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Metal Biosorption by Cyanobacteria Focusing on the Adsorption of Rare Earth Elements

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It is not in doing what you like, but in liking what you do that is the secret of happiness.

- Sir James Matthew Barrie -

I Abstracts

Chapter I

In the first chapter, the metal adsorption by the cyanobacterium *Calothrix brevissima* was analyzed in closer detail. Here, for the first time, water-soluble compounds that form complexes with Rare Earth Elements and other metals were isolated from cyanobacterial biomass using chromatographic methods. The isolation and further purification of these compounds resulted in two different fractions separated by their molecular weight with a cut-off of 10 kDa. Sugar analysis with an HPLC and GC-MS-based method indicated that the fraction with a molecular weight below 10 kDa likely consists of smaller fragments of the compounds enriched in the high molecular weight fraction, as both fractions were composed of similar monomeric sugar building blocks. Adsorption experiments on the binding specificity of the isolated compounds indicated a competition for the same binding sites between cobalt, lead, and terbium. In contrast, alkaline and alkaline earth metals, more specifically, sodium, potassium, magnesium, and calcium, showed no competition with terbium during complex formation. FT-IR analysis revealed that functional groups involved in the adsorption process are predominantly sulfate and hydroxyl groups. The formed complexes were stable, even at pH values between 6.7 and 9.5.

This study demonstrated how specific polysaccharide structures from *Calothrix brevissima* contribute to Rare Earth Element adsorption by chelate formation. While some metal cations were able to compete for the same binding sites, the isolated compounds displayed high affinity for Rare Earth Elements.

Chapter II

In the second chapter, the potential for the enrichment of Rare Earth Elements of 12 cyanobacterial strains in an adsorption–based process was investigated. Based on 16S rRNA genes, a phylogenetic analysis was performed, revealing a high genetic diversity within the tested strains. A screening for the maximum adsorption capacity of lanthanum, cerium, neodymium, and terbium was performed, resulting in the selection of five candidate strains that were further investigated. Parameters that influence the adsorption properties of the biomass, i.e., pH value, metal concentration, and incubation time, were tested. These experiments showed that all tested cyanobacterial biomasses were able to adsorb metals at low concentrations, which was best described by fitting data points according to the Langmuir-model. Equilibrium adsorption capacity was reached within a few minutes, indicating fast adsorption kinetics. The optimum pH value for

highest metal uptake was at a pH of approximately 5. Experiments on binding specificity indicated a stronger affinity of the biomass for lead and aluminum than for cerium. However, at concentrations below 2 mM, tested biomass showed better adsorption for cerium. Furthermore, the analysis of metal concentrations in the tested solutions strongly indicated an ion-exchange mechanism in which adsorbed metal cations replace ions of the elements sodium, potassium, magnesium, and calcium on the cell surface.

In this study, the metal-sorption characteristics of novel, mainly uncharacterized cyanobacteria were examined. Biomass derived from those cyanobacteria could be used in future biosorption-based processes for the recovery of Rare Earth Elements from industrial wastewater streams. In the context of process development, critical parameters for metal uptake by the cyanobacterial biomass were optimized, and the dominant chemical mechanisms for metal binding were characterized.

II Zusammenfassungen

Kapitel I

Im ersten Kapitel wurde die Metalladsorption durch das Cyanobakterium *Calothrix brevissima* näher analysiert. Dabei wurden erstmals wasserlösliche Verbindungen, die mit Seltenen Erden und anderen Metallen Komplexe bilden, mit chromatographischen Methoden aus der Biomasse isoliert. Die Isolierung und weitere Aufreinigung dieser Verbindungen ergab zwei verschiedene Fraktionen, die anhand ihres Molekulargewichts bei einem Cut-off von 10 kDa getrennt wurden. Die Zuckeranalyse mit einem HPLC- und GC-MS-basierten Verfahren zeigte, dass die Fraktion mit einem Molekulargewicht unter 10 kDa wahrscheinlich aus kleineren Fragmenten der Verbindungen besteht, die in den Fraktionen mit hohem Molekulargewicht angereichert sind, da beide Fraktionen aus ähnlichen Monosaccharid-Bausteinen zusammengesetzt waren. Adsorptionsexperimente zur Bindungsspezifität der isolierten Verbindungen zeigten eine Konkurrenz um die gleichen Bindungsstellen zwischen Cobalt, Blei und Seltenen Erdelementen. Im Gegensatz dazu zeigten Alkali- und Erdalkalimetalle, insbesondere Natrium, Kalium, Magnesium und Calcium, keine Konkurrenz mit Seltenen Erdelementen während der Komplexbildung. Eine FT-IR-Analyse zeigte, dass überwiegend Sulfat- und Hydroxylgruppen als funktionelle Gruppen am Adsorptionsprozess beteiligt sind. Die gebildeten Komplexe waren selbst bei pH-Werten zwischen 6,7 und 9,5 stabil.
Diese Studie zeigte auf, wie bestimmte Polysaccharidverbindungen aus der Biomasse von *Calothrix brevissima* zur Adsorption von Seltenen Erden durch Chelatbildung beitragen. Obwohl einige Metallkationen um die gleichen Bindungsstellen konkurrierten, zeigten die isolierten Verbindungen eine hohe Affinität zu den getesteten Seltenen Erdelementen.

Kapitel II

Im zweiten Kapitel wurde das Potenzial von 12 Cyanobakterienstämmen zur Anreicherung von Seltenen Erdelementen in einem adsorptionsbasierten Prozess untersucht. Basierend auf 16S-rRNA-Genen wurde eine phylogenetische Analyse durchgeführt, die eine hohe genetische Diversität innerhalb der getesteten Stämme offenbarte. Nach einem Screening auf die maximale Adsorptionskapazität für Lanthan, Cer, Neodym und Terbium wurden fünf vielversprechende Stämme ausgewählt, die anschließend genauer untersucht wurden. Getestet wurden Parameter, die einen Einfluss auf die Adsorptionseigenschaften der Biomasse haben: pH-Wert, Metallkonzentration und Inkubationszeit. Diese Experimente zeigten, dass die Biomasse aller

getesteten Cyanobakterien in der Lage war, Metalle bereits bei niedrigen Konzentrationen zu adsorbieren. Basierend auf den Messdaten, wurde das Adsorptionsverhalten dabei am besten durch das Langmuir-Model beschrieben. Die Adsorptionskapazität der Biomasse erreichte innerhalb weniger Minuten einen Gleichgewichtszustand. Des Weiteren lag der optimale pH-Wert für die höchste Metallaufnahme bei ca. pH 5. Versuche zur Bindungsspezifität zeigten eine stärkere Affinität von Blei und Aluminium zur Biomasse als Cer. Bei niedrigen Metallkonzentrationen unter 2 mM zeigte die getestete Biomasse jedoch eine bessere Adsorption für Cer. Darüber hinaus deutete die Analyse der Metallkonzentrationen in den getesteten Lösungen stark auf einen Ionenaustauschmechanismus hin, bei dem adsorbierte Metallkationen andere Ionen der Elemente Natrium, Kalium, Magnesium und Calcium auf der Zelloberfläche ersetzen.

In dieser Studie wurden die Metallsorptionseigenschaften neuartiger, weitgehend unerforschter Cyanobakterien untersucht. Die Biomasse dieser Cyanobakterien könnte in Zukunft für biosorptionsbasierte Verfahren zur Rückgewinnung von Seltenen Erdelementen aus industriellen Abwasserströmen verwendet werden. Im Hinblick auf eine Prozessentwicklung wurden kritische Parameter, die die Metallaufnahme durch die Cyanobakterien-Biomasse beeinflussen, optimiert und die maßgebenden chemischen Mechanismen der Metallbindung charakterisiert.

