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March 11, 2024

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Tobias Daxecker
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Mathias Standhartinger
Verfasser

Unterschrift

Abstract

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Introduction

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Contents

Abstract	iii
Introduction	iv
1 Introduction	2
1.1 Problem Setting ^{TD}	2
1.2 Contributions ^{MS}	3
1.3 Structure of this Thesis ^{MS}	3
2 System Overview	4
2.1 Detection and Measurement of REE concentration ^{TD}	4
2.1.1 Precipitation Reactions	4
2.1.2 Arsenazo III Assay	5
2.2 Bacteria ^{MS}	5
2.2.1 Methylorum extorquens	5
2.2.2 Cultivation	5
2.2.3 Lanmodulin ^{TD}	5
2.3 Protein Extraction/IR-Spectrometry ^{MS}	6
2.3.1 Cell Lysis	6
2.3.2 SDS-PAGE	6
3 Detection and Measurement of REE concentration^{TD}	7
3.1 Precipitation Reactions	7
3.1.1 Cer Precipitation Reaction	7
3.1.2 Neodymium Precipitation Reaction	8
3.2 Arsenazo III Assay	9
3.2.1 Arsenazo III	9
3.2.2 Probe Preparation	11
3.2.3 Measuring REE Concentration	11
4 Bacteria^{MS}	13
5 Protein Extraction/IR-Spectrometry^{MS}	14
6 Evaluation^{MS}	15
7 Project Management	16
7.1 Planning	16
7.2 Evaluation ^{TD}	16
7.3 Timesheet	18
7.3.1 Tobias Daxecker	18
7.3.2 Mathias Standhartinger	18

8 Future Work^{MS}	19
9 Related Work^{TD}	20
10 Conclusion^{TD}	22
Acknowledgements	23
List of Figures	23
Bibliography	24
CV	27

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].

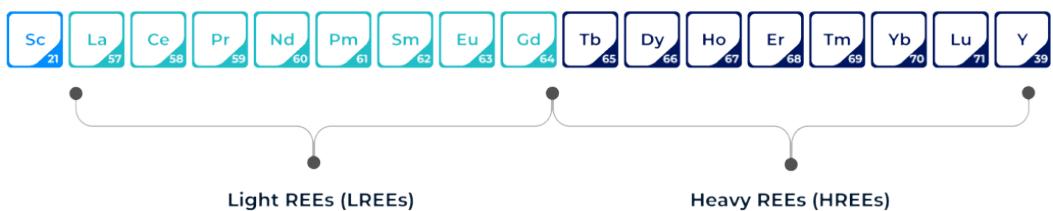


Figure 1.1: List of all rare earth elements. Those 17 elements can be further categorized into the light rare earth elements (LREEs) and the heavy rare earth elements (HREEs). Picture from REIA / Argus Media.

1.1 Problem Setting^{TD}

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 CO₂— 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^{MS}

1.3 Structure of this Thesis^{MS}

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^{TD}

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].

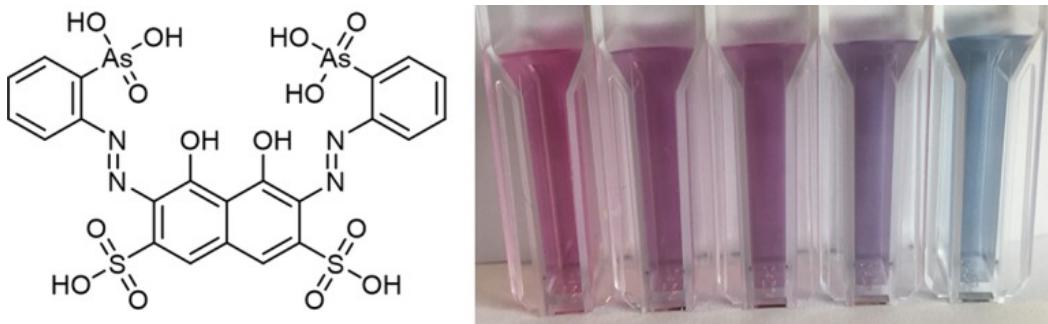


Figure 2.2: Structure of arsenazo III. On the right you can see how the color of the dye changes with the levels of REE concentration. Picture from "Facile Arsenazo III-based assay for monitoring rare earth element depletion from cultivation media for methanotrophic and methylotrophic bacteria" Hoogendorn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

2.2.1 Methylorubrum extorquens

2.2.2 Cultivation

2.2.3 Lanmodulin^{TD}

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 Ln^{III} or Ce^{III} ions [9]. However, the mechanisms that include LanM are not understood as a whole to this day.

