



Höhere technische Bundeslehranstalt  
und Bundesfachschule  
im Hermann Fuchs Bundesschulzentrum



# REECYPRO

## Diplomarbeit

Schulautonomer Schwerpunkt  
Bionik

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February 28, 2024

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# Abstract

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# Introduction

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# Contents

<b>Abstract</b>	iii
<b>Introduction</b>	iv
<b>1 Introduction</b>	2
1.1 Problem Setting <sup>TD</sup> . . . . .	2
1.2 Contributions <sup>MS</sup> . . . . .	3
1.3 Structure of this Thesis <sup>MS</sup> . . . . .	3
<b>2 System Overview</b>	4
2.1 Detection and Measurement of REE concentration <sup>TD</sup> . . . . .	4
2.1.1 Precipitation Reactions . . . . .	4
2.1.2 Arsenazo III Assay . . . . .	5
2.2 Bacteria <sup>MS</sup> . . . . .	5
2.2.1 Methylorum extorquens . . . . .	5
2.2.2 Cultivation . . . . .	5
2.2.3 Lanmodulin <sup>TD</sup> . . . . .	5
2.3 Protein Extraction/IR-Spectrometry <sup>MS</sup> . . . . .	6
2.3.1 Cell Lysis . . . . .	6
2.3.2 SDS-PAGE . . . . .	6
<b>3 Detection and Measurement of REE concentration<sup>TD</sup></b>	7
3.1 Precipitation Reactions . . . . .	7
3.1.1 Cer Precipitation Reaction . . . . .	7
3.1.2 Neodymium Precipitation Reaction . . . . .	7
3.2 Arsenazo III Assay . . . . .	7
3.2.1 Probe Preparation . . . . .	7
3.2.2 Measuring REE Concentration . . . . .	8
<b>4 Bacteria<sup>MS</sup></b>	9
<b>5 Protein Extraction/IR-Spectrometry<sup>MS</sup></b>	10
<b>6 Case Study</b>	11
<b>7 Evaluation<sup>MS</sup></b>	12
<b>8 Project Management</b>	13
8.1 Planning . . . . .	13
8.2 Evaluation <sup>TD</sup> . . . . .	13
8.3 Timesheet . . . . .	14
8.3.1 Tobias Daxecker . . . . .	14

8.3.2 Mathias Standhartinger . . . . .	14
<b>9 Future Work<sup>MS</sup></b>	<b>15</b>
<b>10 Related Work<sup>TD</sup></b>	<b>16</b>
<b>11 Conclusion</b>	<b>17</b>
<b>Acknowledgements</b>	<b>18</b>
<b>Listings</b>	<b>18</b>
<b>List of Figures</b>	<b>19</b>
<b>Bibliography</b>	<b>20</b>
<b>CV</b>	<b>23</b>

# 1 Introduction

Rare Earth Elements (REEs) play a critical role in modern-day life. They are used in nearly every device that uses electrical power to operate. A few examples where REEs are essential are: lasers, computer monitors, electric motors, electric generators, high-power magnets, liquid crystal displays (LCDs), solar panels and many more [1].

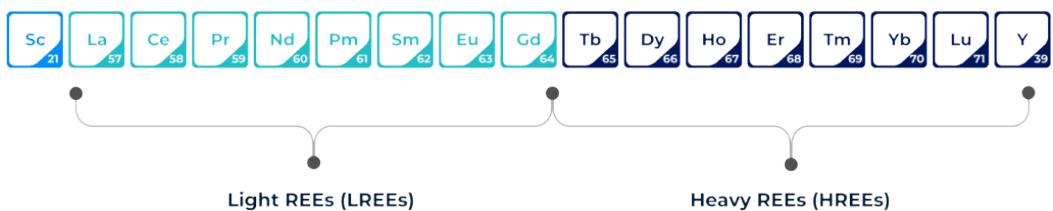


Figure 1.1: List of all rare earth elements. Those 17 elements can be further categorized into the light rare earth elements (LREEs) and the heavy rare earth elements (HREEs). Picture from REIA / Argus Media.

