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# REECYPRO

## Diplomarbeit

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Bionik**

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# Abstract

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# Introduction

In 2021, around five million tonnes of electronic waste were generated in the EU alone, but less than 40 per cent were recycled. This waste often contains valuable metals, but currently most of them are disposed. Some of these disposed metals are the so-called rare earth elements. The rare earths are critical for every electronic device but they are only used in small quantities, so that conventional recycling is not a economically feasible possibility.

For every new smartphone, for example, new rare earths must be mined. This happens mostly in countries where compliance with human and environmental rights are questionable. The following refining of the rare earths is a very energy-consuming, environmentally harmful and climate damaging process. For a single tonne of neodymium, the most used rare earth element, some 75 tonnes of CO<sub>2</sub> are emitted. But the problems do not stop there. There are only a few places on earth where rare earths are mined, because it is mostly not economically viable because of China, who dominates the market.

The largest mine for rare earths is located in China and additionally, China is the largest producer of refined rare earths, which is then used elsewhere to produce electronics. This means that the world's current supply of rare earth elements is largely monopolized by a country, which does not adhere to human and environmental rights.

A solution could be the recycling of rare earths from electronic waste. However, currently established methods are either very expensive, damaging to the environment or use a lot of energy.

A promising alternative could be the usage of bacteria to recover the rare earths. Bacteria have the advantages that they do not need a lot of energy to grow and they only need inexpensive resources to be able to grow.

In this thesis, we tested if, and how rare earth elements can be recycled from electronic waste using bacteria. We outline how this process works and we report our findings from the actual realisation of this process.

# Contents

<b>Abstract</b>	<b>iii</b>
<b>Introduction</b>	<b>iv</b>
<b>1 Introduction</b>	<b>2</b>
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 Theoretical Background</b>	<b>4</b>
2.1 System Overview . . . . .	4
2.1.1 Detection and Measurement of REE concentration <sup>TD</sup> . . . . .	4
2.1.2 Methylorum extorquens . . . . .	6
2.2 Detection and Measurement of REE concentration <sup>TD</sup> . . . . .	8
2.2.1 Precipitation Reactions . . . . .	8
2.2.2 Arsenazo III Assay . . . . .	11
2.3 Methylorum extorquens . . . . .	15
2.3.1 Taxonomy <sup>MS</sup> . . . . .	15
2.3.2 Methanol Metabolism <sup>MS</sup> . . . . .	17
2.3.3 Lanmodulin <sup>TD</sup> . . . . .	19
2.3.4 Growth <sup>MS</sup> . . . . .	20
2.3.5 Lysis <sup>MS</sup> . . . . .	21
2.4 Protein Extraction and Analysis <sup>MS</sup> . . . . .	22
2.4.1 Protein Extraction . . . . .	22
2.4.2 IR-Spectrometry . . . . .	24
<b>3 Experimental Section</b>	<b>27</b>
3.1 Cultivation of Bacteria <sup>MS</sup> . . . . .	27
3.2 Protein Extraction <sup>MS</sup> . . . . .	28
3.3 Protein Analysis <sup>TD</sup> . . . . .	28
3.3.1 IR-Spectrometry . . . . .	28
3.3.2 SDS-PAGE . . . . .	29
3.4 Arsenazo-III Assay <sup>TD</sup> . . . . .	31
<b>4 Results and Discussion</b>	<b>32</b>
4.1 Cultivation of Bacteria <sup>MS</sup> . . . . .	32
4.2 Protein Analysis <sup>TD</sup> . . . . .	32
4.3 Arsenazo-III Assay <sup>TD</sup> . . . . .	32

<b>5 Project Management</b>	<b>33</b>
5.1 Planning . . . . .	33
5.2 Evaluation <sup>TD</sup> . . . . .	33
5.3 Timesheet . . . . .	35
5.3.1 Tobias Daxecker . . . . .	35
5.3.2 Mathias Standhartinger . . . . .	38
<b>6 Future Work<sup>MS</sup></b>	<b>40</b>
<b>7 Related Work<sup>TD</sup></b>	<b>41</b>
<b>8 Conclusion<sup>TD</sup></b>	<b>44</b>
<b>Acknowledgements</b>	<b>45</b>
<b>List of Figures</b>	<b>45</b>
<b>Bibliography</b>	<b>47</b>
<b>CV</b>	<b>51</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].

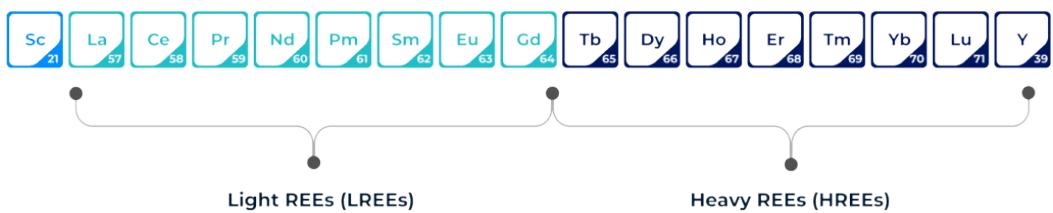


Figure 1.1: List of all rare earth elements. Those 17 elements can be further categorized into the light rare earth elements (LREEs) and the heavy rare earth elements (HREEs). Picture from REIA / Argus Media.

## 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>

To combat the issues mentioned above, we worked on a way to recycle REEs without the need for large amounts of energy or resources. By using bacteria that produce a special amino acid that allows us to bind the REEs in electronic waste, we achieved just that. Due to the bacteria not needing significant amounts of energy, we managed to remain eco-friendly and cost-efficient. The recycling process works by washing shredded electronic waste with our bacteria solution. After changing the pH value of said solution we can get the REEs back in their pure forms. This process works on a scientific level in a laboratory but could also be used on an industrial scale using large bioreactors and washing tanks.

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

## 2 Theoretical Background

In order to understand the process of the recovery of rare earth elements from electronic waste with bioaccumulation, the key procedures and techniques are described in this chapter.

### 2.1 System Overview

In the following section, all used methods and the most important concepts are briefly summarized.

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

##### 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 figure 2.1 with an orange-red precipitate.

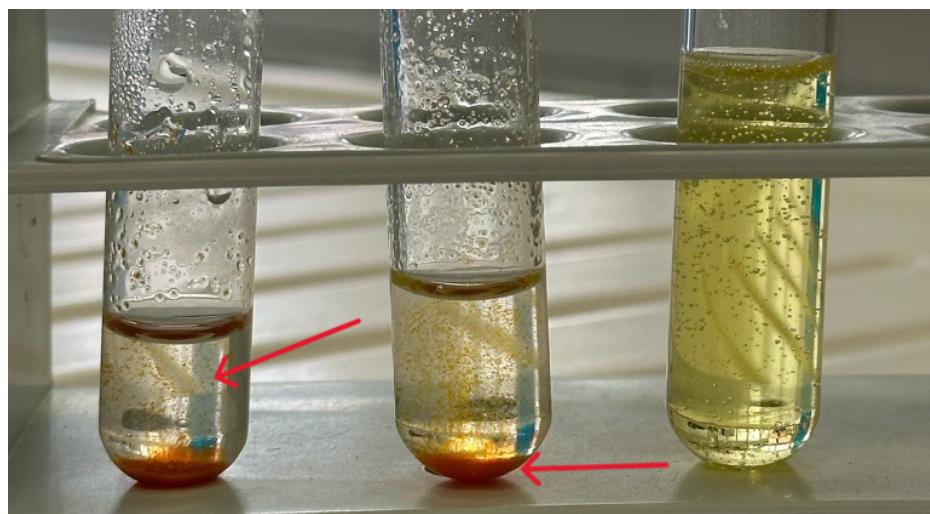


Figure 2.1: Precipitation of a successful REE detection reaction. The red arrows point to the orange-red precipitation.

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.

### 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].

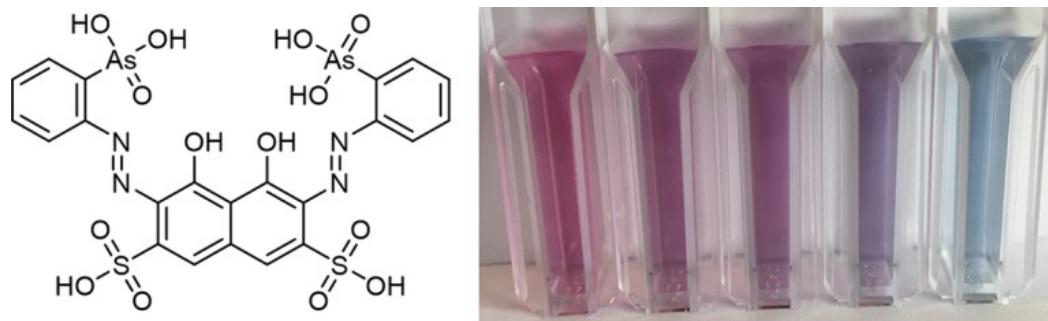


Figure 2.2: Structure of arsenazo III. The color change of the dye depending on the REE concentration is shown on the righthandside. Picture from "Facile Arsenazo III-based assay for monitoring rare earth element depletion from cultivation media for methanotrophic and methylotrophic bacteria" Hoogendorn 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.

### 2.1.2 *Methylorum extorquens*

#### General information<sup>MS</sup>

Utilizing a special strain of bacteria called *Methylorum extorquens*, we can extract these REEs from electronic waste. This works because the aforementioned bacteria produce an amino acid called lanmodulin which has the unique property of binding to REEs [8]. This technique allows us to wash REEs out of electronic waste in a similar way that surfactants wash the dirt out of laundry.