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IV Contents

V List of Abbreviations

ATR	attenuated total reflection
cm	centimeter
DAD	diode array detector
dH_2O	demineralized water
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EDX	energy dispersive X-ray
EI	electron ionization
EPS	extracellular polymeric substances
ESR	electron spin resonance
eV	electronvolt
FT-IR	Fourier-transform infrared spectroscopy
g	gram
GC	gas chromatography
gDNA	genomic deoxyribonucleic acid
HPLC	high-pressure liquid chromatography
HSAB	hard and soft acids and bases
ICP-OES	inductively coupled plasma optical emission spectrometry
ITS	internal transcribed spacer
L	liter
M	molar
min	minute
MSTFA	N-methyl-N-(trimethylsilyl)trifluoroacetamide
mS	millisiemens
MS	mass spectrometry
µL	microliter

µM	micromolar
m/z	mass-to-charge ratio
NCBI	National Center for Biotechnology Information
nm	nanometre
NMR	nuclear magnetic resonance
PCR	polymerase chain reaction
rcf	relative centrifugal force
REE	Rare Earth Elements
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
SAG	Culture Collection of Algae at Göttingen University
SEM	scanning electron microscopy
TEM	transmission electron microscopy
TMCS	trimethylchlorosilane
TMS	trimethylsilyl
V	volume
XAFS	X-ray adsorption fine spectroscopy
XAS	X-ray absorption spectroscopy
XPS	X-ray photoelectron spectroscopy
°C	degree celsius
% (w/v)	percent weight per volume

1. Introduction

The accumulation of toxic metals in the environment caused by industrial effluents and mining operations is a growing concern. Numerous methods have been developed for the decontamination of industrial wastewater, including membrane processes, chemical precipitation, flocculation, electroplating, or ion exchange.[1,2] However, these conventional technologies have drawbacks, such as the generation of toxic sludge or high operational costs due to the utilization of non-regenerable materials.[3] More eco-friendly approaches for metal recovery have been explored in recent years to improve the environmental impact. To that end, industrial waste streams also have been considered as an alternative source for precious elements with limited supply, such as gold, platinum, or Rare Earth Elements.[4,5]

In that context, the utilization of biological materials in biosorption-based processes has gained increased attention as a low-cost and environmentally friendly method for metal recovery from diluted wastewater streams.[6]

Biosorption

Biosorption can be defined as a physicochemical process in which substances passively bind to the surface of biological materials. The term “sorption“ refers to both absorption and adsorption.[7] The meaning of absorption is the incorporation of substances in one physical state into another substance of a different state of matter (e.g., liquids being absorbed by a solid matrix).[8] Adsorption, on the other hand, is a process in which a substance is accumulated on the surface of a solid. In this context, the substance that is being accumulated is called “sorbate”, while the solid material is called “sorbent”.[9]

Biosorption can take place on the surface of both living and dead biological materials.[10] In addition, living organisms may have active transport mechanisms for transferring metal ions from the cell surface into the cell, which is called bioaccumulation.[11] This process is a secondary step that occurs after the adsorption of metals on the cell surface and is generally slower.[12] Moreover, bioaccumulation is considered a more expensive and challenging process, as dead biomass is not affected by toxic effects of adsorbed substances and can theoretically be reused.[13] For these reasons, the metabolism-independent process of biosorption is usually considered to be more suitable for industrial applications than bioaccumulation within the cell.

Biosorption process

A biosorption-based process starts with incubating biomass that is used as biosorbent in water containing the target metal elements (Figure 1). Here, the biomass can either be stirred in a tank[14,15] or immobilized and packed into a column[16,17]. Depending on the adsorption properties of the biomass and the environmental parameters during the process, specific metal ions are removed from the solution and bind to the surface of the biosorbent. Afterwards, the metal-enriched biomass is separated from the aqueous solution, for example, via sedimentation[18], centrifugation[19], or filtration[14]. In systems that are based on immobilized biomass, this separation step is not necessary. Lastly, metals can be recovered with destructive methods, such as pyrolysis[20] or burning[21,22], resulting in metal-rich ashes, or by selective elution of adsorbed metal ions. The latter approach is more desirable from an economic perspective, as the biomass can be reused after a regeneration step. The release of adsorbed metal ions from biomass can be achieved, for example, by changing the pH value [23] or the addition of metal-complexing substances, such as citric acid or ethylenediaminetetraacetic acid (EDTA).[24,25] A major downside of biosorbents in that context is their relatively poor long-term stability. The adsorption properties of biological materials usually deteriorate after a few adsorption-desorption cycles.[26,27] This aspect is being researched on extensively in order to achieve improvements in industrial applicability.[28,29]

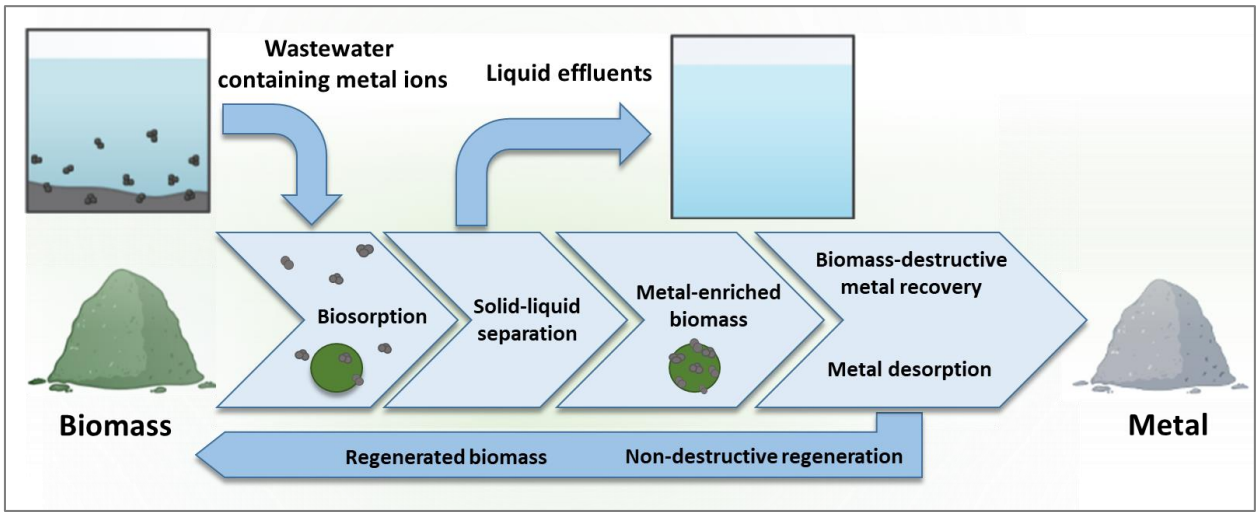


Figure 1: Schematic overview of a biosorption process for the recovery of metal ions from aqueous solutions; figure was created with BioRender.com

Applications of biosorption

Biosorption-based processes have been used for the removal of various chemical pollutants from aqueous solutions. Those pollutants include, for example, dyes, pharmaceuticals, pesticides, and metals.[30–32] For metals, especially the removal of heavy metals from wastewater streams has been the focus of many research projects. In that context, most studies focused on key environmental pollutants, such as lead, copper, mercury, cadmium, chromium, and arsenic, as well as radioactive actinides like uranium.[33–36] Nevertheless, the recovery of other elements, including precious metals such as gold, platinum, and palladium, has also been investigated in the past.[37]