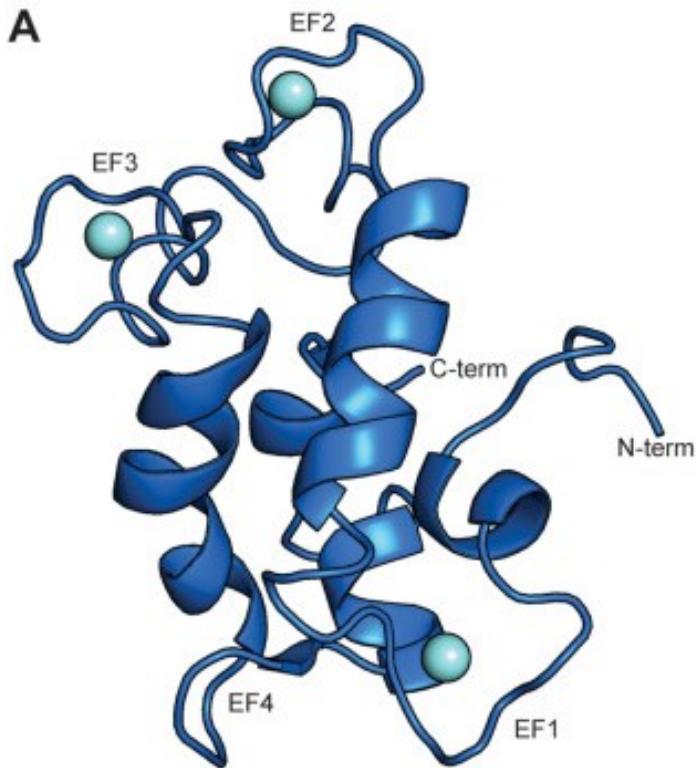


Figure 2.3: Graphical visualization of Lanmodulins structure. EF indicates the EF hands, this is where the REEs can bind to the protein. In this visualization, the turquoise-colored spheres are Y^{III} ions which are bound to the EF hands. Picture from "The biochemistry of lanthanide acquisition, trafficking 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 Ln^{III} and other lanthanoids over Ca^{II} which is usually associated with these EF-hands [10].

2.3 Protein Extraction/IR-Spectrometry^{MS}

2.3.1 Cell Lysis

2.3.2 SDS-PAGE

3 Detection and Measurement of REE concentration^{TD}

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, a qualitative and quantitative analysis of elements in a probe is usually done with inductively coupled plasma mass spectroscopy (ICP-MS) or atom absorption spectroscopy (AAS). However, as the ICP-MS and AAS use machines that are very, very expensive, these methods were not an option as they 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].