Figure 2.3: *Methylorum extorquens* in a petri dish.

These bacteria reside in common soil, plant leaves, and dust and can also form symbiotic relationships with some plants. The bacteria appear orange or pink when cultivated on a solid or in a liquid medium. *Methylorum extorquens* utilizes methanol as an energy and carbon source, which is why we had to put methanol in our nutrient media.

#### 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.

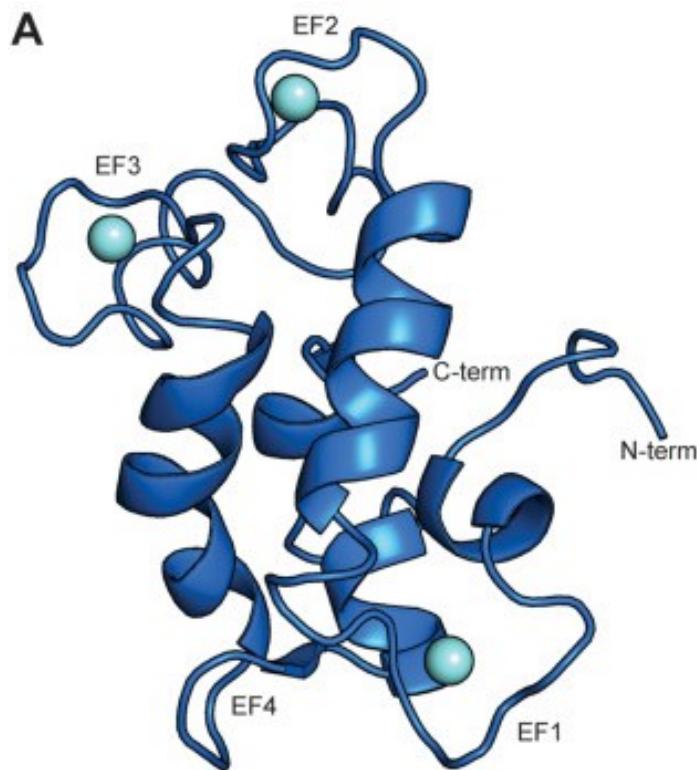


Figure 2.4: Graphical visualization of Lanmodulins structure. EF indicates the EF-hands, this is where the REEs can bind to the protein. In this visualization, the turquoise-colored spheres are  $\text{Y}^{\text{III}}$  ions which are bound to the EF-hands. Picture from "The biochemistry of lanthanide 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].

**Cell Lysis<sup>MS</sup>**

**Protein Extraction<sup>MS</sup>**

**IR-Spectrometry<sup>MS</sup>**

**SDS-PAGE<sup>MS</sup>**

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

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

In modern chemistry, qualitative and quantitative analysis of elements in a sample 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.

### 2.2.1 Precipitation Reactions

#### Cer Precipitation Reaction

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

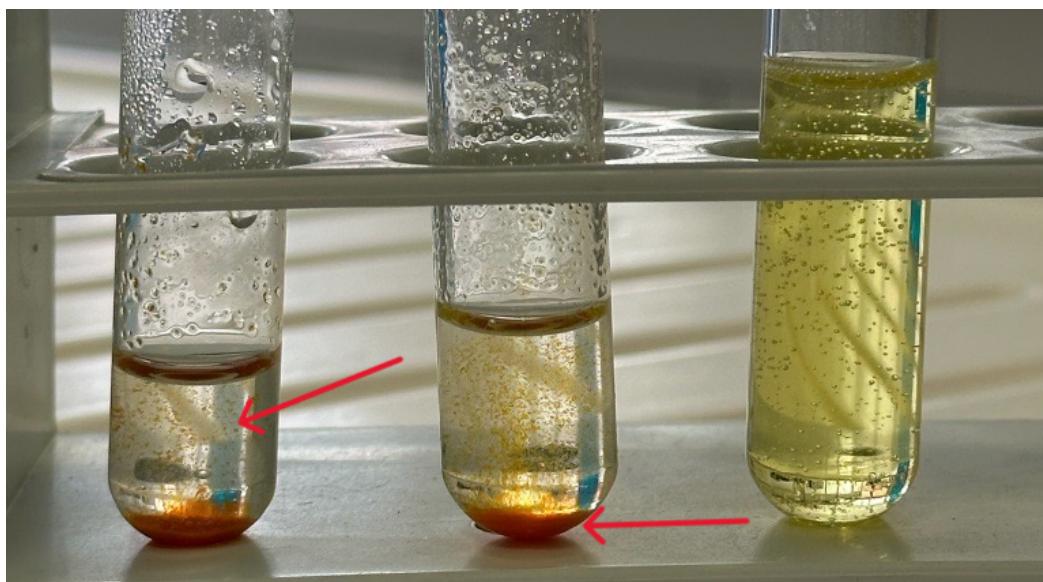


Figure 2.5: Precipitation of a successful cer detection reaction. The test tube on the righthandside does not show any precipitation because the sample was deionized water.

Cer in the aforementioned states forms complexes together with  $\text{H}_2\text{O}_2$ . 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.

### 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].

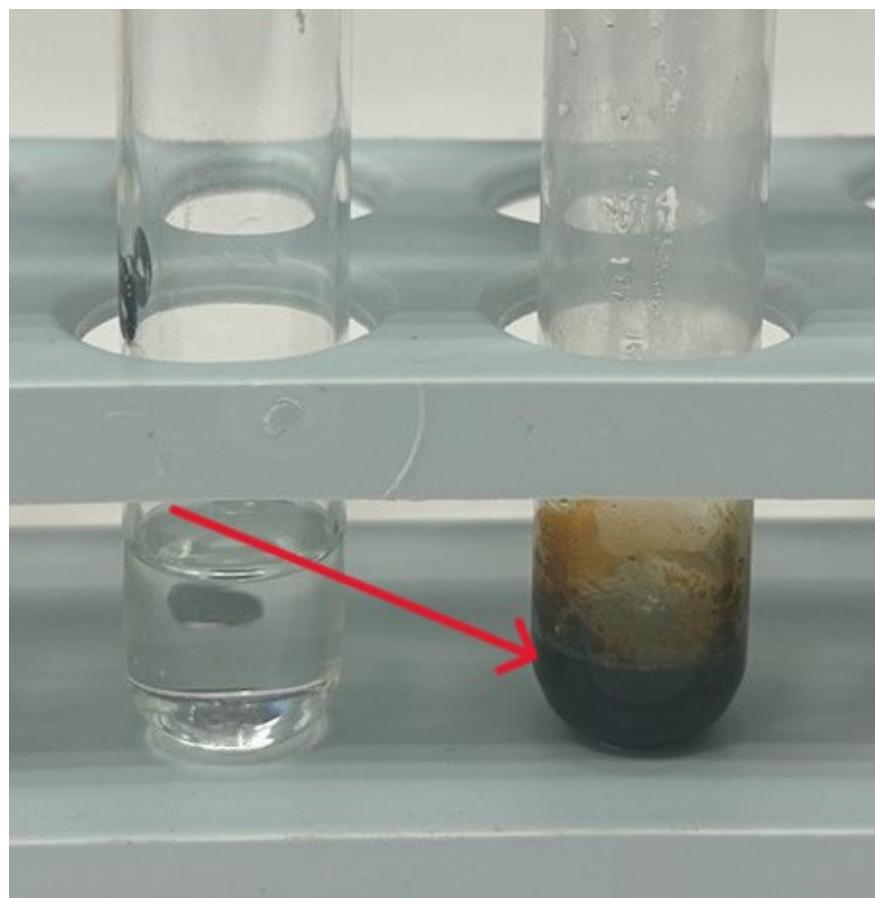


Figure 2.6: Neodymium detection reaction. Neodymium is contained in the right sample. The blue precipitate is clearly visible.

### 2.2.2 Arsenazo III Assay

#### 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].

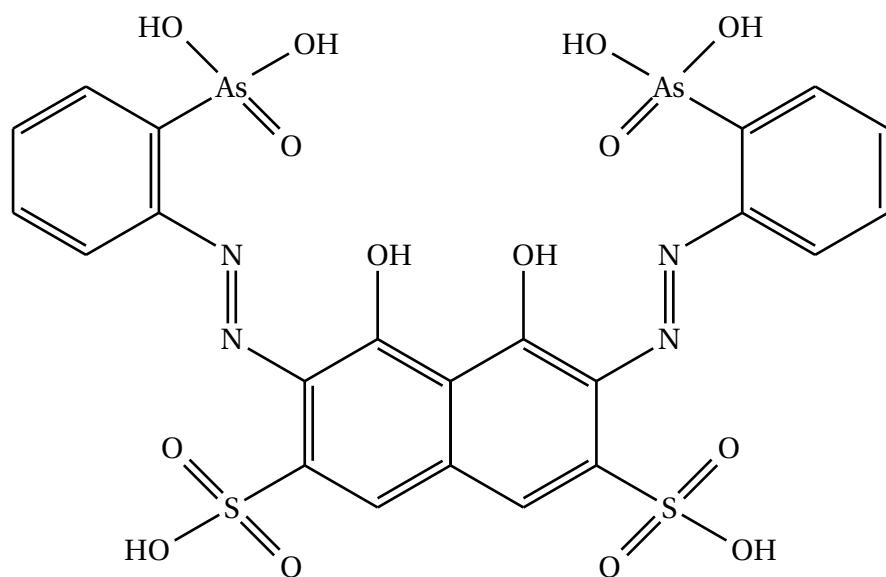


Figure 2.7: Structure of 2,7-bis(2-arsenophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid. Or, in its abbreviated form, arsenazo III.