## 1.1 Problem Setting<sup>TD</sup>

Given the importance of REEs in the modern world, it is evident that the demand for them is increasing quickly. In the coming years, as the use of electronic devices increases, many of them will become electronic waste. It is vital for the world's future supply of rare earth elements to recycle them from this waste.

Currently used recycling methods for REEs are mostly damaging to the environment and very costly [2]. Therefore, only around one percent of the global REEs supply is from recycled sources [3]. The rest comes from mining, which brings its own challenges. Rare earth ores (REOs) often contain radioactive elements which adds more complexity to the processing of the ores. Also, the extraction of REEs is done by using a process called flotation which produces large amounts of waste water. This waste water is highly problematic, as it often contains radioactive minerals, acids and toxic agents [4].

The processing of REOs does not only damage the environment, but it also contributes to climate change. As an example, 75 tonnes of CO<sub>2</sub>— equivalents are emitted for every tonne of newly refined Neodymium [5].

There are already thousands of tonnes of electronic waste that contain significant amounts of REEs. Recycling them would reduce the need of mining new REOs and therefore reduce the environmental impact of new electronic devices. Sadly, there is no easy and environmentally friendly process to recycle REEs on an industrial scale.

## 1.2 Contributions<sup>MS</sup>

## 1.3 Structure of this Thesis<sup>MS</sup>

## 2 System Overview

In order to understand the process of the recovery of rare earth elements from electronic waste with biosorption, the key procedures and techniques are described briefly in the following section.

### 2.1 Detection and Measurement of REE concentration<sup>TD</sup>

#### 2.1.1 Precipitation Reactions

A relatively simple proof if a probe contains REEs is a precipitation reaction. The precipitation reactions work because the rare earths form greater complexes with other molecules which have a different color than the surrounding solution [6]. As an example, a Ce precipitation reaction is shown in 2.1 with an orange-red precipitate.

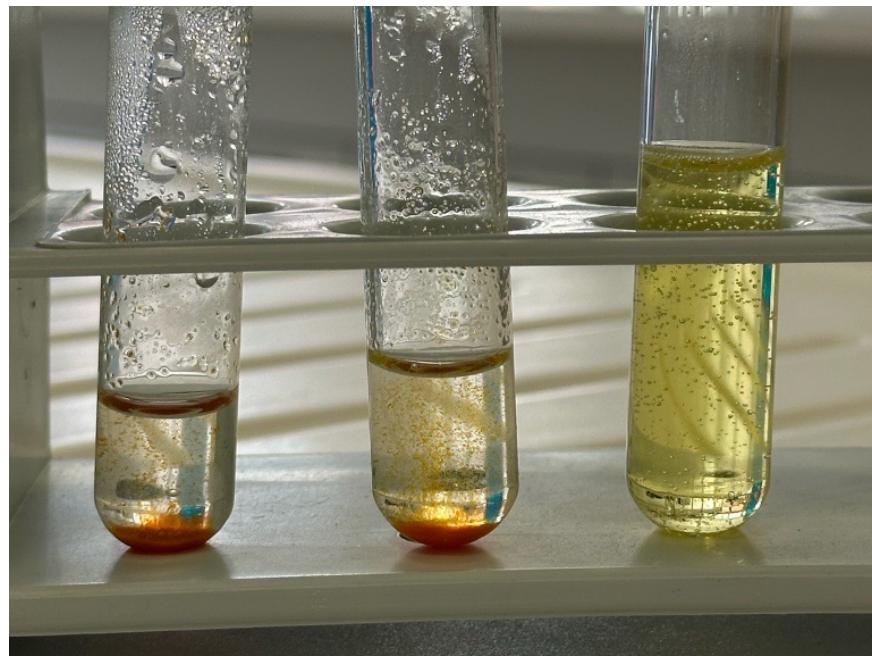


Figure 2.1: Precipitation of a successful REE detection reaction. The test tube on the righthandside does not show any precipitation because the probe was deionized water.

However, you must be careful because of the REEs chemical similarity, the detection of a specific REE is not always possible with these precipitation methods. A precipitation reaction might also not be sensitive enough for your use case. So it could be possible that your probe contains rare earths, but you were not able to detect them.