Compared to chemically synthesized resins with defined composition and tailor-made functional groups for metal recovery, biosorbents are inferior in long-term stability, adsorption capacity, and binding specificity. However, the decisive advantages of biosorbents for applications in metal recovery are cost-efficient production, simple applicability, and availability in large quantities. In that context, especially waste products from agricultural production processes or animal farming have been intensively investigated as biosorbents for metal sequestration. Plant-derived biological materials tested for biosorption are, for example, fruit peels[38–40], husks[41,42], or brans[43–45]. Furthermore, animal waste materials include crab shells[46], egg shells[47], or chicken feathers.[48]

Biological waste materials can be modified with chemical or physical methods to improve their adsorption properties.[49] However, this usually increases the overall process costs and reduces their competitive edge against artificially synthesized sorbent materials. There has to be a cost-benefit analysis to determine if modifications of biosorbents are economically justified. To that end, alternative sources for the production of biosorbent materials with superior sorption properties have been explored in the past, including phototrophic microorganisms.[50]

Applications of cyanobacteria-based biosorption for metal recovery

Cyanobacteria are a diverse group of photosynthetic prokaryotes that can be found in various seawater, freshwater, and terrestrial habitats.[51,52] Due to their high abundance, they account for the majority of biologically sequestered trace metals in aquatic environments.[53] Cyanobacteria offer various benefits over other microorganisms, such as distinct cell wall compositions with usually a high mucilage volume with high metal binding affinity and a large surface:volume ratio.[54,55] Their ability to adsorb metals is associated with the presence of high-affinity metal-binding groups on the cell surface.[53] Many cyanobacteria can be cultivated on a large scale, facilitating the production of low-cost biomass for applications in biosorption processes.[56]

At present, several cyanobacteria have been investigated for their potential use in metal recovery.[57] Compared to biomass from other microorganisms, such as bacteria or yeasts, cyanobacterial biomass often exhibited promising sorption properties for future applications in metal removal from aqueous solutions.[58] Although living cyanobacterial biomass has been demonstrated to be effective in metal adsorption[59,60], biosorption technology based on the utilization of dead biomass has several major advantages.[61] Dead cells, for instance, are not constrained by toxic effects of metals ions and have no requirement for suitable growth conditions or nutrient supply.[62] Hence, the use of dead biomass is more versatile and allows less demanding process designs. Dried biomass can be embedded in different matrix materials, for example, alginate, polyurethane, or silica gel, to improve processability and reduce the loss of biomass.[63,64] The physical entrapment of biomass inside a polymeric matrix can give additional mechanical strength, rigidity, and porosity to the biosorbent.[65]

Mechanisms and functional groups involved in biosorption

The adsorption of metal ions generally leads to the accumulation of metals on the surface of the biosorbent. In the past, various mechanisms have been proposed to explain this phenomenon. It is likely that multiple mechanisms are simultaneously involved in the biosorption process.[35] However, depending on the chemical composition of the biosorbent and the correlated adsorption properties, specific mechanisms can be more dominant.[66,67] Frequently listed mechanisms occurring during the attachment of metals to the biosorbent surface are electrostatic attraction, complexation or chelation, ion exchange, and surface precipitation [68–70] (Figure 2).

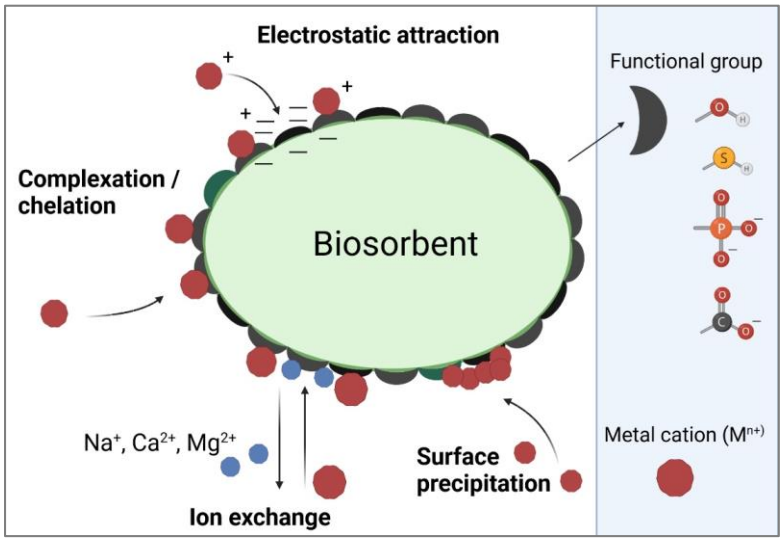
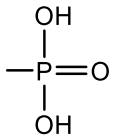
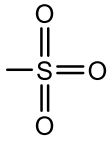


Figure 2: Schematic overview of proposed mechanisms for biosorption of metal-ions;[69] figure was created with BioRender.com

Numerous functional groups have been identified to interact with metal ions during metal adsorption. An incomprehensive list of functional groups involved in biosorption processes is shown in Table 1.[71] Most of these functional groups are negatively charged under regular environmental conditions or have free electron pairs, which facilitate the interaction with positively charged metal ions. The presence and relative abundance of specific functional groups at the cell surface can significantly influence the binding specificity and overall adsorption capacity of biosorbents.[72]

Table 1: Selected functional groups involved in biosorption processes [71]

Functional groups	Structural formula	Occurrence in selected biomolecules
Amine	$-NH_2$	chitosan, amino acids
Hydroxyl	$-OH$	polysaccharides, uronic acids, amino acids
Carbonyl	$C=O$	proteins, peptides
Carboxyl	$-COOH$	polysaccharides, uronic acids, amino acids
Imine	$=NH$	amino acids
Phosphonate		phospholipids
Sulfonate		sulfated polysaccharides
Sulfhydryl	$-SH$	amino acids

Factors influencing biosorption

The uptake of substances by biological materials is complex and can be influenced by many factors. Besides cell viability and biomass composition, a number of different environmental parameters can impact a biosorption-based process. Those parameters can be monitored and regulated in a technical application and are vital for the development of an industrial process.

pH value

One of the most important environmental parameters for the adsorption of metal ions is the pH of the metal-containing solutions. The pH value substantially influences the solubility of metals, for example, by speciation or the formation of metal hydroxides,[73,74] which can lead to precipitation and significantly reduce metal adsorption.[3] For the recovery of metals in a biosorption-based process, solutions with a neutral or alkaline pH are therefore not applicable in most cases.