Arsenazo III was first synthesized in 1959 [14]. In comparison to arsenazo I and II, it possesses two functional arsено groups (see figure 2.7). 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 (see figure 2.8).

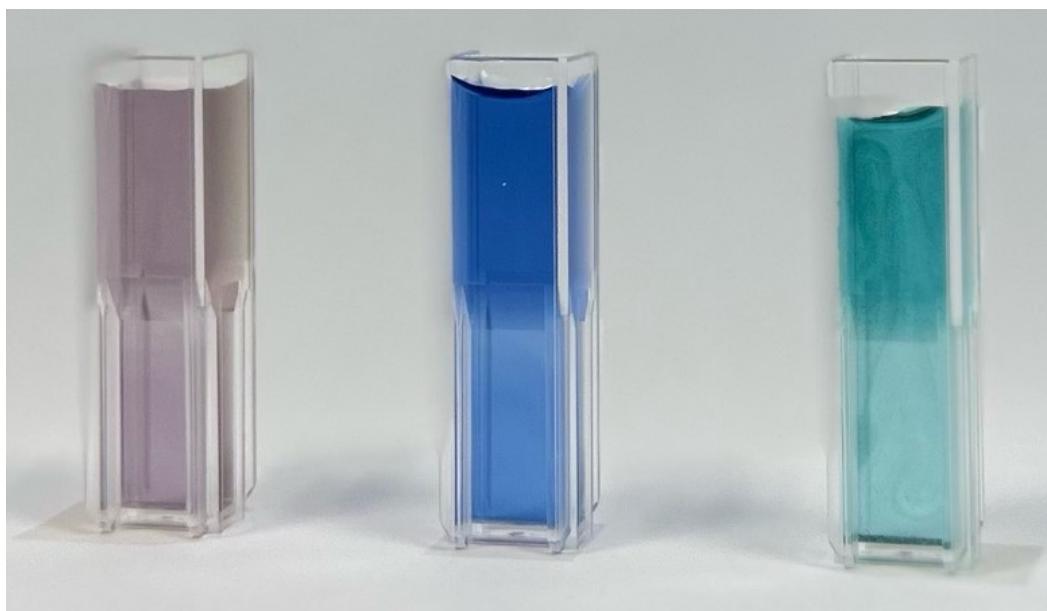


Figure 2.8: Example for different colors of arsenazo III with different samples. The contents of the cuvettes are (from left to right):  $\text{FeCl}_3$ ,  $\text{CuSO}_4$ ,  $\text{NdCl}_3$ . All are mixed with  $10\mu\text{L}$  of  $10\text{mM}$  arsenazo III.

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 figure 2.9). The other arsene group is most likely not used to form these stable complexes.

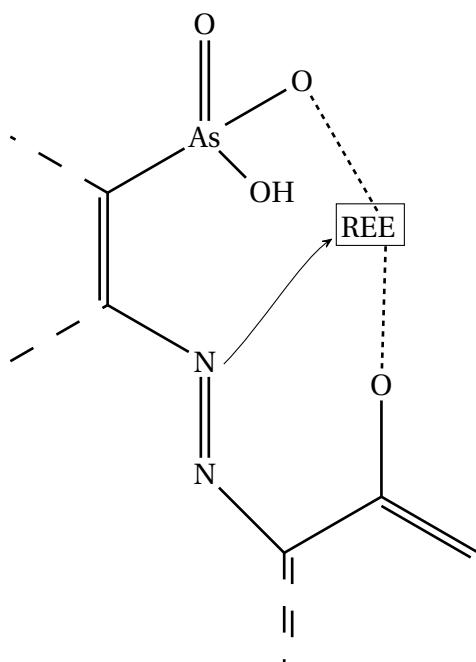


Figure 2.9: An arsenazo III complex with an atom of a rare earth element.

## 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.

## 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. 2.10).

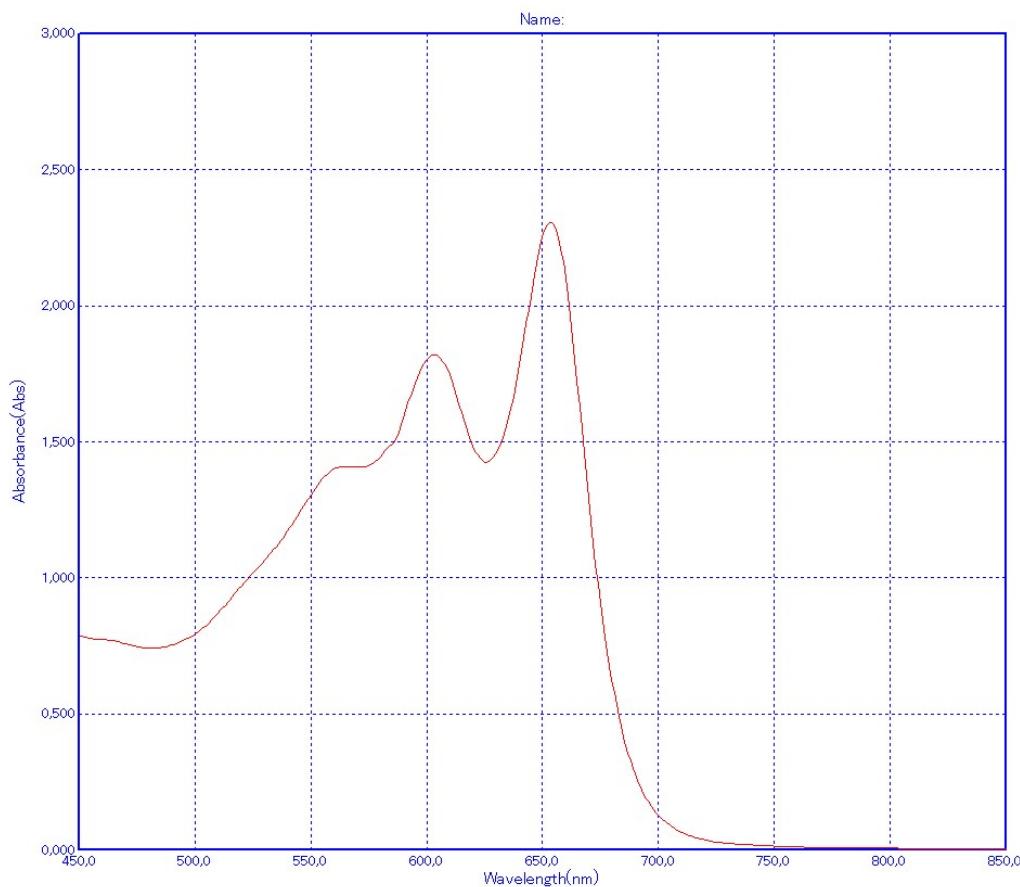


Figure 2.10: Example of a spectrum of an arsenazo III assay. The peak at around 650nm is the product of a complex formed by one rare earth atom and one arsено group.

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  $\mu$ L of the sample and 10  $\mu$ L 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  $\mu$ L 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 $\mu$ 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.

## 2.3 *Methylorubrum extorquens*

### 2.3.1 Taxonomy<sup>MS</sup>

#### Phylum Pseudomonia

*Pseudomonadota* is a major phylum of gram-negative bacteria (information about gram-negative bacteria will follow further down). They are incredibly diverse, encompassing pathogens, free-living species, nitrogen-fixing bacteria, and many more. *Pseudomonadota* exhibit a large range of shapes and sizes as well as metabolisms and habitats which will also be discussed further down. The diversity of *Pseudomonadota* makes them play a major role in the world's nutrient cycling ranging from crucial ecological relationships with humans to simple things such as nitrogen fixation [19]. *Pseudomonadota* includes five classes but only the class *Alphaproteobacteria* is of importance for us.

#### Class Alphaproteobacteria

*Alphaproteobacteria* is a highly diverse class of bacteria belonging to the phylum *Pseudomonadota*. They are named after the first letter of the Greek alphabet (alpha) due to being one of the first major lineages to diverge within the *Proteobacteria* phylum.

This class is incredibly varied, encompassing bacteria with a range of lifestyles including phototrophs (light-using), methanotrophs (methane-utilizing), symbionts (mutually beneficial relationships with other organisms), and pathogens (disease-causing).

Soil, water including cold deep-sea vents, hot springs, and symbiotic relationships even with humans are natural habitats of *Proteobacteria* [20].

**Rhizobium:** These bacteria form a symbiotic partnership with legumes, such as peas and soybeans. *Rhizobium* colonizes the legume's root nodules and fixes atmospheric nitrogen into a usable form that is essential for plant growth.

**Wolbachia:** This widespread genus of bacteria lives symbiotically within insects and other arthropods. *Wolbachia* can manipulate the host's reproduction in various ways, sometimes even influencing sex ratios or protecting the host from viruses.

**Rickettsia:** This genus includes several species that are obligate intracellular pathogens, meaning they can only live and reproduce inside the cells of a host organism. *Rickettsiae* causes various human diseases, including typhus fever and Rocky Mountain spotted fever.

**Magnetococcus:** These magnetotactic bacteria contain magnetosomes, specialized organelles that allow them to align and move along magnetic fields.

#### Order Hyphomicrobiales

*Hyphomicrobiales* can utilize single-carbon compounds like methanol as an energy source, the bacterium *Methylorubrum extorquens* does this, for example.

*Hyphomicrobiales* produce carotenoid pigments and therefore appear pink or orange in colonies. These colonies are aerobic, which means they require oxygen for growth. They inhabit a large variety of environments including soils, plant surfaces, root structures, water and dust.