### 2.1.2 Arsenazo III Assay

A better and more versatile method to detect rare earths in a probe is the so-called Arsenazo III Assay. With this assay, it is not only possible to detect if rare earths are present, but it is also possible to determine the concentration of REEs [7].

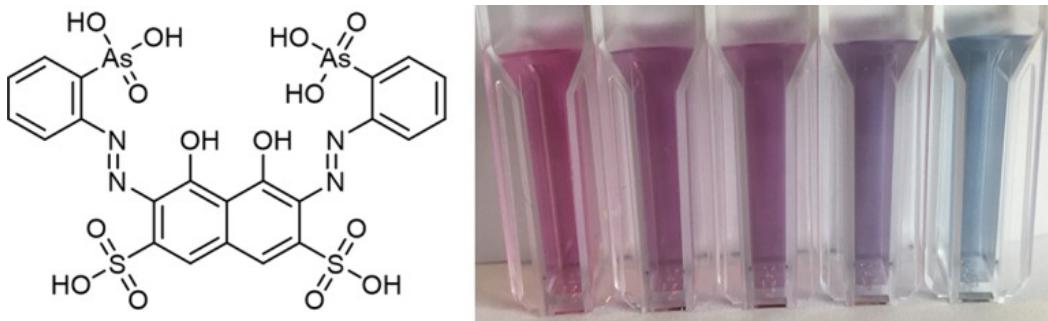


Figure 2.2: Structure of Arsenazo III. On the right you can see how the color of the dye changes with the levels of REE concentration. Picture from "Facile Arsenazo III-based assay for monitoring rare earth element depletion from cultivation media for methanotrophic and methylotrophic bacteria" Hoogendorn et al. [7].

Arsenazo III is a metallochromic dye. This means that the dye changes its color depending on the presence of metal ions (for example: 2.2). A second crucial characteristic is that the color of an Arsenazo III solution is also dependent on the concentration of some metal ions. The metal ions and the Arsenazo III molecule form complexes which block some certain frequencies of light. This property can be used to determine the concentration of rare earths in a probe.

## 2.2 Bacteria<sup>MS</sup>

### 2.2.1 Methylorubrum extorquens

### 2.2.2 Cultivation

### 2.2.3 Lanmodulin<sup>TD</sup>

Lanmodulin (LanM) is a protein produced by *M. extorquens*, a lanthanide-utilizing bacteria [8]. LanM is not essential for the growth or survival of *M. extorquens*, and it is only produced when the bacteria are in a medium with presence of  $\text{Ln}^{\text{III}}$  or  $\text{Ce}^{\text{III}}$  ions [9]. However, the mechanisms that include LanM are not understood as a whole to this day.