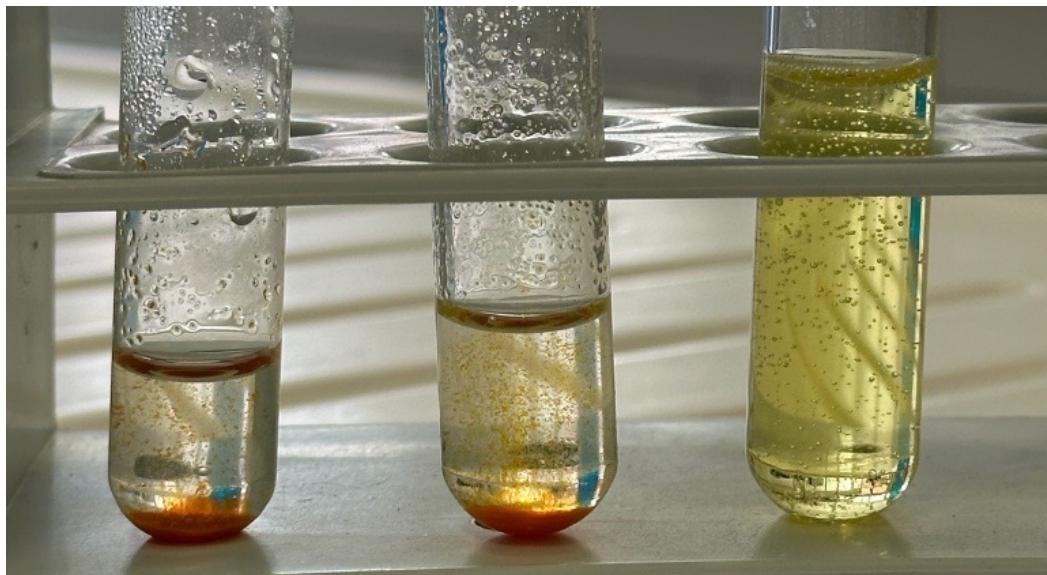


Figure 3.1: Precipitation of a successful cer detection reaction. The test tube on the righthandside does not show any precipitation because the sample was deionized water.

Cer in the aforementioned states forms complexes together with H_2O_2 . The complexes are called cer peroxide hydrates. Their chemical formulas are $\text{Ce}(\text{OH})_2(\text{OOH})$ and $\text{Ce}(\text{OH})_3(\text{OOH})$. 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].

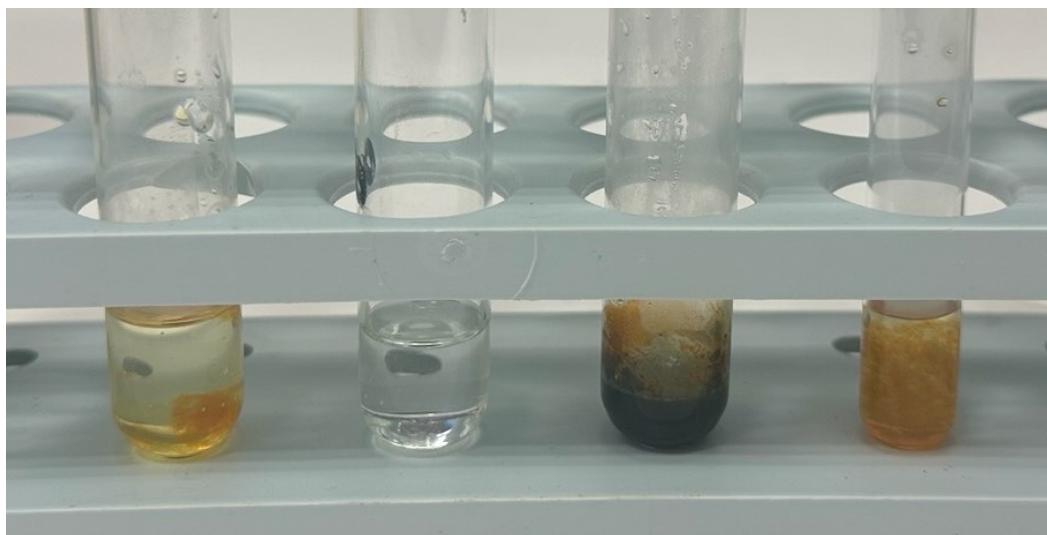


Figure 3.2: Neodymium detection reaction. Neodymium is contained in the sample of the third test tube (left to right). The blue precipitate is clearly visible.

3.2 Arsenazo III Assay

3.2.1 Arsenazo III

The arsenazo III assay is based on the dye arsenazo III or ASIII [7]. It is often used to detect calcium, uranium and a lot of other metals, including rare earth elements [12, 13].