They also play important ecological roles in their habitats, like plant-microbe interactions when metabolizing methanol on plant leaves or carbon and nitrogen cycling in various environments [21].

### Genus *Methylochromobacter*

They use specialized pathways to break down methanol for energy and to create biomass. This metabolic capability has potential applications in Bioremediation, which means that this bacteria can clean up methanol-contaminated areas. This family of bacteria is also able to produce valuable chemicals from methanol [22].

Bacteria of the genus *Methylochromobacter* are rod-shaped or slightly bent and show pink or orange pigmentation like every genus that belongs to the order *Methylobacterium*.

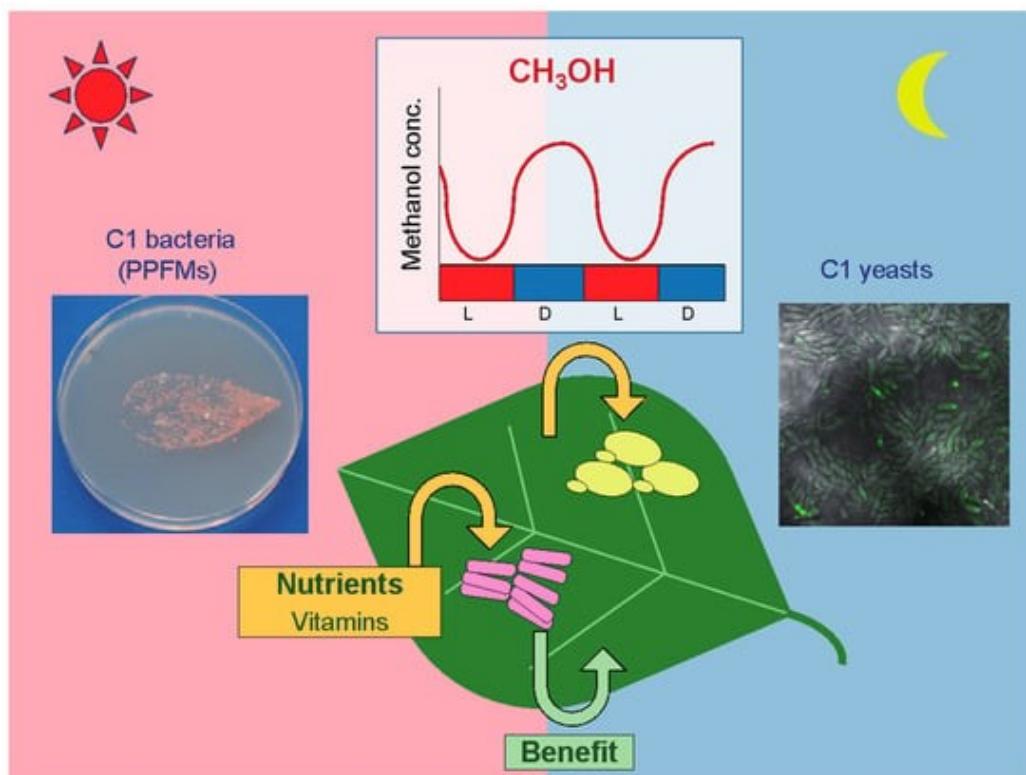


Figure 2.11: Pink *Methylochromobacter extorquens* on a leaf utilizing the plant's nutrients.

### Species *Extorquens*

In our thesis, the *extorquens* bacterium species holds immense significance as it displays all the key characteristics of the aforementioned groups to which it belongs. The *Methy-*

*lorubrum extorquens* strain is unique in its ability to utilize methanol or methane as its sole source of carbon and energy. Additionally, this bacterium has the capability to metabolize various compounds such as acetate, pyruvate, and succinate, which are converted to energy. This makes the *Methylochlorobacter extorquens* strain a particularly fascinating subject for further research and analysis.



Figure 2.12: *M. extorquens* in a sealed petri dish.

### 2.3.2 Methanol Metabolism<sup>MS</sup>

*Methylochlorobacter extorquens* exhibits the ability to utilize the simple alcohol methanol CH<sub>3</sub>OH as its only source of carbon and energy. This metabolism is explained in three steps:

#### 1. Initiation: Oxidation of Methanol

- Location: Periplasm (the space between the inner and outer cell membranes)
- Enzymes:
  - Methanol dehydrogenase (MposX):

- \* XoxF1: Requires lanthanides for activity, oxidizing methanol to formaldehyde (HCHO) and releasing H<sup>+</sup>.
- \* XoxF2: Less dependent on lanthanides, potentially involved in regulating methanol uptake.
- Importance: Formaldehyde is a toxic intermediate, requiring rapid conversion for *M. extorquens*' survival [23].

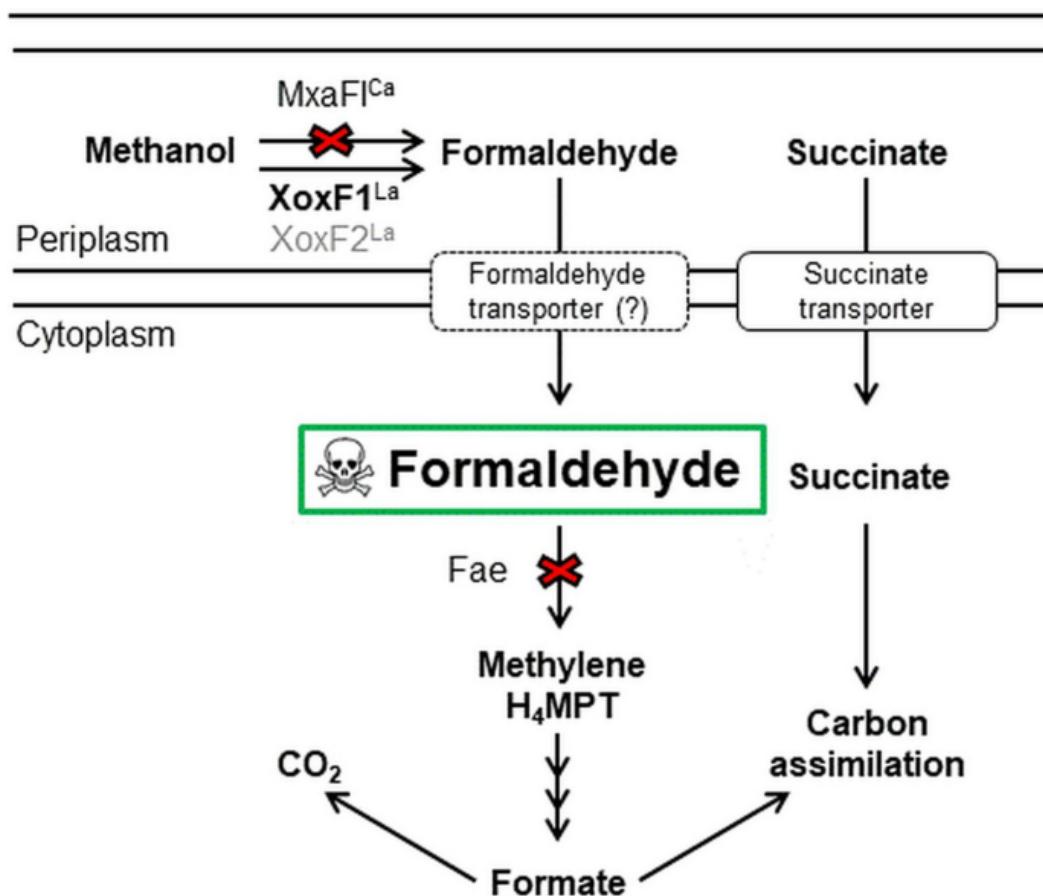


Figure 2.13: Schematic of the metabolic processes to oxidizing methanol to formaldehyde which is reduced or eliminated and used for growth by cells.

## 2. Capturing the Essence: Fixation of Formaldehyde

- Molecule: Dephosphotetrahydromethanopterin ( $\text{dH}_4\text{MPT}$ ) acts as a one-carbon carrier.
- Enzyme: Formaldehyde-activating enzyme ( $\text{Fae}$ ) catalyzes the reaction, attaching formaldehyde to  $\text{dH}_4\text{MPT}$ .
- Significance: Enables the transport of formaldehyde into the cytoplasm for further metabolism [23].

## 3. Carbon Assimilation: The Serine Cycle Takes Over

- Location: Cytoplasm
- Pathway:
  - a) Formate dehydrogenase: Oxidizes the formaldehyde-dH<sub>4</sub>MPT complex, generating formate (HCOO).
  - b) Formate acetyltransferase: Condenses formate with acetyl-CoA, forming S-acetyl-CoA.
  - c) Serine hydroxymethyltransferase: Transfers the one-carbon unit from S-acetyl-CoA to glycine, forming serine.
  - d) Serine transaminase: Converts serine to pyruvate, a key metabolic intermediate.
- Importance: The serine cycle efficiently converts the one-carbon unit from methanol into usable cellular building blocks [23].