The pH value also determines the properties of functional groups on the surface of biosorbents, in particular, their concentration of negative charges. In general, the adsorption capacity for metal ions usually is lowest at highly acidic conditions.[75,76] The reason for this phenomenon is the increased portion of positively charged active sites at the cell surface under acid conditions, which repel positively charged metal ions. On the other hand, high pH values can cause precipitation of many metal elements. In that context, the optimum pH for the removal of metals is different depending on the biosorbent and its dominant functional groups that are involved in the adsorption process.[77] In general, if the pH of metal solutions is lowered from 6 to 2, the adsorption of metals declines significantly for the majority of biomass types.[78] Below pH values of 2, there is usually no relevant metal removal from aqueous solutions.[78] For most algae-based biosorption processes, the optimum has been reported between 5 and 6.[79]

Temperature

Although adsorption reactions are usually exothermic,[80] depending on the type of metal ion and biosorbent, a biosorption process can be both exothermic and endothermic.[81] The operational temperature however, has no significant effect on metal sorption between 20°C and 35°C.[82] Moreover, a biosorption process usually is not operated at high temperatures because of increased operational costs and reduced stability of the biological material that is used as biosorbent.[83]

Contact time

The contact time of the biosorbent and sorbate has no direct effect on biosorption capacity, but it can act as a limiting factor. Biosorbents reach maximum adsorption capacity when their free binding sites are fully saturated. Depending on the chemical composition of the biosorbent and the interacting metal cations, the occupation of free binding sites exhibits different reaction kinetics to reach an equilibrium state. Sufficient data sets obtained from kinetic studies allow the description of metal adsorption with different models, such as pseudo-first order or pseudo-second order.[84]

Initial metal concentration

The initial metal concentration in aqueous solutions is an important factor for the adsorption of metals on biomass. In general, there is a positive correlation between metal concentration and metal uptake following a typical saturation curve. Hence, the optimum percentage of metal removal is usually achieved at low initial metal concentrations. However, under the premise that there is sufficient biosorbent material, metal uptake increases with elevated initial metal concentrations. The metal uptake of biosorbents at different metal concentrations is described by

isotherm experiments. Isotherms indicate the amount of metal bound to the surface of a biosorbent as a function of the metal concentration in a given solution.[85] As such, adsorption isotherms are an essential indicator for estimating the feasibility of specific materials in applications on wastewater with a known metal concentration and composition. Isotherm curves are determined by varying the initial metal concentration at a constant temperature. Typically, the metal adsorption capacity increase as the metal concentration rises and reaches saturation at higher concentrations.[86]

Various models are used to fit isotherm data points and describe the affinity of biosorbents for specific elements.[87] Two isotherm models that are frequently used in the context of metal biosorption are the Freundlich model and the Langmuir model.[88,89] Those models were initially developed for the adsorption of gases and have some assumptions that are not applicable to biosorbents, such as the same affinity of all binding sites for the sorbate. In contrast, biological materials comprise multiple functional groups with different metal affinities that can be further affected by environmental factors like pH or temperature. Nonetheless, from a practical point of view, these models can give a useful indication of the biosorbents´ affinity for metals at specific concentrations.

Competing ions

Industrial wastewater streams usually comprise various metal elements in elevated concentrations. Research on various biosorbent materials has shown, that different metal cations can compete for the same binding sites during biosorption.[90,91] Thus, the adsorption of specific target elements is influenced by the presence of competing ions and their concentration in the aqueous effluents. In addition, the binding specificity of biosorbents is determined by their chemical composition and abundance of functional groups that interact with the metal ions at the surface.[92]

For the development of a biosorption-based process, the composition of the aqueous solutions, the chemical features of the biosorbent, and the process parameters, such as the pH value, have to be considered, as these factors influence binding specificity and adsorption capacity. The interplay between these factors is very complex and has to be determined experimentally. Depending on the process parameters and the presence of competing ions in the wastewater, the same biosorbent material can exhibit different binding affinities for specific metal elements.[93,94]

Rare Earth Elements

The research presented in this work focused predominately on the potential application of cyanobacterial biomass to recover Rare Earth Elements (REE) from aqueous solutions via biosorption.

REE encompass 17 metal elements consisting of scandium, yttrium, lanthanum and 14 additional elements of the lanthanides series: cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutetium.[95] The adjective “rare” is deceptive since it does not correlate with geological rarity but originated from the occurrence of REE as a mixture of elements in minerals in generally low concentrations. The proportion of lanthanum, cerium, or neodymium of the upper continental crust, for example, is estimated at 31 µg g^{-1}, 63 µg g^{-1}, and 27 µg g^{-1}, respectively.[96] Hence, their abundance can be compared to nickel, copper, or zinc. Nonetheless, other lanthanides, such as terbium, are actually rare, with an estimated proportion of 0.7 µg g^{-1}.[96]

Chemical properties and applications

Chemically, REE exhibit very similar chemical features, such as high electropositivity and the predominance of the 3+ oxidation state.[97] There are some exceptions, as cerium or europium, for example, can be stable in a 4+ or 2+ oxidation state.[98,99] Electrons of elements belonging to the lanthanide series gradually occupy the 4f valence orbitals. At the 3+ oxidation state, those 4f orbitals are still shielded by 5s and 5p orbitals and only have little influence on the chemical reactivity of the elements.[100] However, the occurrence of electrons in the 4f orbitals induces distinct and unique optical and magnetic properties, which make lanthanides a critical resource for many applications in modern high-technology sectors.[101,102] REE are strong Lewis acids with a constant decrease in the size of their atomic and ionic radii through the lanthanide series caused by the gradual occupancy of the 4f valence orbitals.[95] This phenomenon is called “lanthanide contraction”. The contracted nature of the 4f valence orbitals results in an almost entirely ionic chemical bonding behavior.[103] During complex formation, REE usually form complexes with high coordination numbers and weak metal-ligand bonds that are mediated by steric effects.[103,104]

Numerous applications in the high-technology sector are dependent on the utilization of REE. For example, the most abundant REE cerium is used in various catalysts playing a vital part in the chemical processes occurring in converters.[105] Lanthanum is extensively used in cameras, optical lenses, lighting applications, and the production of batteries that are used in electric vehicles.[106,107]

The element neodymium is used to create strong magnets found in wind turbines and electric motors.[108] Furthermore, neodymium-containing magnets have applications in computer hard

drives.[109] Terbium, on the other hand, is mainly required for the production of visual displays or lighting technologies.[110]

Rare Earth Element production

Despite their widespread distribution, there are only few deposits with sufficiently high concentrations for an economically profitable production.[111] In most cases, REE are produced as a co-product or by-product of other minerals.[112,113] Due to their low concentrations in the starting raw materials and the following complex and energy-intensive purification, REE usually cannot be produced profitably in a stand-alone mining operation. A large portion of the globally produced REE, for example, is obtained from side streams of iron mining in the Bayan Obo mine in China.[114] For many years, China had a monopoly on REE production. After China imposed export restrictions on REE in 2010, the price of REE skyrocketed and the strategic importance of these elements for many key industries became obvious.[115] Since then, many countries have made efforts to establish their own REE production to become more economically and politically independent. At present, 60-70% of the total REE production is still located in China.[116,117] The annual global production of REE in 2022 was approximately 300,000 metric tons.[117] In 2020, the European Commission listed REE as critical resources in the “Study on the EU's list of Critical Raw Materials”.[118] In that context, REE were assessed to have the highest supply risk.