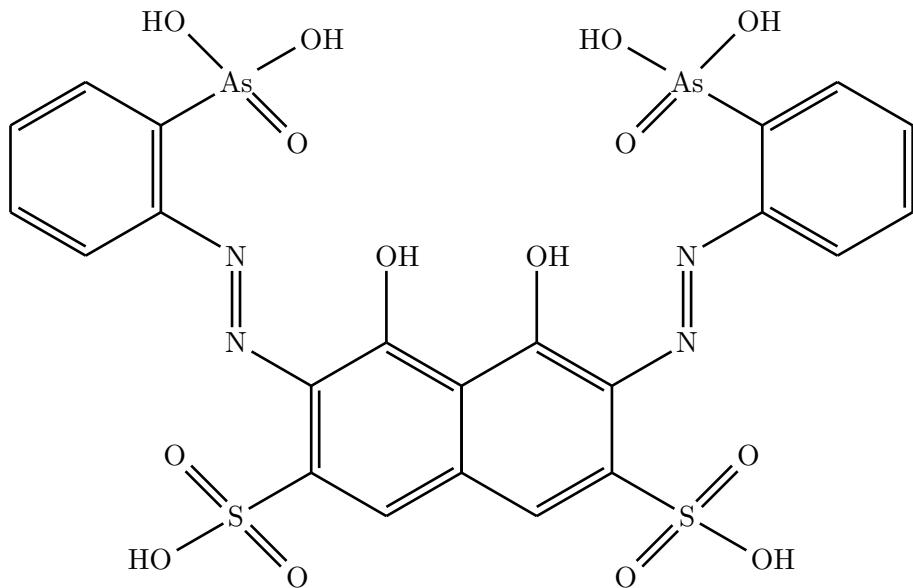


Figure 3.3: Structure of 2,7-bis(2-arsenophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid. Or, in its abbreviated form, arsenazo III.

Arsenazo III was first synthesized in 1959 [14]. In comparison with arsenazo I and II, it possesses two functional arsено groups (see fig.3.3). The arsenazo III dye has the property to change its color based on the pH and the presence of some elements. Normally, the dye has a pinkish-crimson color, but when, for example, thorium is present, the color changes to green. For other elements, other colors have been reported, such as blue for calcium or violet-blue and also green for rare earth elements.

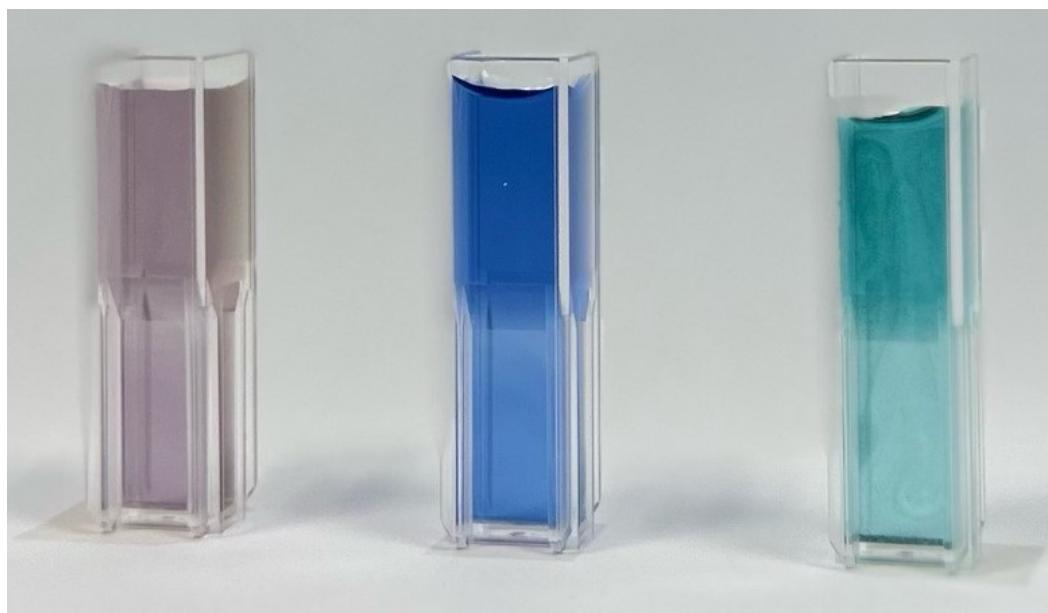


Figure 3.4: Example for different colors of arsenazo III with different samples. The contents of the cuvettes are (from left to right): FeCl_3 , CuSO_4 , NdCl_3 . All are mixed with $10\mu\text{L}$ of 10mM arsenazo III.

The color change happens, because the arsenazo III forms complexes with certain elements. Arsenazo III and rare earths and some other metals form 1:1 complexes [15, 16]. This means that for every molecule of arsenazo III, one rare earth element atom was bound (see fig. 3.5). The other arseno group is most likely not used to form these stable complexes.