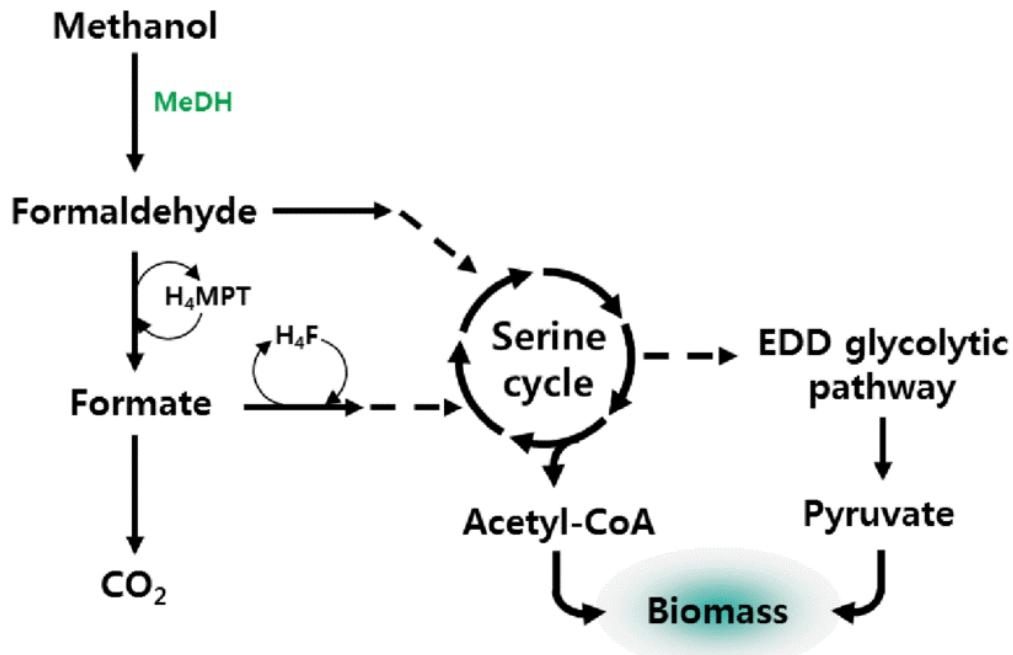


Figure 2.14: *Methylococcus extorquens* metabolizing methanol

### 2.3.3 Lanmodulin<sup>TD</sup>

Lanmodulin (LanM) is a protein that was discovered in 2018 in the bacteria *Methylococcus extorquens* [8]. The molecule is around 12kDa in size, and it possesses unique properties, even when compared to other similar proteins. Lanmodulin contains four of the so-called EF-hands. These hands are normally used to sense Ca<sup>II</sup> ions. Lanmodulin, however, is able to bind Ln<sup>III</sup> and other lanthanide ions (which most of the rare earth elements belong to) to this EF-Hands, not only Ca<sup>II</sup> [10].

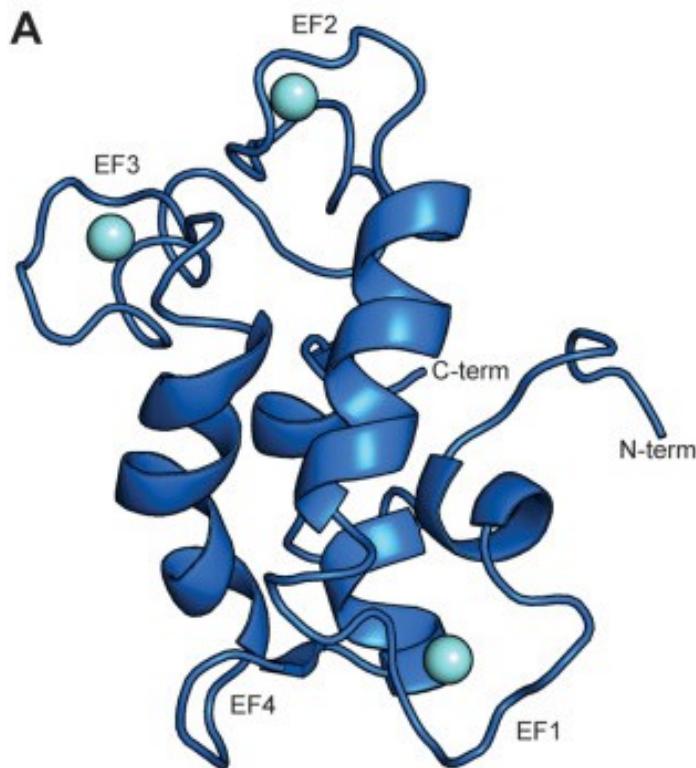


Figure 2.15: Graphical visualization of Lanmodulins structure. EF indicates the EF-hands, this is where the REEs can bind to the protein. In this visualization, the turquoise-colored spheres are  $\text{Y}^{\text{III}}$  ions which are bound to the EF-hands. Picture from "The biochemistry of lanthanide acquisition, trafficking and utilization", Emily R. Featherston and Joseph A. Cotruvo [9].

The second interesting property originates from the ability to bind lanthanide ions. It was found that LanM does not only bind lanthanide ions, but it even favors them to bind to its EF-hands. The affinity for the lanthanides is around  $10^8$  times higher than for  $\text{Ca}^{\text{II}}$ . This means that, in a solution with, for example,  $\text{Ln}^{\text{III}}$  and  $\text{Ca}^{\text{II}}$  ions, only very few calcium ions will bind to the LanM.

When LanM binds the lanthanide ions, something interesting happens: it undergoes a transformation. It changes its shape and morphs into a sphere-like structure, which contains the ions inside. However, how and why exactly lanmodulin does this, is the subject of ongoing research [24].

#### 2.3.4 Growth<sup>MS</sup>

*Methylorum extorquens* thrives at temperatures between 30°C and 35°C, making it a mesophilic bacteria. To promote its optimal growth, the bacteria was placed in a swivel incubator set to this temperature. Additionally, the nutrient solution needs to be slightly acidic to neutral, with a pH range of 6.5-7.5, to further enhance growth. Because *M. extorquens* is an aerobic bacteria, the solution in which it is cultivated must be able to

exchange gas and absorb oxygen. This is achieved by sealing the Erlenmeyer flask with a piece of sterile cotton that allows oxygen to pass through while keeping other bacteria and fungi out.



Figure 2.16: Swivel incubator with temperature control used for cultivating *M. extorquens*.

Under optimal conditions, the exponential growth phase of *M. extorquens* typically lasts six to eight hours, during which the number of cells increases rapidly. However, this growth phase comes to a halt due to a lack of nutrients or waste product accumulation, resulting in the stationary phase. After this point, the viability and number of cells gradually decrease, known as the death phase.

### 2.3.5 Lysis<sup>MS</sup>

In order to obtain Lanmodulin and REEs, it is necessary to break open the cell walls of the bacteria. This process, known as lysis, can be accomplished using a variety of techniques - either mechanical or enzymatic. Mechanical methods include bead beating, French press lysis, and shock freezing, while enzymatic lysis can be achieved through lysozyme treatment, which utilizes an enzyme that specifically breaks down bacterial cell walls. For this project, a combination of shock freezing and cell wall disruption using an ultrasonic bath was selected.

## 2.4 Protein Extraction and Analysis<sup>MS</sup>

### 2.4.1 Protein Extraction

To control the binding of REEs, lanmodulin has to be extracted from bacteria cells. This is done with the following steps:

#### Lysis: Breaking the Cellular Wall

As already discussed in 2.3.5 lysis involves breaking open the bacterial cells to access the intracellular proteins. This is achieved through our chosen methods:

- **Shock frosting**

Shock frosting, also known as freeze-thaw lysis, is a physical method used to disrupt bacterial cells by subjecting them to rapid temperature changes. The process involves freezing the bacterial culture rapidly at low temperatures like -80°C in our case, followed by thawing them at medium temperatures about 38°C to not coagulate the protein. The rapid freezing causes the formation of ice crystals, which physically disrupt the cell membrane. Upon thawing, the ice crystals melt, leading to further mechanical stress on the cellular structures, eventually resulting in cell lysis and the release of intracellular components.

In the case of *Methylo rubrum extorquens*, shock frosting was an effective way to disrupt cellular integrity, releasing intracellular proteins like lanmodulin.



Figure 2.17: Enterprise freezer that reaches -80°C for shock frosting bacteria.

- **Sonication:**

Sonication, or ultrasonication, is a mechanical method used to disrupt bacterial cell walls through the application of high-frequency sound waves. In this method, the bacterial culture is subjected to ultrasonic waves generated by a sonicator probe or bath. The high-frequency waves create alternating cycles of compression and rarefaction within the culture medium, generating cavitation bubbles. These bubbles undergo rapid expansion and collapse, generating intense shear forces and microstreaming, which disrupt the cell membrane and cell wall.

Sonication is particularly effective for lysing tough bacterial cell walls, including those of gram-negative bacteria like *Methylorum extorquens*. However, it is essential to optimize sonication parameters such as amplitude, duration, and temperature to avoid excessive heating and denaturation of sensitive intracellular components. Sonication was the final step for effective lysis and disruption of *M. extorquens* cell walls.



Figure 2.18: Ultrasonic bath used to disrupt cell walls.

### Cell Debris Removal

After cell disruption, the lysate (cell extract) contains a mixture of cellular components, including proteins, membranes, and nucleic acids. To isolate the proteins, unwanted components were removed by centrifugation. The lysate is spun at high speeds in a centrifuge. This separates the heavier cell debris (pellet) from the soluble components (supernatant) containing the proteins.

Throughout this process, protein concentration and purity were monitored using techniques like SDS-PAGE and IR-Spectrometry respectively, more on these methods and

their effectiveness later. To keep the amino acids intact, the epi containing the bacteria needed to be cooled all the time. This prevented protease from consuming and therefore destroying the amino acids of our interest.



Figure 2.19: Centrifuge with cooling function for inhibiting protease.

### 2.4.2 IR-Spectrometry

Infrared (IR) spectroscopy has established itself as a cornerstone analytical technique across diverse scientific fields. Its ability to elucidate the structural characteristics of molecules stems from the fundamental interaction between atoms and infrared radiation, providing invaluable insights into the presence of functional groups and the unique vibrational fingerprint of each molecule.