Due to their similar physical and chemical properties, the purification of REE on an industrial scale is challenging and energy intensive[119]. Chemical leaching of REE is the most common extraction method from mineral concentrates.[120,121] There is a wide range of methods used for REE recovery, including chemical precipitation,[122,123] ion exchange,[124] flotation,[125,126] electrodialysis,[127] or solvent extraction.[128,129] In that context, in industrial processes, solvent extraction is the most widely used method for the separation and purification of REE.[130]

A study published in 2013 estimated the released amounts of greenhouse gases per kg REE oxide and the major contributing factors during REE processing.[131] According to this data, the production of samarium, europium, and gadolinium releases approximately 55 kg of CO_2 per kg REE oxide. For elements that are more abundant, such as lanthanum or cerium, approximately 9 kg of CO_2 per kg REE oxide is released. In comparison, the carbon footprint for the production of steel is estimated between 0.82 and 2.1 kg of CO_2 per kg.[132] The main contributing factors to the overall energy demand for REE purification are the energy input during the process itself (e.g. steam generation or electricity) and the utilization of various chemicals, such as hydrochloric acid.[131] Furthermore, conventional REE production typically has a negative local impact on the environment, such as the accumulation of radioactive elements, soil acidification, eutrophication,

or freshwater consumption.[133] In the broad context of sustainability, more eco-friendly alternative REE production methods need to be implemented in the future. In spite of commercial and political incentives to retrieve REE from local sources, less than 1% of REE are currently being recycled.[134] Although industrial wastewater streams contain significant amounts of REE, their total concentration is still relatively low. Furthermore, the efficiency of most standard metal recovery systems is not adequate to recycle REE cost-effectively.[134]

Potential ecotoxicity of Rare Earth Elements

Most industrial wastewater treatment processes focus on the removal of toxic heavy metals, such as lead, mercury, or cadmium. The necessity for the recovery of those elements is universally acknowledged as their harmful or toxic impact on the environment and human health is extensively documented and understood.[135,136] For REE, on the other hand, there is little data on their environmental impact if they accumulate beyond natural levels. In the past, there was no incentive to study this aspect, as their concentration is usually negligibly low. However, with a significant increase in industrial production and applications in modern technology, the release of REE into the environment, for example by mining or metallurgical operations, increased dramatically.[137] This circumstance is further amplified with the utilization of REE in medicinal products and agriculture, for example as intravenous radio-contrast agents during magnetic resonance imaging or animal feed additives.[138,139] Recent studies indicate that the accumulation of REE in the environment could have a negative impact on various ecosystems.[140] REE can, for example, accumulate in some plants, such as switchgrass or radish, and have adverse effects on their growth.[141] Furthermore, there are indications that long-term exposure to increased REE-levels might have a negative impact on human health.[142] In addition to economic motives, the recovery of REE for industrial effluents could therefore also have environmental incentives.[143]

Commonly used industrial wastewater treatment systems are not specifically designed for the removal of REE from aqueous solutions. Hence, standard recycling processes generally operate with poor efficiency for REE recovery.[144] As cyanobacteria have been demonstrated to adsorb metals from highly diluted aqueous solutions effectively, there is potential for applications in REE sequestration via biosorption.[145]

2. Materials and Methods

The following paragraph represents an overview of the most important materials, methods, and procedures used in this thesis. Further, detailed information is presented in the respective materials and methods parts and the supplementary data sections of the corresponding manuscripts included in this thesis.

Chemicals and reagents

Components for cultivation media and metal adsorption experiments were purchased from Sigma-Aldrich (Taufkirchen, Germany), Carl Roth GmbH & Co. KG (Karlsruhe, Germany), or Merck KGaA (Darmstadt, Germany).

Media composition and stock solutions

BG11 medium (according to Stanier et al. 1971)[146]

The pH value was adjusted to 7.1 before autoclaving. To avoid precipitation, trace elements and calciumchlorid solutions were either sterile filtrated or autoclaved separately and added to the final medium after autoclaving.

Table 2: BG11 medium

Component	**Quantity for 1 L**
$NaNO_3$	1.50 g
K_2HPO_4 x 3 H_2O	0.040 g
$MgSO_4$ x 7 H_2O	0.075 g
Na_2CO_3	0.020 g
Fe Citrate solution	1 mL
EDTA solution	1 mL
Ca solution	1 mL
Trace elements solution	1 mL

Table 3: Fe Citrate solution for BG11 medium

Component	**Quantity (g L^{-1} dH_2O)**
Citric acid	6.00
Ferric ammonium citrate	6.00

Table 4: EDTA solution for BG11 medium

Component	**Quantity (g L^{-1} dH_2O)**
$Na_2EDTA \times 2\ H_2O$	1.00

Table 5: Calcium chloride solution for BG11 medium

Component	**Quantity (g L^{-1} dH_2O)**
$CaCl_2 \times 2\ H_2O$	36.0

Table 6: Trace elements solution for BG11 medium

Component	**Quantity (g L^{-1} dH_2O)**
H_3B_3	2.86
$MnCl_2 \times 4\ H_2O$	1.81
$ZnSO_4 \times 7\ H_2O$	0.222
$Na_2MoO_4 \times 2H_2O$	0.39
$CuSO_2 \times 5\ H_2O$	0.079
$Co(NO_3)_2 \times 6\ H_2O$	0.0494

Modified Spirulina medium (according to Andersen 2005)[147]

For 1 L of modified Spirulina Medium 500 mL of “Solution I” and 500 mL of “Solution II” were autoclaved separately and aseptically combined after cooling.

Table 7: Solution I for Spirulina medium

Component	**Quantity for 500 mL**
$NaHCO_3$	13.61 g
Na_2CO_3	4.03 g
K_2HPO_4	0.50 g

Table 8: Solution II for Spirulina medium

Component	**Quantity for 500 mL**
$NaNO_3$	2.50 g
K_2SO_4	1.00 g
NaCl	1.00 g
$MgSO_4$ x 7 H_2O	1.00 g
$CaCl_2$ x 2 H_2O	0.04 g
$FeSO_4$ x 7 H_2O	0.01 g
Na_2EDTA x 2 H_2O	0.08 g
Trace elements solution	1 mL

Table 9: Trace elements solution for Spirulina medium

Component	**Stock solution (g L^{-1} dH_2O)**	**Quantity** for 1 L
Na_2EDTA x 2 H_2O	-	0.80 g
$FeSO_4$ x 7 H_2O	-	0.70 g
$ZnSO_4$ x 7 H_2O	1.00	1 mL
$MnSO_4$ x 7 H_2O	2.00	1 mL
H_3BO_3	10.00	1 mL
$Co(NO_3)_2$ x 6 H_2O	1.00	1 mL
Na_2MoO_4 x 2 H_2O	1.00	1 mL
$CuSO_4$ x 5 H_2O	0.005	1 mL

Cyanobacterial strains and their cultivation

Eight strains used in this thesis were isolated from environmental samples by the research group of Prof. Dr. Michael Lakatos at the University of Applied Sciences Kaiserslautern. *Synechococcus elongatus* UTEX 2973 was obtained from the Culture Collection of Algae at the University of Texas in Austin. *Calothrix brevissima* SAG 34.79, *Limnospira maxima* SAG 49.88, and *Limnospira platensis* SAG 85.79 were bought from the Culture Collection of Algae at Göttingen University.

Table 10: Overview of cultivated cyanobacteria

Strain	Strain number	Cultivation vessel	Medium
Scytonema hyalinum	02.01	submerse cultivation	BG11
Nostoc. sp.	20.02	submerse cultivation	BG11
Komarekiella sp.	89.12	submerse cultivation	BG11
Komarekiella sp.	90.01	submerse cultivation	BG11
Desmonostoc muscorum	90.03	submerse cultivation	BG11
Reptodigitus sp.	92.01	submerse cultivation	BG11
Phormidium autumnale	97.20	submerse cultivation	BG11
Symphyonema bifilamentata	97.28	submerse cultivation	BG11
Synechococcus elongatus	UTEX 2973	2.7 L stirred photobioreactor	BG11
Calothrix brevissima	SAG 34.79	500 mL shaking flask and 2.7 L stirred photobioreactor	BG11
Limnospira maxima	SAG 49.88	500 mL shaking flask	modified Spirulina medium
Limnospira platensis	SAG 85.79	500 mL shaking flask	modified Spirulina medium

The cyanobacterial biomass, that was used for the execution of adsorption experiments, was produced in different cultivation systems. *Scytonema hyalinum* 02.01, *Nostoc. sp*. 20.02, *Komarekiella sp*. 89.12*, Komarekiella* sp. 90.01, *Desmonostoc muscorum* 90.03, *Reptodigitus* sp.