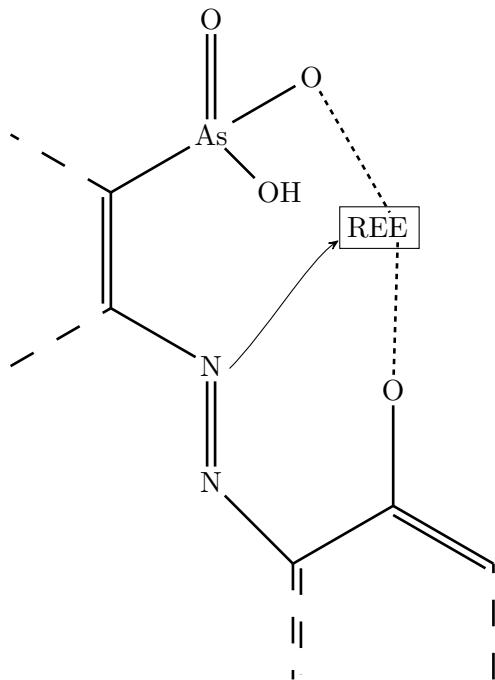


Figure 3.5: An arsenazo III complex with an atom of a rare earth element.

3.2.2 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 2.7 to 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.

3.2.3 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 [17].

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

The transmittance is then used to calculate the absorbance A using the following formula [18].

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

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 (see fig. 3.6).

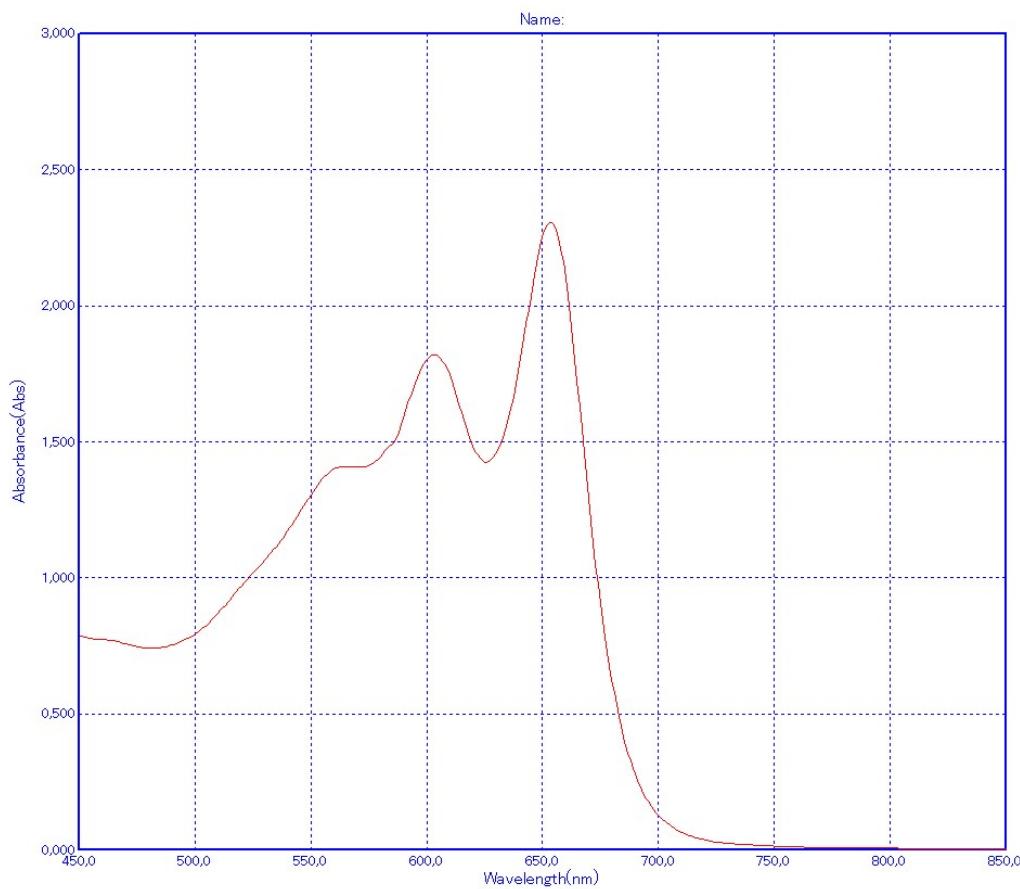


Figure 3.6: Example of a spectrum of an arsenazo III assay. The peak at around 650nm is the product of a complex formed by one rare earth atom and one arsено group.

