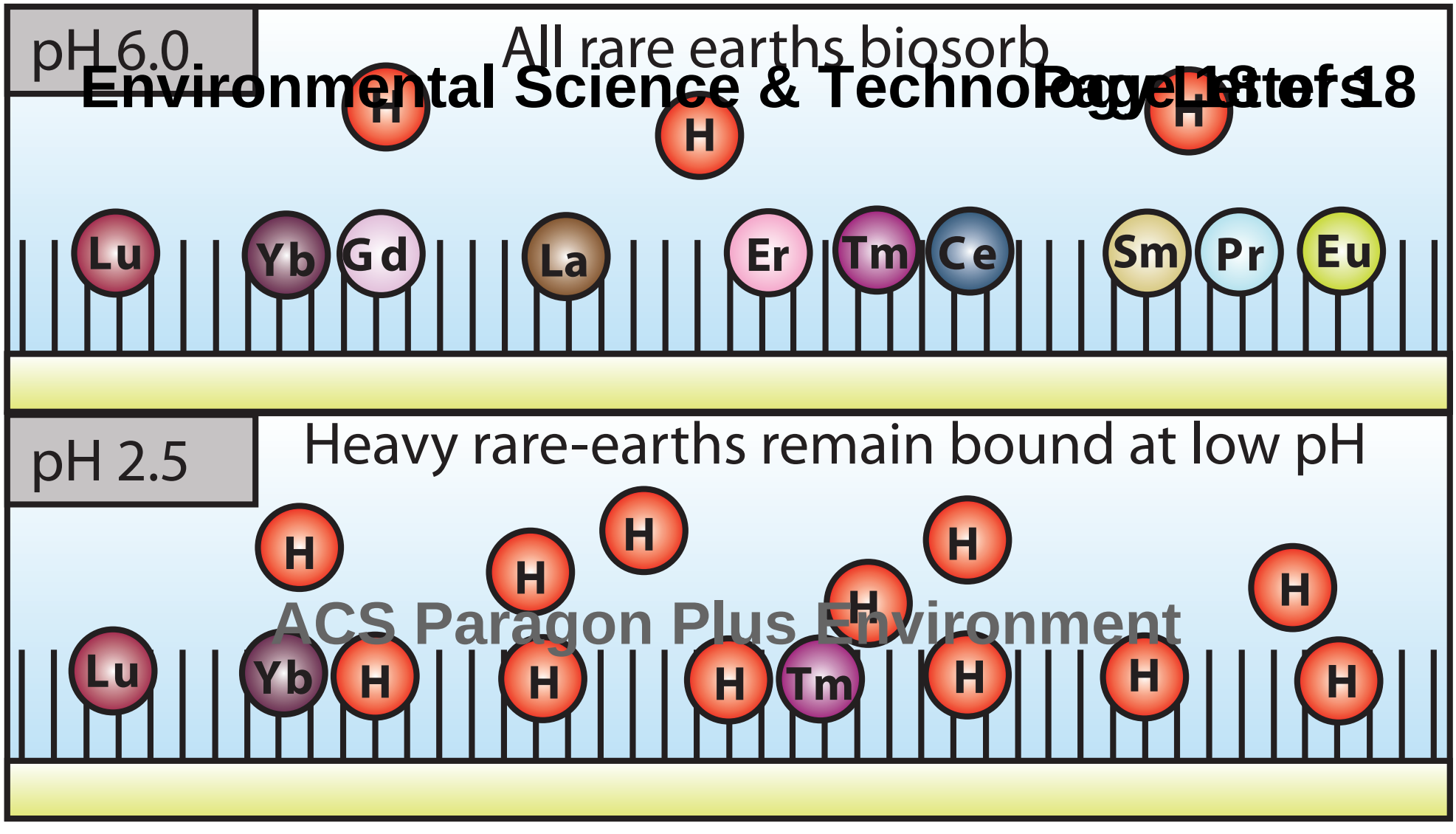
Lanthanide








pH 6.0
All rare earths biosorb
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Heavy rare-earths remain bound at low pH
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