92.01, *Phormidium autumnale* 97.20 and *Symphyonema bifilamentata* 97.28 were grown in cultivation flasks as submersed cultures. *Calothrix brevissima* SAG 34.79, *Limnospira maxima* SAG 49.88 and *Limnospira platensis* SAG 85.79 were cultivated in 500 mL shaking flasks. *Synechococcus elongatus* UTEX 2973 and *Calothrix brevissima* SAG 34.79 were cultivated in an Infors Lab5 stirred photobioreactor system (Infors HT, Bottmingen, Switzerland).

Extraction and isolation of biomass-derived metal chelators

For a more detailed investigation of metal binding properties displayed by the cyanobacterium *Calothrix brevissima,* metal interacting components were isolated from dried biomass. A schematic overview of the extraction and isolation process for the metal chelators is shown in Figure 3.

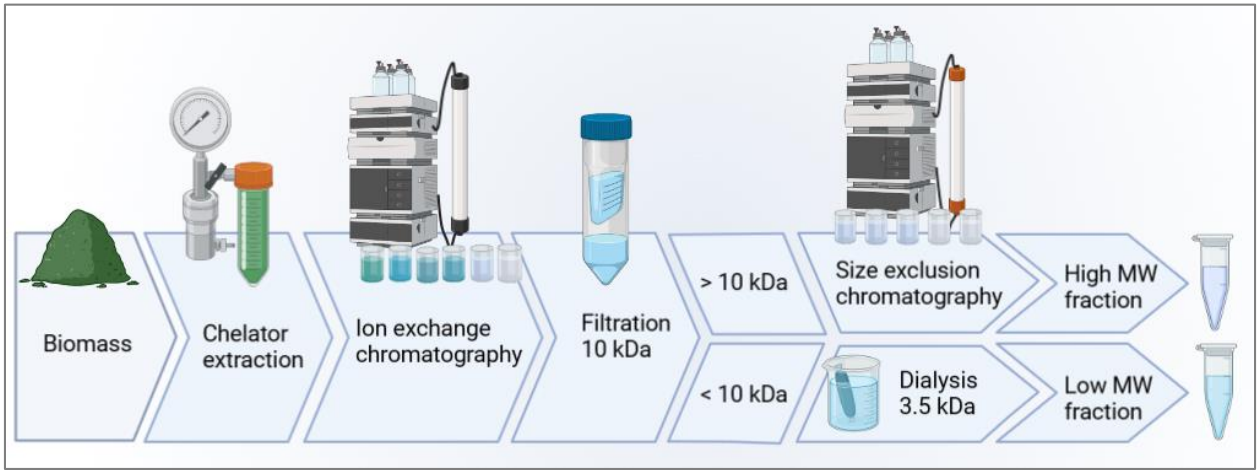


Figure 3: Schematic workflow for the extraction and isolation of biomass-derived metal chelators, resulting in a low molecular weight and a high molecular weight fraction (cut-off 10 kDa); figure was created with BioRender.com

At first, cells were disrupted using a high-pressure homogenizer (Avestin Emulsiflex B15, Avestin Europe, Germany) set to 2,000 bar. 250 mg of dry biomass was suspended in 15 mL of deionized water and passed through the device three times. Afterwards, the disrupted cells were incubated at 50 °C under constant shaking to extract water-soluble compounds. After 1 h the undissolved residue was separated by centrifugation at 10,000 rcf for 10 min (Eppendorf 5810R centrifuge, Eppendorf, Hamburg, Germany). The resulting supernatant was filtered with a 0.45 µm syringe filter (Filtrapur S, Sarstedt Inc, US) and a 0.2 µm membrane filter in a vacuum vial (Whatman, GE Healthcare, Chicago, US). The filtered sample was injected into a chromatography system

(NGC Chromatography System, Bio-Rad) with a 5 mL sample loop, two pumps, mixer, UV/Vis detector, conductivity detector, and an automated fraction collector. The separation was performed using a glass column with Q-Sepharose fast flow (GE Healthcare, Chicago, US). In order to pass the sample through the column and allow negatively charged molecules to bind, 12 mL of demineralized water was pumped into the system. Afterwards, a NaCl-gradient (0-1 M) was applied for 15 min, followed by regeneration of the resin by a 1 M NaCl solution for another 15 min. For all steps, the flow rate was set to 0.6 mL min^{-1}. During the chromatography run, fractions of 2.5 mL were collected and probed for terbium luminescence sensitization according to a protocol described by Jurkowski et al..[148] Fractions that displayed an interaction with terbium were pooled and further purified using a 10 kDa centrifugal filter concentrator (Centriprep, Merck, Darmstadt, Germany).

The fraction below the cut-off limit of 10 kDa was desalted by applying a dialysis against demineralized water until a conductivity of 0.01 mS cm^{-1}. For this purification step, a membrane (Spectra/Pro, Spectrum Laboratories Inc., US) with a cut-off at 3.5 kDa was used. Moreover, the fraction retained by the 10 kDa filter was further purified with a size exclusion chromatography using the same, previously described, chromatographic system. For this purpose, a Tricorn Superdex 75 (GE Healthcare, Chicago, US) column was used and the flow was set to 0.5 mL min^{-1}. Subsequently, fractions of 2.5 mL were collected automatically and tested for terbium sensitization using the previously mentioned protocol by Jurkowski et al..[148] Thereafter, all fractions that showed an interaction with terbium were collected and dried for storage with a HT-4 Atlas Evaporator (GeneVac Ipswich, United Kingdom) linked to a VC 3000 Vapour Condensator (GeneVac, Ipswich, United Kingdom).

Analytics

Chemical hydrolysis

To release monomeric carbohydrate building blocks from the samples´ constituting polymeric carbohydrates, each sample was hydrolyzed with 1 % sulfuric acid in an autoclave for 1 h at 121 ℃ at 1 bar. Afterwards, each sample was centrifuged at 10,000 rcf for 10 min. Following hydrolysis, the solutions were neutralized with calcium carbonate to a pH of 7. Precipitated calcium salt was removed by centrifugation at 10,000 rcf for 10 min after neutralization. The supernatant was frozen at -20 °C for 48 hours. Subsequently, the samples were heated to 5 °C and centrifuged at 10,000 rcf for 30 min to remove any residual precipitate.

HPLC analysis

Sugar analysis was carried out using an HPLC system (Infinity II LC 1260, Agilent technologies, Waldbronn, Germany) equipped with an autosampler, quaternary pump, column oven, DAD, and a Shodex RI detector (Showa Denko Europe GmbH, Munich, Germany). Prior to injection, each sample was filtered using Modified PES 500 µL Centrifugal Filters (VWR, Ismaning, Germany) with a cut-off of 10 kDa. Afterwards, the monomeric sugar mixture resulting from chemical hydrolysis was analyzed using the HPLC system previously described. The monomers were analyzed using a Rezex ROA-Organic Acid H+ (8 %) ion-exclusion column (300 mm, 7.8 mm internal diameter; Phenomenex LTD, Aschaffenburg, Germany) applying an isocratic separation with 5 mM sulfuric acid at a flow rate of 0.5 mL min^{-1} at a temperature of 70 °C.