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. The dual measurement is necessary for rare earth concentrations of more than $2\mu\text{mol/L}$, 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^{MS}

5 Protein Extraction/IR-Spectrometry^{MS}

6 Evaluation^{MS}

7 Project Management

7.1 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	obsolete
MS_4	Binding of LanM to Rare Earth Elements	29.02.2024
MS_5	Separation of Rare Earths from LanM	n/d

7.2 Evaluation^{TD}

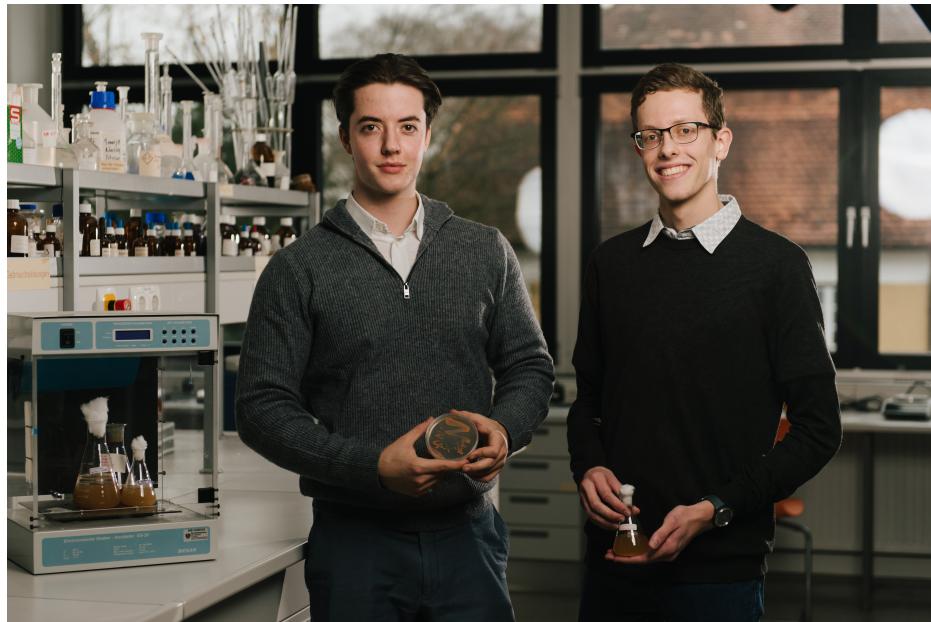


Figure 7.1: The project team

When we started to conduct some research for the project in the summer break, we also simultaneously began 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.

Another problem that we encountered was that we simply could not do our project the way we had planned at the beginning. Due to limited financial resources and equipment, we

could not carry out our planned work. A lot of methods we tried out did not produce the expected or reliable results. When we ran into these problems, we had to change how we want to achieve our planned goals. This also meant that one of our planned milestones (MS_3 Detection of LanM, see section 7.1) is completely obsolete, because this step is simply not necessary anymore.

After we had tried our new approach, we finally achieved promising results. This brought fresh air into the project because we saw that progress was being made. After weeks of repeated failure, we found new motivation to keep going.

Our new approach requires less expensive resources and is simpler to carry out. Overall, this made our project better, and it did not change our main goal. The transformation from our first approach to the other would not have been possible if we had not used agile project management methods.

7.3 Timesheet

7.3.1 Tobias Daxecker

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Ort, Datum

Tobias Daxecker

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7.3.2 Mathias Standhartinger

Braunau/Inn, 11.03.2024
Ort, Datum

Mathias Standhartinger

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8 Future Work^{MS}

9 Related Work^{TD}

There are some other studies that are somewhat close to our work. Most of them have the same basic idea at their core. That is to use *M. extorquens* or lanmodulin to recycle rare earth elements.