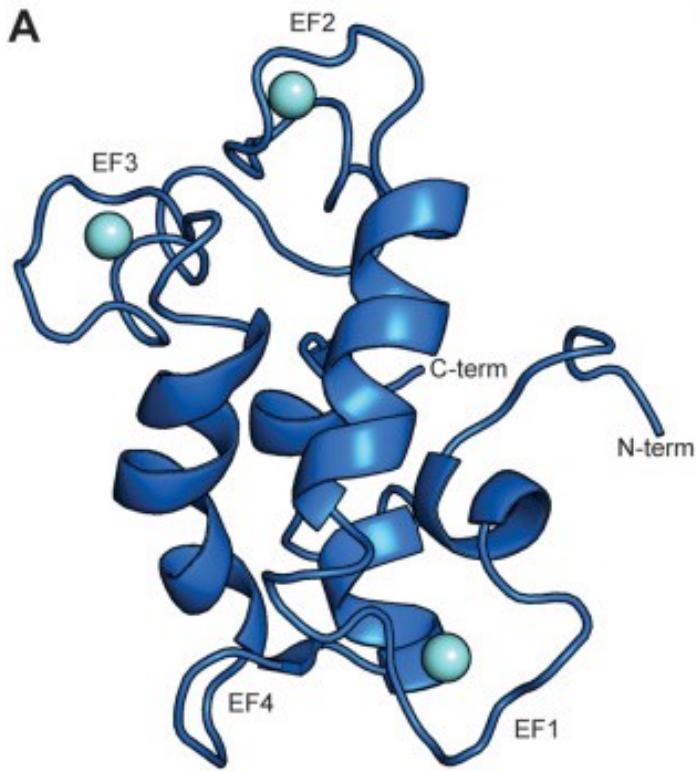


Figure 2.3: Graphical visualization of Lanmodulins structure. EF indicates the EF hands, this is where the REEs can bind to the protein. In this visualization, the turquoise-colored spheres are  $\text{Y}^{\text{III}}$  ions which are bound to the EF hands. Picture from "The biochemistry of lanthanide acquisition, trafficking and utilization", Emily R. Featherston and Joseph A. Cotruvo [9].

The most important characteristic of LanM is, that the molecule is able to bind lanthanide ions, primarily light REEs (LREEs). When LanM does this, it undergoes a transformation from a disordered state to a compact form of itself. The REEs are hereby bound to the so-called EF hands which favor to bind to  $\text{Ln}^{\text{III}}$  and other lanthanoids over  $\text{Ca}^{\text{II}}$  which is usually associated with these EF-hands [10].

## 2.3 Protein Extraction/IR-Spectrometry<sup>MS</sup>

### 2.3.1 Cell Lysis

### 2.3.2 SDS-PAGE

## 3 Detection and Measurement of REE concentration<sup>TD</sup>

The detection of rare earth elements in a probe is a crucial step in our work. It allows us to quantify the effectiveness of our process.

In modern chemistry, quantification of elements in a probe is usually done with inductively coupled plasma mass spectroscopy or ICP-MS. However, as the ICP-MS uses machines which are very, very expensive, this was not an option as it exceeded our limited financial resources by far. Instead, we had to search for other methods to detect and quantify rare earths.

In our work, we used two precipitation reactions and one method to quantify the concentration of REEs.

### 3.1 Precipitation Reactions

#### 3.1.1 Cer Precipitation Reaction

The precipitation reaction for cer works by utilizing the oxidation states +III and +IV [11, 6].

Cer in the aforementioned states forms complexes together with H<sub>2</sub>O<sub>2</sub>. The complexes are called cer peroxide hydrates, and their chemical formulas are Ce(OH)<sub>2</sub>(OOH) and Ce(OH)<sub>3</sub>(OOH). These complexes fall out of the solution as a red-brown colored precipitate.

#### 3.1.2 Neodymium Precipitation Reaction

The reaction to detect neodymium is a bit more complicated. It also uses the +III oxidation state of neodymium. The neodymium reacts with acetic acid to form neodymium acetate. As the last step, iodide is given to the solution which forms a blue-colored complex together with the neodymium acetate [6].

### 3.2 Arsenazo III Assay

The Arsenazo III assay is based on the dye Arsenazo III or ASIII [7]. It is normally used to detect Ca<sub>2+</sub>, but it is also sensitive for other metals [12].

#### 3.2.1 Probe Preparation

To get reliable and correct results, the sample must be prepared beforehand. This happens by adjusting the pH level of the sample solution to around 2.8. This ensures that only rare

earth ions interact with the Arsenazo III dye. Another advantage of this acidic level is that the ions of the rare earths dissolve better from the sample.

### 3.2.2 Measuring REE Concentration

The measuring of the concentration of the rare earths works with a UV-Vis-spectrometer. This is a device, that can produce light with a single wavelength. The light goes through the sample and the light intensity is measured. When the intensity of the outgoing light  $I$  is set in relation to the intensity of the ingoing light  $I_0$ , the emerging result is the transmittance  $T$  [13].

$$T = \frac{I}{I_0}$$

The transmittance is then used to calculate the absorbance  $A$  using the following formula [14].

$$A = \log T^{-1} = \log \frac{I_0}{I}$$

The absorbance is the output of the UV-Vis-spectrometer. It is possible to measure just the absorbance at one single wavelength with the device. However, it can also measure the absorbance from a series of wavelengths and plot the result to a spectrum. For the Arsenazo III assay, the absorbance at the wavelength of around 650 nm is important.