GC-MS analysis

Sample material was processed with a trimethylsilyl (TMS) derivatization for a GC-MS-based identification of carbohydrate constituents. The derivatization was performed following a modified version of a previously described protocol.[149] First, 50 µL of pyridine were added to each sample. Afterwards, 50 µL of MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) with 1% TMCS (Trimethylchlorosilane) were added and the samples were incubated in a water bath at 50 °C for 1 h. Then, GC-MS analysis was carried out using a modified version of a previously described protocol by Ringel et al.[150]. The samples were analyzed using a Trace GC-MS Ultra system with DSQII (Thermo Fisher Scientific, Waltham, US). One microliter (1/10 split ratio) of each sample was injected by a TriPlus autosampler onto an SGE BPX5 column (30 m, I.D. 0.25 mm, film 0.25 µm) with an injector temperature of 280 °C. As a carrier, helium gas was used with a flow rate of 0.8 mL min^{-1}. The initial oven temperature was set to 70 °C for 2 min. Subsequently, the temperature was ramped to 290 °C with a rate of 5 °C min^{-1} and then kept constant for 4 min. The MS data was recorded at 70 eV (EI). All masses were recorded in positive mode in a range between 50 and 650 m/z.

ICP-OES analysis

The quantification of metals in analyzed solutions was achieved via ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) using an Agilent 725 Series ICP Optical Emission Spectrometer, (Agilent Technologies Inc., Santa Clara, US). The standards used for calibration were a TraceCERT® Rare earth element mix with 16 elements from Sigma-Aldrich (Sigma-Aldrich, Taufkirchen, Germany) and a Certipur® ICP multi-element standard solution IV from Merck (Merck KGeA, Darmstadt, Germany) with 23 elements.

Luminescence analysis

To investigate the interaction of terbium with metal chelating compounds, a luminescence-based method that was published by Jurkowski et al.[148] was applied. Solutions containing the investigated samples were transferred into a quartz glass 96 multiwell plate (Hellma Analytics, Müllheim, Germany) and placed in an EnSpire multiplate reader (PerkinElmer, Waltham, US). Subsequently, the samples were excited with a wavelength of 230 nm and the emission spectrum from 460 to 570 nm was recorded. The displacement of terbium ions by other metal ions was observed by comparing the emission signal intensities at 544 nm.

FT-IR analysis

In this study, IR spectroscopy was used to identify functional groups in cyanobacteria biomass, as well as the isolated EPS samples and to detect possible interactions with metal cations. IR spectra were recorded using an FT-IR spectrometer (Nicolet iS50R, Thermo Fisher Scientific, Waltham, US) equipped with an iS50 ATR (attenuated total reflection) multi-range, diamond sampling station. For each sample, IR spectra were obtained in a range from 400 – 4,000 cm^{-1}.

DNA-analysis

Prior to DNA-analysis, approximately 50 mg of biomass were collected from cultures during stationary growth phase. This biomass was used for gDNA extraction with the DNeasy PowerSoil Pro Kit (Qiagen, Hildesheim, Germany) following the manufacturer's instructions. Using the primers Wil1 (AGAGTTTGATCCTGGCTCAG) and Wil18 (TTTGCGGCCGCTCTGTGTGCCTAGGTATCC)[151] and ready-to-go PCR mini beads (GE Healthcare, Chicago, US) in a MiniAmp Plus Thermal Cycler (Thermo Fisher Scientific, Waltham, US), the 16S–23S ITS gene region was amplified by PCR in a 50 µL reaction. The resulting PCR products were examined by gel electrophoresis using 1 % (w/v) agarose and the E-Gel Power Snap Electrophoresis System (Invitrogen, Waltham, US). Subsequently, the PCR products of the expected length were purified with a NucleSpin™ Gel and PCR Clean-up Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the manufacturer´s instructions. Afterwards, the samples were sent for Sanger sequencing to Genewiz/Azenta (Germany GmbH, Leipzig, Germany) using the primers Wil1 (AGAGTTTGATCCTGGCTCAG), Wil4 (AGGCAGCAGTGGGGAA), Wil5 (CTGCTGCCTYCCGTA), Wil10 (GAATTGACGGGGRCCC), Wil11 (CCGTCAATTYYTTTRAGTTT), Wil16 (AAGGAGGTGATCCAGCCGCA), and Wil18 (TTTGCGGCCGCTCTGTGTGCCTAGGTATCC)[151].

Phylogenetic analysis

The sequences generated by Sanger sequencing were assembled using the Geneious Prime (v2021.0.1) software package (Biomatters Limited, Auckland, New Zealand). Afterwards, the sequences were compared to already submitted sequences of strains from public culture collections using the BLAST tool of the National Center for Biotechnology Information (NCBI) GenBank.

Subsequently, a phylogenetic analysis of the assembled 16S rRNA gene sequences was carried out together with related sequences of cyanobacterial strains cited from GenBank, including *Gloeobacter violacaeus* as outgroup. For the gene alignment, the Muscle algorithm in Mega X [152] was applied. Based on the lowest Akaike information criterion value, the evolutionary model that was best suited for the database was used and calculated in Mega X, which was the RGT G+I model of nucleotide substitutions. The generated phylogenetic tree was statistically verified by using the maximum likelihood method with a bootstrap value of 1,000, calculated with Mega X. Additionally, Bayesian inference (BI) phylogenetic analyses, with two runs of eight Markov chains, were executed for one million generations with default parameters with MrBayes 3.2.1 [153]. Before the end of each run, the analysis reached stationarity (average standard deviation of split frequencies between runs < 0.01).

Biosorption experiments

Metal adsorption experiments were carried out based on a previously described methodology by Heilmann et al..[154,155] All tested biomass samples were washed three times with demineralized water to remove any residual media components that could interfere with the following experiments. Then, the washed biomass was lyophilized after being frozen at -80 °C. Adsorption experiments were performed by incubating 10-20 mg of lyophilized biomass in 2 mL metal solutions with a predetermined concentration. By analyzing and comparing the metal concentration before and after incubation, the metal adsorption was determined. Each sample was centrifuged for 5 min at 10,000 rcf at room temperature. Subsequently, the metal concentration in the supernatant was measured.

Determination of adsorption capacities

The adsorption capacity for metals was determined by incubating dry biomass in 10 mM metal solutions with an initial pH of 5 ± 0.2 for 3 h under constant shaking at room temperature. Subsequently, the metal uptake was determined by dividing the changes in metal concentration

by the amount of incubated biomass (see equation 1).

$$Q = \frac{n_i - n_f}{m} = \frac{(c_i - c_f) \times V}{m} \quad (1)$$

with Q = adsorption capacity, n_i = initial amount of substance, c_f = final amount of substance after incubation, c_i = initial metal concentration, c_f = final metal concentration after incubation, V = volume, and m = weight of biomass

Influence of initial pH value

Similar to the method previously described, the impact of the initial pH value on metal adsorption was examined. The experiments were conducted ranging from pH 1 to 6 due to the formation of insoluble REE hydroxides at pH values above 7.[74,155] NaOH and HCl were used to adjust the pH of the metal solutions that were applied to the biomass.

Adsorption kinetics

The experiments on adsorption kinetics were conducted by adjusting the incubation time of cerium(III)nitrate solutions with a concentration of 10 mM and an initial pH value of 5±0.2. Samples were taken after an incubation time of 2 min, 5 min, 15 min, 30 min, and 60 min.