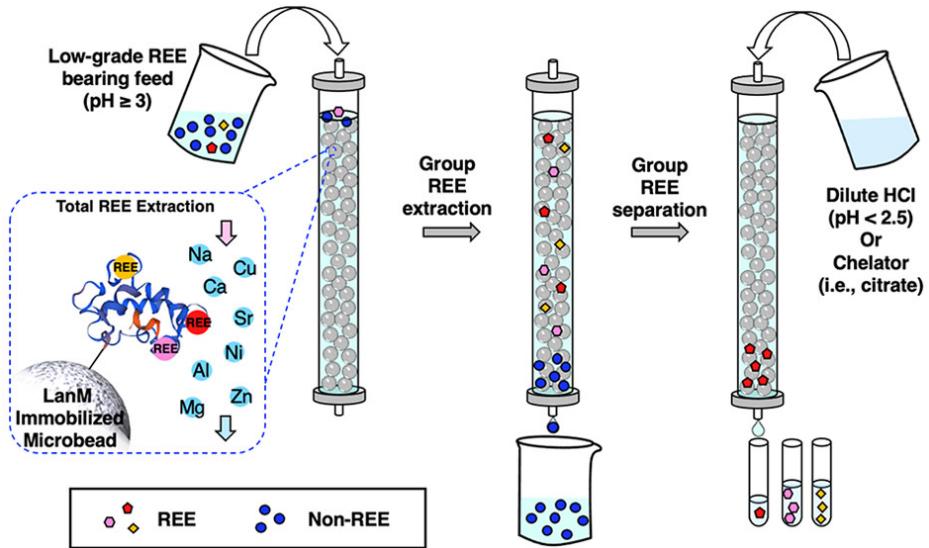


Figure 9.1: Overview of the work which inspired this thesis. Picture from "Bridging Hydrometallurgy and Biochemistry: A Protein-Based Process for Recovery and Separation of Rare Earth Elements", Dong et al. [19].

An example for the usage of only lanmodulin would be the work of Dong et al. [19]. Their approach was to take lanmodulin and attach it to a microbead (a small sphere made of agarose). The product of this procedure is the immobilized lanmodulin. They made a lot of the immobilized lanmodulin and put it into a column. Afterwards, they let a solution which contained ash from a coal power plant, which in turn contained some REEs, flow through the column. The REEs get caught by lanmodulin, and every other metal flows freely through the whole column. After that, they washed their column, and then they began separating the different rare earths. They achieved this by giving solutions with different pH values into their column. Lanmodulin releases only some certain rare earths at a certain pH which is useful for separating them. When every rare earth has been extracted, the column can be cleaned and even be reused for the next recycling process.

This is a very clever process that even inspired this thesis. However, this work is not easy to reproduce. It requires costly chemicals and machinery, which only a company or a university can afford. Therefore, it was not feasible at our school. What must also be taken into consideration is that they used a genetically modified bacteria which produced the lanmodulin. This step alone would take too long to achieve for a diploma thesis.

Good et al. took another approach, which is surprisingly similar to our work. Their basic idea was to let *M. extorquens* grow in a solution which contains electronic waste and find methods to increase the yield of this recycling method [20]. This approach is fairly similar to our own work. However, this work did not inspire us because the paper was first published on December 27th 2023, when we already had worked three months on our project.

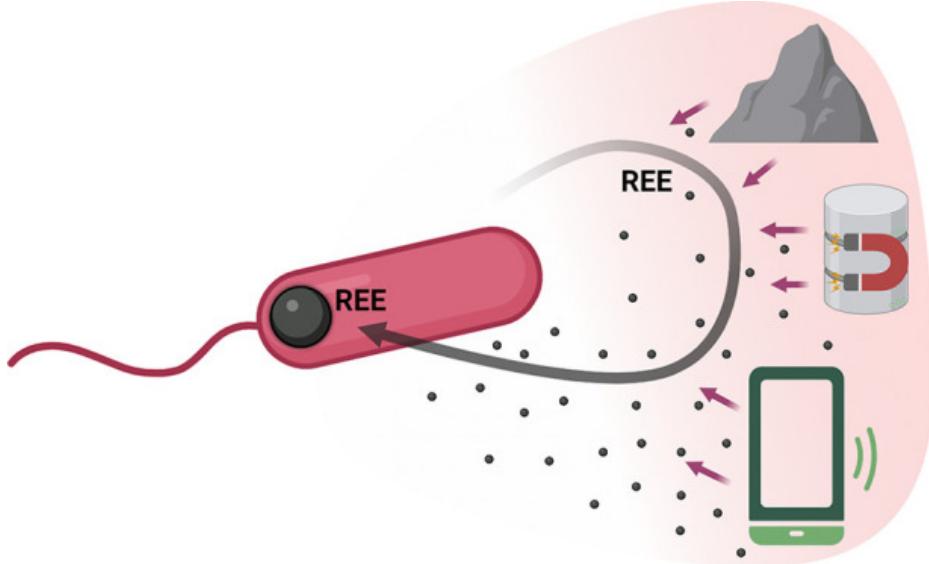


Figure 9.2: Very simplified abstract of the work from Good et al. Picture from "Scalable and Consolidated Microbial Platform for Rare Earth Element Leaching and Recovery from Waste Sources", Good et al. [20].