#### Theory:

The theoretical foundation of IR spectroscopy is based on the well-established principle that molecules can undergo vibrational motion when exposed to specific frequencies of infrared radiation, the radiation matching the molecule's frequency is absorbed. This radiation occupies a distinct region within the electromagnetic spectrum, encompassing wavelengths ranging from approximately  $2.5 \mu\text{m}$  to  $16 \mu\text{m}$ . Unlike ultraviolet-visible (UV-Vis) spectroscopy, which excites electrons to higher energy levels, the energy associated with infrared radiation is insufficient for electronic transitions. Instead, it triggers vibrations within the covalent bonds of the molecule.

The concept of vibrational modes is important to understanding IR spectroscopy. These modes represent the various ways in which the atoms within a molecule can vibrate relative to each other. These atoms are connected by bonds. Stronger bonds, characterized by higher bond energies, vibrate at higher frequencies, while weaker bonds exhibit lower vibrational frequencies. This distinctive correlation between bond type and vibrational frequency forms the cornerstone of IR spectroscopy.

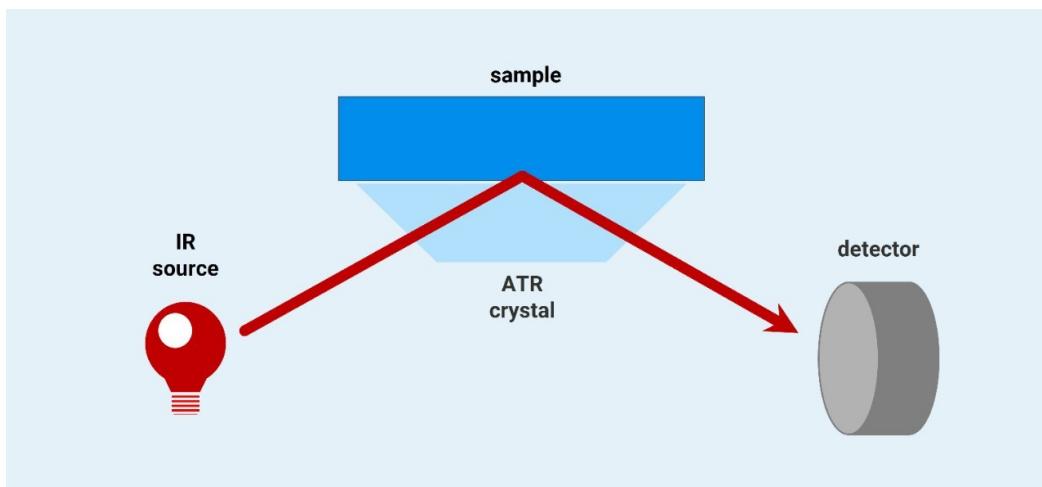


Figure 2.20: Schematic of the reflection of IR light in an ATR (Attenuated Total Reflectance) Spectrometer

### The Infrared Spectrometer

This instrument houses a light source that emits a broad spectrum of infrared radiation. The prepared sample, in either solid, liquid, or gas phase, is positioned within the path of this radiation. As the infrared light traverses the sample, specific frequencies corresponding to the molecule's vibrational modes are absorbed. The remaining radiation is subsequently reflected and detected, and a spectrum is generated. This spectrum typically presents the intensity of transmitted or absorbed light (y-axis) versus the frequency or wavelength (x-axis).

#### Modes:

**Transmission Mode:** In this mode, the infrared radiation directly transmits through the sample. The resulting spectrum depicts the frequencies absorbed by the molecule, and it is the most commonly employed mode for solid and liquid samples.

**Reflection Mode:** This mode finds particular utility when analyzing samples that are challenging to prepare as thin films for transmission measurements. In this case, the infrared radiation is directed onto the sample's surface, and the reflected light is captured. The spectrum reflects the molecule's vibrational modes based on the analysis of the reflected frequencies. For the analysis of *Methylorubrum extorquens*, this mode was chosen simply because of the available equipment.

#### Deciphering the Spectrum:

The IR spectrum generated from the experiment bears a resemblance to a unique fingerprint for each molecule. The x-axis typically displays the wavelength. The y-axis represents the intensity of the absorption (or transmittance). The spectrum usually shows diverse peaks, each corresponding to a specific vibrational mode within the molecule. The location of a peak indicates the type of bond vibration that occurred, while the peak's intensity reflects the strength of the absorption.

By drawing upon extensive reference databases and theoretical calculations, the IR spectrum can be interpreted to glean valuable information about the sample. The presence of specific peaks can confirm the existence of particular functional groups, such as carbonyls ( $\text{C}=\text{O}$ ), alkenes ( $\text{C}=\text{C}$ ), and alcohols ( $\text{O}-\text{H}$ ). The relative intensities of these peaks can even provide insights into the molecule's environment and interactions with neighboring molecules.

The analysis of proteins and rare earth elements using the IR-Spectrometer was rather sobering. Partly due to large alcohol and water peaks in the spectrum which originated from the nutrition solution and cleaning pads used for the bacteria and the IR-Spectrometer.

# 3 Experimental Section

In the following chapter, we describe the work we carried out and why we did it. We begin with methods for cultivating bacteria, which are followed by the extraction of proteins from *M. extorquens*. Afterwards, we describe how we analyzed the proteins. In the end, we show you how we did the arsenazo-III assay.

## 3.1 Cultivation of Bacteria<sup>MS</sup>

Preparation of solid nutrient solution for petri dishes:

**Materials:**

- Peptone 2,5g
- Meat Extract 1,5g
- Agar 7,5g
- H<sub>2</sub>O 500mL
- Scale
- Autoclave bottle
- Spatula

**Execution:**

- Weigh all the necessary ingredients
- Fill the autoclave bottle with around 100ml of water
- Add the dry ingredients to the autoclave bottle
- Mix the dry ingredients with water
- Add the remaining water to the bottle
- Shake until mixed

Finalizing the solid nutrient solution to be poured into petri dishes:

**Materials:**

- Petri dishes

- Autoclave
- Autoclave indicator tape
- Sterile workbench

**Execution:**

- Add a piece of indicator tape on the cap of the autoclave bottle
- Autoclave the nutrient solution at 121°C and 1 bar for 15 minutes
- After autoclaving the nutrient solution swiftly pour it into the petri dishes
- Leave the nutrient solution to harden
- Flip the petri dishes carefully on their cover and put them in the fridge

## 3.2 Protein Extraction<sup>MS</sup>

## 3.3 Protein Analysis<sup>TD</sup>

### 3.3.1 IR-Spectrometry

The IR-Spectrometry was done using an ATR-IR-Spectrometer. This meant that we could place the sample directly onto the device, without additional sample preparation.

The following table gives an overview of the lysed samples of *M. extorquens* we measured, what treatments we gave the bacteria to grow in and when we measured the samples after the treatment.

Type of sample	Treatment	Weeks of growth after treatment
Proteins dissolved in water	no treatment	0, 1 and 2
Cell paste	no treatment	0, 1 and 2
Proteins dissolved in water	0,5mL of Ce-solution	0, 1 and 2
Cell paste	0,5mL of Ce-solution	0, 1 and 2
Proteins dissolved in water	1mL of NdFeB-magnet, dissolved in water	0, 1 and 2
Cell paste	1mL of NdFeB-magnet, dissolved in water	0, 1 and 2

Table 3.1: Types of samples measured with IR-Spectrometry.

We put a drop of a sample onto the ATR-IR-Spectrometer and measured the IR spectrum. Before each measurement, we cleaned the ATR crystal with a drop of ethanol on a soft tissue.

### 3.3.2 SDS-PAGE

The SDS-PAGE was carried out as it is described in the guide of the manufacturer of our electrophoresis cell, Bio-Rad Laboratories, Inc. [25], with some slight modifications.

#### Gel Casting:

##### Materials and Methods:

Ingredient	Volumina for Stacking Gel	Volumina for Resolving Gel
30% Acrylamide	1,485mL	4,5mL
0,5M Tris-HCl pH6,8	3,78mL	-
1,5M Tris-HCl pH8,8	-	3,75mL
10% SDS	150µL	150µL
diH <sub>2</sub> O	9mL	5,03mL
TEMED	18µL	7,5µL
10% APS	90µL	75µL

Table 3.2: Ingredients for SDS-PAGE gel.

- Mix all ingredients except for TEMED and APS
- Add TEMED and APS to the resolving gel
- Transfer resolving gel into the gel cassette
- Add ethanol on top, to ensure that the stacking gel binds to the resolving gel
- Wait until the resolving gel has polymerized
- When the resolving gel is firm, pour off the ethanol
- Put the gel comb into the gel cassette, so that one end is between the glasses
- Add TEMED and APS to the stacking gel
- Fill the stacking gel into the gel cassette
- Make sure that no air bubble is between the tines of the comb
- Press the comb gently into the fluid gel
- Wait until the stacking gel has polymerized

We increased the amount of TEMED and APS by 20 percent compared to the manufacturer's guide, because oftentimes the resolving gel did not polymerize.

#### Running buffer:

##### Materials and Methods:

- Tris Base 30,30g

- Glycine 144,70g
- SDS 10,00g
- Mix all ingredients with 1000mL of deionized water

**Probe Preparation:**

**Materials and Methods:**

Laemmli-Buffer:

- 0,5M Tris-HCl pH6,8 3,75mL
- Glycerol 100% 7,5mL
- 1,0% Bromophenol blue 0,3mL
- 10% SDS 6,0mL
- diH<sub>2</sub>O to 30mL
- Mix all ingredients
- When the buffer is yellow, add Tris base until it is blue

Probe Preparation:

- Mix the sample with the same amount of Laemmli-Buffer
- Heat the mixture to 95°C for 5min

We did not add  $\beta$ -mercaptoethanol to the sample/buffer mixture, because the chemical was not available at that time.