The final measurement is done with a 1 mL cuvette. Half of it is filled with a phosphate-citrate buffer to ensure a correct pH level. Afterwards, 490 µL of the sample and 10 µL of the Arsenazo III dye are added to the cuvette. The solutions in the cuvette have to be mixed, and then a spectrum from 500 nm to 800 nm is recorded. The absorbance at 650 nm is noted. This is later used for calculation of the concentration. Then, 20 µL of Arsenazo III are again added and mixed into the cuvette. The spectrum and the value at the wavelength of 650 nm are again recorded. This last step is necessary for rare earth concentrations of more than 2 µmol/L, because it was found that these values suit better for higher concentrations.

These measurements are not only done with the samples but also with solutions that contain a known concentration of rare earths. The values can then be used to calculate a calibration line which in turn gives us the concentration of the samples.

## **4 Bacteria<sup>MS</sup>**

## 5 Protein Extraction/IR-Spectrometry<sup>MS</sup>

## 6 Case Study

## 7 Evaluation<sup>MS</sup>

# 8 Project Management

## 8.1 Planning

Nº	Milestone	Date of Achieval
MS_1	Cultivation of Bacteria	09.11.2023
MS_2	Extraction of LanM	07.12.2023
MS_3	Detection of LanM	n/d
MS_4	Binding of LanM to Rare Earth Elements	n/d
MS_5	Separation of Rare Earths from LanM	n/d

## 8.2 Evaluation<sup>TD</sup>

When we started to conduct some research for the project in the summer break, we also began simultaneously to plan the work with agile project management methods. As it turned out, doing the project management this way was really helpful. During our work, we encountered a lot of obstacles which we had not thought of before, which resulted in a slower progress than we had previously expected. Using an agile project board made it very easy for us to keep track of all of our work. Even though on some days we had to add more tasks to the *Todo* or *In Progress* than to the *Finished* section.

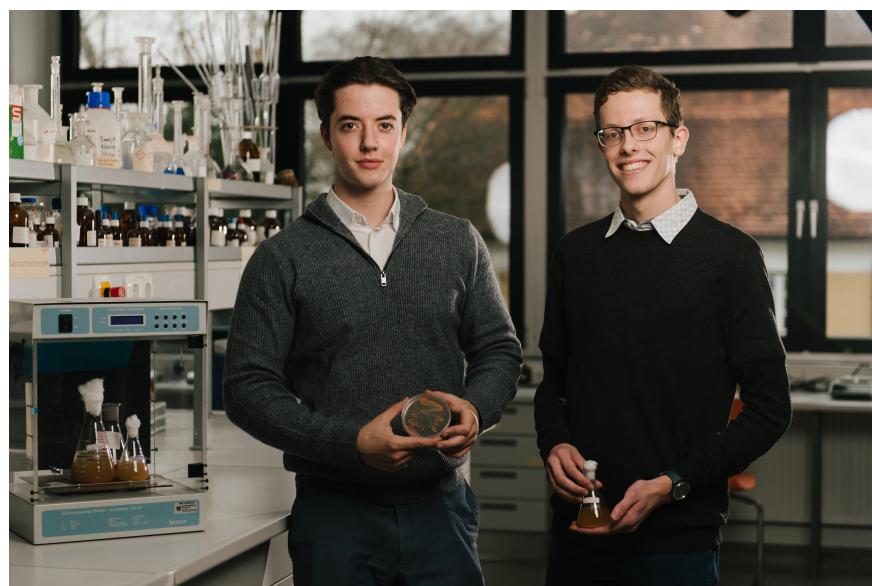


Figure 8.1: The project team

### 8.3 Timesheet

#### 8.3.1 Tobias Daxecker

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#### 8.3.2 Mathias Standhartinger

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## 9 Future Work<sup>MS</sup>