The main difference to our work is their technological advantage. They used a genetically modified strain of *M. extorquens* AM1, which is called $\Delta mxaF$. They deleted the gene *mxaF* to ensure that the growth of the bacteria is dependent on the uptake of rare earths. This led to a higher rare earth uptake capacity per bacteria.

Another remarkable difference is that they did not only let the bacteria grow with crushed magnets, but also with a crushed smartphone. This did have, interestingly, no significant impact on the growth of *M. extorquens* AM1 $\Delta mxaF$, according to their study.

After that, they improved the yield by adding an organic acid to the bacteria's growth medium. This helped to extract the rare earths from the crushed magnet (and smartphone). What also boosted their yield was that they genetically engineered *M. extorquens* AM1 $\Delta mxaF$ even further.

We can conclude that our project was done with the minimum of resources you can use to achieve some good results. Compared to the above-mentioned studies, we had very limited resources and only basic laboratory equipment, so we could do only basic work. What the other studies achieved is really great, but we showed that it is possible to be part of the newest developments of science without expensive materials and equipment.

10 Conclusion^{TD}

The process of recycling of rare earths from e-waste using bacteria is a more eco-friendly and energy efficient way than currently established recycling methods. In our project, we achieved to carry out this process and to determine its efficiency. Hereby, it is important to know that we only measured the natural capacity of *M. extorquens* without any additional changes.

In brief, our project can be summarized as follows: We found a way to efficiently recycle rare earth elements from e-waste. This works with the bacteria *Methylorum extorquens*, which has the ability to use rare earth elements in its metabolism. This property of the bacteria is essential because the e-waste is simply given in a crushed form to the culture medium. The rare earths accumulate naturally in the bacteria. The bacteria can then be opened to recover the rare earths.

<Beschreibung von Ergebnisse / Effizienz>

We learned a lot during the time of this project, because neither of us had previous knowledge in the field of microbiology. This meant that we had to research everything from the ground up. In the beginning, we thought we would do a lot of things differently than we do now. But after three months of work, we came to a dead end because our school lacked the required equipment. This had the consequence that we had to pivot our work in a new direction. Afterward, we finally managed to achieve results.

The key method, which we discovered late in the project was the arsenazo-III assay. This assay is a method to determine the concentration of rare earth elements in a sample. Without this method, we would not have achieved any results at all, because all the other methods we tried did not work well enough.

What is also noteworthy is that we learned that at any given time something unexpected can happen, which ruins the work of a whole day.

Acknowledgements

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 Y^{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
3.1	Precipitation of a successful cer detection reaction. The test tube on the righthandside does not show any precipitation because the sample was deionized water.	7
3.2	Neodymium detection reaction. Neodymium is contained in the sample of the third test tube (left to right). The blue precipitate is clearly visible.	8
3.3	Structure of 2,7-bis(2-arsenophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid. Or, in its abbreviated form, arsenazo III.	9
3.4	Example for different colors of arsenazo III with different samples. The contents of the cuvettes are (from left to right): FeCl_3 , CuSO_4 , NdCl_3 . All are mixed with 10 μL of 10mM arsenazo III.	10
3.5	An arsenazo III complex with an atom of a rare earth element.	10
3.6	Example of a spectrum of an arsenazo III assay. The peak at around 650nm is the product of a complex formed by one rare earth atom and one arsено group.	12
7.1	The project team	16
9.1	Overview of the work which inspired this thesis. Picture from "Bridging Hydrometallurgy and Biochemistry: A Protein-Based Process for Recovery and Separation of Rare Earth Elements", Dong et al. [19].	20
9.2	Very simplified abstract of the work from Good et al. Picture from "Scalable and Consolidated Microbial Platform for Rare Earth Element Leaching and Recovery from Waste Sources", Good et al. [20].	21

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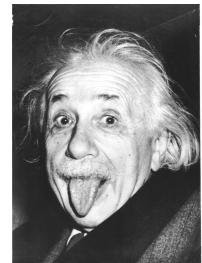
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