**Performing Gel Electrophoresis:**

**Materials and Methods:**

- Insert the gel(s) with removed comb(s) into the running module
- Fill the gel box with the running buffer to around three quarters
- Insert 30 $\mu$ L of protein marker into one well
- Insert 15 $\mu$ L of sample into the remaining wells
- Fill the gel box completely with running buffer
- Put the lid onto the box and plug in the electrodes
- Let the SDS-PAGE run for 60min at 100V

Gel Staining:

- \_\_\_\_\_
- \_\_\_\_\_
- Mix all ingredients
- Remove the glass front plate gently from the gel
- Cut the stacking gel carefully off
- Remove the gel cautiously from the spacer plate
- Put the gel into the staining solution
- Let the gel stain overnight

Gel Destaining:

- \_\_\_\_\_
- \_\_\_\_\_
- Mix all ingredients
- Rinse the stained gel under deionized water
- Put the stained gel into the destaining solution
- Let the gel destain overnight
- After the gel destained, rinse it with deionized water
- The gel can be stored in a fridge in deionized water

### 3.4 Arsenazo-III Assay<sup>TD</sup>

## **4 Results and Discussion**

**4.1 Cultivation of Bacteria<sup>MS</sup>**

**4.2 Protein Analysis<sup>TD</sup>**

**4.3 Arsenazo-III Assay<sup>TD</sup>**

# 5 Project Management

## 5.1 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

## 5.2 Evaluation<sup>TD</sup>

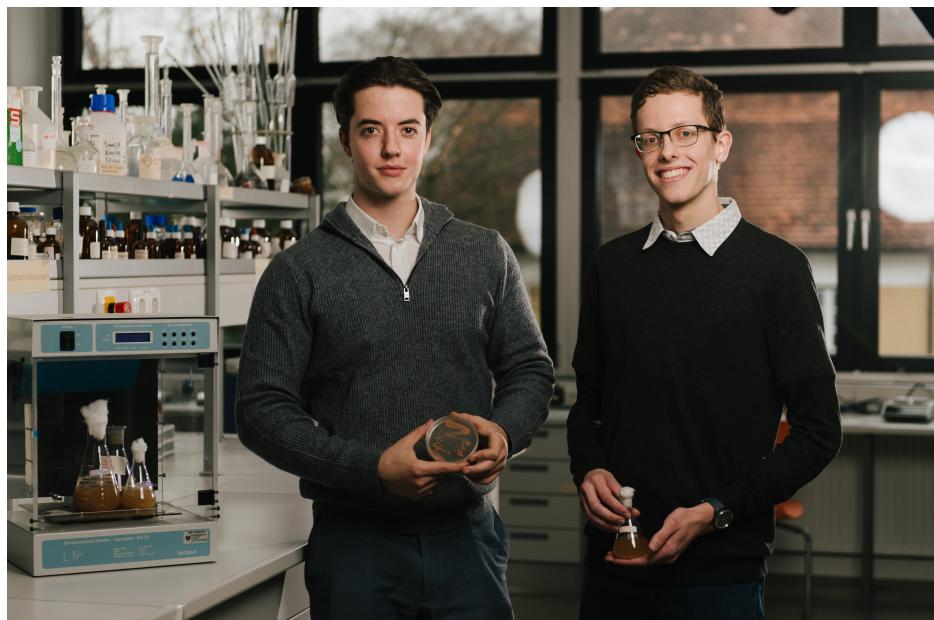


Figure 5.1: The project team

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 5.1) 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.

## 5.3 Timesheet

### 5.3.1 Tobias Daxecker

Date	Work	Time in hours
03.08.2023	Research	3,00
18.08.2023	Research	2,00
24.08.2023	Research	2,00
03.09.2023	Research	1,00
06.09.2023	Research	2,00
07.09.2023	Research	4,00
08.09.2023	Production of nutrition media	6,00
22.09.2023	Set up of MS Teams team	1,00
28.09.2023	Fact sheet, milestones	1,00
02.10.2023	Research	1,00
06.10.2023	Freezing of bacteria	1,00
09.10.2023	Research	1,00
10.10.2023	Research plan draft, research	2,00
15.10.2023	Research plan draft, research	3,00
16.10.2023	Research plan draft, research	1,00
18.10.2023	Research plan draft, research	2,00
24.10.2023	Research plan draft, research	1,00
27.10.2023	Research plan draft, research, newspaper article	3,00
30.10.2023	Newspaper article, writing of diploma thesis, research	3,00
31.10.2023	Writing of diploma thesis, research	4,00
01.11.2023	Writing of diploma thesis, research	6,00
02.11.2023	Writing of diploma thesis, research	5,00
03.11.2023	Writing of diploma thesis, research	5,00
06.11.2023	Writing of diploma thesis, research	2,00
10.11.2023	Destaining of SDS-PAGE	2,00
12.11.2023	Newspaper article, writing of diploma thesis	2,00
14.11.2023	Newspaper article, writing of diploma thesis	2,00
19.11.2023	Writing of diploma thesis, research	6,00
20.11.2023	Cultivation of bacteria, writing of diploma thesis, registration for Jugend Innovativ	3,00
24.11.2023	Destaining of SDS-PAGE, preparation for open house day	1,00
26.11.2023	Writing of diploma thesis, research	4,00
27.11.2023	Submission for ECO Bonus	1,00
03.12.2023	Writing of diploma thesis, research	3,00
10.12.2023	Writing of diploma thesis, research	5,00
11.12.2023	Writing of diploma thesis, research	1,00

Date	Work	Time in hours
28.12.2023	Writing of diploma thesis, research, project report Jugend Innovativ	3,00
29.12.2023	Writing of diploma thesis, research, project report Jugend Innovativ	2,00
01.01.2024	Writing of diploma thesis, research	3,00
02.01.2024	Writing of diploma thesis, research	4,00
03.01.2024	Writing of diploma thesis, research	4,00
04.01.2024	Writing of diploma thesis, research	3,00
12.01.2024	Writing of diploma thesis, research, project report Jugend Innovativ	1,00
14.01.2024	Writing of diploma thesis, research, project report Jugend Innovativ	3,00
15.01.2024	Writing of diploma thesis, research, project report Jugend Innovativ	1,00
16.01.2024	Writing project report for Jugend Innovativ	3,00
17.01.2024	Writing project report for Jugend Innovativ	2,00
22.01.2024	Writing project report for Jugend Innovativ	1,00
23.01.2024	Writing project report for Jugend Innovativ	2,00
24.01.2024	Writing project report for Jugend Innovativ, review of the project report	1,00
26.01.2024	Writing project report for Jugend Innovativ	2,00
27.01.2024	Writing project report for Jugend Innovativ	6,00
27.01.2024	Writing of diploma thesis	1,00
28.01.2024	Writing project report for Jugend Innovativ	1,00
30.01.2024	Writing project report for Jugend Innovativ	1,00
16.02.2024	Execution of an arsenazo-III assay	5,00
20.02.2024	Writing of diploma thesis	2,00
21.02.2024	Writing of diploma thesis	5,00
22.02.2024	Writing of diploma thesis	3,00
23.02.2024	Writing of diploma thesis	3,00
26.02.2024	Writing of diploma thesis	1,00
28.02.2024	Writing of diploma thesis	1,00
03.03.2024	Writing of diploma thesis	4,00
04.03.2024	Writing of diploma thesis	5,00
05.03.2024	Writing of diploma thesis	1,00
06.03.2024	Creation of a cost plan	1,00
08.03.2024	Presentation for job exchange	1,00
10.03.2024	Presentation for job exchange, Writing of diploma thesis	2,00
11.03.2024	Presentation for job exchange, Writing of diploma thesis	1,00
17.03.2024	Writing of diploma thesis	7,00

**Total sum of free time work hours: 178,00**

Braunau/Inn, 20.03.2024

*Ort, Datum*

Tobias Daxecker

*Unterschrift*

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### 5.3.2 Mathias Standhartinger

Date	Work	Time in hours
03.08.2023	Research	3,00
18.08.2023	Research	2,00
24.08.2023	Research	2,00
03.09.2023	Research	1,00
06.09.2023	Research	2,00
07.09.2023	Research	4,00
08.09.2023	Production of nutrition media	6,00
06.10.2023	Freezing of bacteria	1,00
18.10.2023	Research plan draft, Research	2,00
03.11.2023	Design of project poster	2,00
03.11.2023	Registration for contests	2,00
10.11.2023	Destaining of SDS-Page	2,00
13.11.2023	Planning of a video for Jugend Innovativ	2,00
14.11.2023	Newspaper article, writing of diploma thesis	2,00
20.11.2023	Cultivation of bacteria, writing of diploma thesis	2,00
24.11.2023	Destaining of SDS-PAGE, preparation for open house day	1,00
29.12.2023	Writing of diploma thesis, research	2,00
15.01.2024	Writing of a script for the project video	2,00
16.01.2024	Writing project report for Jugend Innovativ	1,00
23.01.2024	Writing of diploma thesis, preparation for video shoot	4,00
24.01.2024	Writing project report for Jugend Innovativ	2,00
24.01.2024	Preparation for video shoot	1,00
25.01.2024	Follow up of video shoot	1,00
27.01.2024	Design project report for Jugend Innovativ	4,00
28.01.2024	Design project report for Jugend Innovativ	2,00
29.01.2024	Correction of the project report	1,00
05.02.2024	Preparation of video shoot	2,00
07.02.2024	Writing of a script for the project video and preparation for video shoot	4,00
16.02.2024	Execution of an arsenazo-III assay	5,00
19.02.2024	Search for Stockfootage and preparation of video cut	2,00
23.02.2024	Writing of diploma thesis	2,00
21.02.2024	Writing of diploma thesis	4,00
26.02.2024	Writing of diploma thesis	1,00
28.02.2024	Writing of diploma thesis	1,00
01.03.2024	Writing of diploma thesis	3,00
02.03.2024	Writing of diploma thesis	4,00
04.03.2024	Writing of diploma thesis	3,00