## 10 Related Work<sup>TD</sup>

## 11 Conclusion

# Acknowledgements

# Listings

# List of Figures

1.1	List of all rare earth elements. Those 17 elements can be further categorized into the light rare earth elements (LREEs) and the heavy rare earth elements (HREEs). Picture from REIA / Argus Media. . . . .	2
2.1	Precipitation of a successful REE detection reaction. The test tube on the righthandside does not show any precipitation because the probe was deionized water. . . . .	4
2.2	Structure of Arsenazo III. On the right you can see how the color of the dye changes with the levels of REE concentration. Picture from "Facile Arsenazo III-based assay for monitoring rare earth element depletion from cultivation media for methanotrophic and methylotrophic bacteria" Hoogendorn et al. [7].	5
2.3	Graphical visualization of Lanmodulins structure. EF indicates the EF hands, this is where the REEs can bind to the protein. In this visualization, the turquoise-colored spheres are $\text{Y}^{\text{III}}$ ions which are bound to the EF hands. Picture from "The biochemistry of lanthanide acquisition, trafficking and utilization", Emily R. Featherston and Joseph A. Cotruvo [9]. . . . .	6

# Bibliography

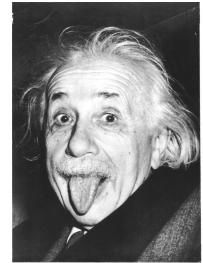
- [1] Daniele Paderni, Luca Giorgi, Vieri Fusi, Mauro Formica, Gianluca Ambrosi, and Mauro Micheloni. Chemical sensors for rare earth metal ions. *Coordination Chemistry Reviews*, 429:213639, 2021.
- [2] Yoshiko Fujita, Scott K. McCall, and Daniel Ginosar. Recycling rare earths: Perspectives and recent advances. *MRS Bulletin*, 47(3):283–288, Mar 2022.
- [3] Simon M. Jowitt, Timothy T. Werner, Zhehan Weng, and Gavin M. Mudd. Recycling of the rare earth elements. *Current Opinion in Green and Sustainable Chemistry*, 13:1–7, 2018. Reuse and Recycling / UN SGDs: How can Sustainable Chemistry Contribute? / Green Chemistry in Education.
- [4] Doris Schüler, Matthias Buchert, Ran Liu, Ste-fanie Dittrich, and Cornelia Merz. *Study on rare earths and their recycling*. Darmstadt, Germany, Öko-Institut e.V., 2011.
- [5] IEA. Average ghg emissions intensity for production of selected commodities, 2021.
- [6] Gerhart Jander und Ewald Blasius und Joachim Strähle. *Lehrbuch der analytischen und präparativen anorganischen Chemie*. Stuttgart: Hirzel, 1995.
- [7] Carmen Hogendoorn, Paula Roszczenko-Jasińska, N Cecilia Martinez-Gomez, Johann de Graaff, Patrick Grassl, Arjan Pol, Huub J M Op den Camp, and Lena J Daumann. Facile arsenazo III-based assay for monitoring rare earth element depletion from cultivation media for methanotrophic and methylotrophic bacteria. *Appl. Environ. Microbiol.*, 84(8), April 2018.
- [8] Joseph A. Jr. Cotruvo, Emily R. Featherston, Joseph A. Mattocks, Jackson V. Ho, and Tatiana N. Laremore. Lanmodulin: A highly selective lanthanide-binding protein from a lanthanide-utilizing bacterium. *Journal of the American Chemical Society*, 140(44):15056–15061, 2018.
- [9] Emily R. Featherston and Joseph A. Cotruvo. The biochemistry of lanthanide acquisition, trafficking, and utilization. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1868(1):118864, 2021.
- [10] Erik C. Cook, Emily R. Featherston, Scott A. Showalter, and Joseph A. Jr. Cotruvo. Structural basis for rare earth element recognition by *methylobacterium extorquens* lanmodulin. *Biochemistry*, 58(2):120–125, 2019. PMID: 30352145.
- [11] Axel Schunk. Experiment des Monats Februar 2015 Cer-Nachweis, 2015.
- [12] N.C. Kendrick. Purification of arsenazo iii, a  $\text{Ca}^{2+}$ -sensitive dye. *Analytical Biochemistry*, 76(2):487–501, 1976.
- [13] International Union of Pure and Applied Chemistry (IUPAC). *transmittance*, 3.0.1 edition, 2019.

- [14] International Union of Pure and Applied Chemistry (IUPAC). *absorbance*, 3.0.1 edition, 2019.

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