Date	Work	Time in hours
05.03.2024	Writing of diploma thesis	3,00
06.03.2024	Creation of a cost plan	1,00
06.03.2024	Writing of diploma thesis	4,00
07.03.2024	Writing of diploma thesis	3,00
07.03.2024	Cutting of the project video	4,00
08.03.2024	Presentation for job exchange	1,00
08.03.2024	Cutting of the project video	4,00
11.03.2024	Presentation for job exchange, Cutting of the project video	3,00
13.03.2024	Writing of diploma thesis	3,00
14.03.2024	Writing of diploma thesis	2,00
15.03.2024	Writing of diploma thesis	2,00
16.03.2024	Writing of diploma thesis	3,00

**Total sum of free time work hours: 120,00**

Braunau/Inn, 20.03.2024

Mathias Standhartinger

Ort, Datum

*Unterschrift*

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

To further optimize this endeavor for industrial applicability, several imperative steps must be undertaken. Primarily, there is a critical need to augment the efficiency of bacterial proliferation and the resultant yield of Rare Earth Elements (REEs). Achieving this entails the development of innovative methodologies aimed at curtailing the growth duration and nutrient utilization by *Methylo rubrum extorquens*. Moreover, the primary substrate for bacterial metabolism, methanol could be replaced with its more rudimentary and economically advantageous precursor, methane, which is also amenable to metabolism by *M. extorquens*. To realize the full-scale industrialization of this project, rigorous testing in expansive bioreactor systems is indispensable, alongside the incorporation of diverse forms of electronic waste. Determining the most economically viable category of e-waste necessitates extensive experimental evaluation. Concurrently, alongside the e-waste assessments, there arises a pressing need for the development of a high-capacity, efficient, and durable shredding apparatus tailored to handle various types of e-waste. This undertaking poses multifaceted challenges, particularly in terms of safety and cost considerations. An industrial-grade e-waste shredder must be inherently non-combustible and proficient in processing metal, plastic, fiberglass, and adhesive materials, all while maintaining optimal power consumption levels and facilitating facile maintenance protocols.

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

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.

An example for the usage of only lanmodulin would be the work of Dong et al. [26]. Their approach was to take lanmodulin and attach it to a microbead (a small sphere made of agarose, see bottom left of figure 7.1). 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.

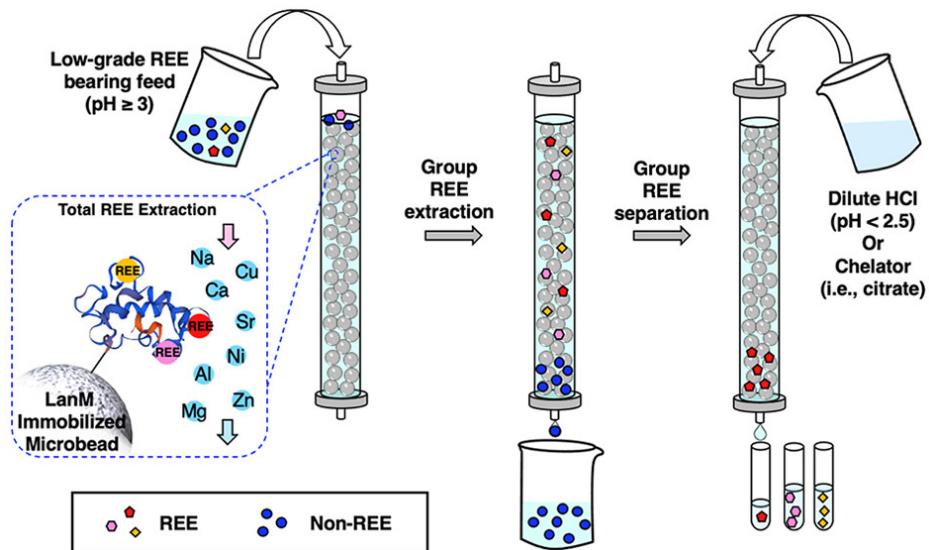


Figure 7.1: Overview of the work which inspired this thesis. Picture from "Bridging Hydrometallurgy and Biochemistry: A Protein-Based Process for Recovery and Separation of Rare Earth Elements", Dong et al. [26].

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 (figure 7.2) and find methods to increase the yield of this recycling method [27]. 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.

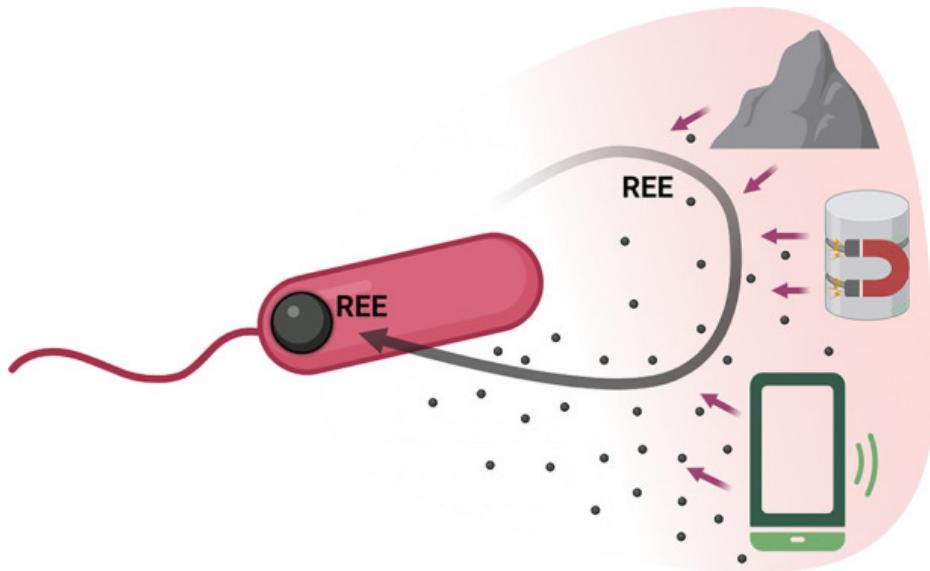


Figure 7.2: Very simplified abstract of the work from Good et al. Picture from "Scalable and Consolidated Microbial Platform for Rare Earth Element Leaching and Recovery from Waste Sources", Good et al. [27].

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.

## 8 Conclusion<sup>TD</sup>

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 *Methylorumbrum 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

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# 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 red arrows point to the orange-red precipitation. . . . .	4
2.2	Structure of arsenazo III. The color change of the dye depending on the REE concentration is shown on the righthandside. Picture from "Facile Arsenazo III-based assay for monitoring rare earth element depletion from cultivation media for methanotrophic and methylotrophic bacteria" Hoogen-dorn et al. [7]. . . . .	5
2.3	<i>Methylorum extorquens</i> in a petri dish. . . . .	6
2.4	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]. . . . .	7
2.5	Precipitation of a successful cer detection reaction. The test tube on the righthandside does not show any precipitation because the sample was deionized water. . . . .	9
2.6	Neodymium detection reaction. Neodymium is contained in the right sample. The blue precipitate is clearly visible. . . . .	10
2.7	Structure of 2,7-bis(2-arsenophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid. Or, in its abbreviated form, arsenazo III. . . . .	11
2.8	Example for different colors of arsenazo III with different samples. The contents of the cuvettes are (from left to right): $\text{FeCl}_3$ , $\text{CuSO}_4$ , $\text{NdCl}_3$ . All are mixed with 10 $\mu\text{L}$ of 10mM arsenazo III. . . . .	12
2.9	An arsenazo III complex with an atom of a rare earth element. . . . .	12
2.10	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 arseno group. . . . .	14
2.11	Pink <i>Methylorum extorquens</i> on a leaf utilizing the plant's nutritiens. . .	16
2.12	<i>M. extorquens</i> in a sealed petri dish. . . . .	17
2.13	Schematic of the metabolic processes to oxidizing methanol to formaldehyde which is reduced or eliminated and used for growth by cells. . . . .	18
2.14	<i>Methylorum extorquens</i> metabolizing methanol . . . . .	19

2.15 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 <sup>III</sup> 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]. . . . .	20
2.16 Swivel incubator with temperature control used for cultivating <i>M. extorquens</i> . . . . .	21
2.17 Enterprise freezer that reaches -80°C for shock frosting bacteria. . . . .	22
2.18 Ultrasonic bath used to disrupt cell walls. . . . .	23
2.19 Centrifuge with cooling function for inhibiting protease. . . . .	24
2.20 Schematic of the reflection of IR light in an ATR (Attenuated Total Reflectance) Spectrometer . . . . .	25
5.1 The project team . . . . .	33
7.1 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. [26]. . . . .	41
7.2 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. [27]. . . . .	42

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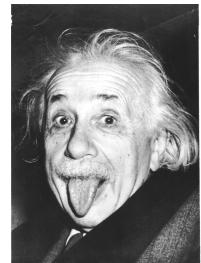
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