Adsorption specificity for cerium

In addition to examining the adsorption capacity in single-element systems, the adsorption specificity was tested with multi-metal solutions containing aluminium, cerium, lead, nickel, and zinc. The experiments were conducted with equimolar mixed-metal solutions with concentrations of 0.5 mM, 1 mM, 2 mM, and 4 mM.

Influence of initial metal concentration

Adsorption isotherms were examined by incubating biomass in metal solutions with cerium(III)-concentrations between 0.5 mM and 10 mM. The samples were incubated at room temperature for 1 hour under constant shaking before being analyzed, as previously stated. The Langmuir and Freundlich model were used to fit the determined data points and describe adsorption isotherms.

3. Research

3.1 Summaries of included publications

Chapter I – Isolation and Investigation of Natural Rare Earth Chelating Agents from Calothrix brevissima – A Step Towards Unraveling the Mechanism of Metal Biosorption

The article "Isolation and Investigation of Natural Rare Earth Metal Chelating Agents From Calothrix brevissima - A Step Towards Unraveling the Mechanisms of Metal Biosorption" was published in the journal Frontiers in Bioengineering and Biotechnology in February 2022 (https://www.frontiersin.org/articles/10.3389/fbioe.2022.833122/full).

Michael Paper shares first authorship of this publication with Wojciech Jurkowski. Michael Paper helped develop the concept, supported the implementation of experiments, evaluated the experimental data, and wrote the manuscript.

The aim of this study was to gain deeper insights into the metal adsorption properties displayed by the cyanobacterium *Calothrix brevissima* by investigating the structural components of the biomass involved in metal absorption. Hence, in this publication, Rare Earth Element-chelating compounds from biomass of the cyanobacterium *C. brevissima* were isolated following a luminescence-based method that was developed and published by Jurkowski et al. in 2020 [148]. Using a preparative FPLC-based process, these compounds were obtained in sufficient quantities for further analysis of their chemical composition and their mechanism of interacting with metal cations. This process resulted in two separate fractions with metal chelating compounds. Both a low molecular weight fraction with components <10 kDa and a high molecular weight fraction >10 kDa were enriched and purified using filtration, dialysis, and size exclusion chromatography.

After chemical hydrolysis, the monomeric sugar composition of isolated compounds was investigated using an HPLC and GC-MS-based analysis. The evaluation of the resulting data showed that both the high and low molecular weight fractions of the biomass-derived chelators are composed of arabinose, xylose, mannose, galactose, and glucose, respectively. This substantiated the assumption that the chelators in the low molecular weight fraction originate from the larger compounds present in the high molecular weight fraction.

For the identification of functional groups involved in metal chelating, an FT-IR analysis was

carried out with the isolated components before and after incubation with terbium(III) nitrate. The comparison of the obtained spectra indicated that sulfate and to a lesser extent hydroxyl groups are involved in the complexation process. Furthermore, the formed complexes were stable even at high pH values of up to 9.5.

To investigate the binding specificity for other metals, the spectrophotometric method previously used for the isolation of the chelating compounds was modified. Different metal cations were applied to compete with terbium ions for free binding sites in the isolated compounds. These experiments indicated that cobalt and lead were able to replace terbium. On the other hand, alkaline earth and alkaline metals did not negatively affect terbium complexation, even at 10-fold excess concentrations.

This study showed that specific polysaccharide structures derived from *Calothrix brevissima* contribute to REE adsorption via chelate formation. Those compounds showed high affinity for REE, but other metal cations could compete for the same binding sites.

Chapter II – Rare earths stick to rare cyanobacteria: Future potential for bioremediation and recovery of rare earth elements

The article "Rare earths stick to rare cyanobacteria: Future potential for bioremediation and recovery of rare earth elements" was published in the journal Frontiers in Bioengineering and Biotechnology in February 2023 (https://www.frontiersin.org/articles/10.3389/fbioe.2023.1130939/full).

The conceptualization of the study and design of the methodological approach was jointly designed by all authors. Planning, execution of experiments, and data validation, with the exception of the phylogenetic analysis, was carried out by Michael Paper. Furthermore, Michael Paper prepared the original draft of the manuscript, which was jointly finalized and reviewed by all authors.

This study aimed to investigate novel cyanobacterial strains for their potential application in biosorption-based enrichment processes for Rare Earth Elements. In total, twelve cyanobacterial strains, including seven terrestrial and five aquatic cyanobacteria, were investigated in this study. As many of the strains were novel environmental isolates, a molecular analysis of their 16S rRNA gene sequences was carried out for all tested cyanobacteria in this study. Based on the resulting sequences, a Maximum Likelihood phylogenetic tree was constructed, revealing a broad genetic diversity within the tested strains. Of the twelve tested cyanobacteria, six novel 16S rRNA gene sequences were discovered and subsequently added to the database GenBank.
Furthermore, all cyanobacterial strains were screened for their maximum adsorption capacity of lanthanum, cerium, neodymium, and terbium. After selecting five candidate strains, parameters that influence the metal uptake of biomass, i.e., pH value, contact time, metal concentration, and binding specificity, were analyzed in more detail using the element cerium. The optimum pH value for the highest metal uptake was observed at pH 5. Kinetic studies showed that the adsorption equilibrium capacity was reached within a few minutes for all tested biomasses, indicating a fast adsorption process. The tested cyanobacterial biomasses could adsorb cerium at low concentrations, which was best described by fitting the determined data points according to the Langmuir-model. Studies on binding specificity indicated a higher affinity of the biomass for lead and aluminum than for cerium. However, at metal concentrations below 2 Mm, tested biomass showed better adsorption for cerium. Furthermore, metal analysis of the tested solution strongly

indicated an ion-exchange mechanism in which adsorbed metal cations replace ions of sodium, potassium, magnesium, and calcium on the cell surface. An FT-IR analysis comparing biomass before and after metal adsorption indicated, that carboxyl and hydroxyl groups are likely involved in the biosorption process.

In this study, the metal-sorption properties of novel, mostly uncharacterized cyanobacteria were investigated. These cyanobacteria could be applied in future biosorption-based processes for the recovery of Rare Earth Elements and the treatment of industrial wastewater. Critical parameters for metal uptake by the cyanobacterial biomass were optimized, and the dominant chemical mechanisms for metal binding were characterized.

3.2 Full-length publications

Isolation and Investigation of Natural Rare Earth Chelating Agents From *Calothrix brevissima* – A Step Towards Unraveling the Mechanisms of Metal Biosorption

frontiers in Bioengineering and Biotechnology

ORIGINAL RESEARCH
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Isolation and Investigation of Natural Rare Earth Metal Chelating Agents From *Calothrix brevissima* - A Step Towards Unraveling the Mechanisms of Metal Biosorption

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In this study water soluble compounds that form complexes with Rare Earth Elements (REE) and other metals were isolated from *Calothrix brevissima* biomass with chromatographic methods for the first time. Molecular characterization showed that the isolated compounds are most likely polysaccharides comprised of arabinose, xylose, mannose, galactose and glucose. FT-IR analysis revealed functional groups involved in the binding mechanism of Tb are likely sulfate- and to a lesser extend hydroxyl-groups. The binding specificity of the isolated compounds was investigated with different metal solutions. Here, ions of the alkali and alkaline earth metals Na, K, Mg and Ca showed no competition for Tb-binding even at 10-fold excess concentration. Ions of the elements Co and Pb on the other hand replaced Tb at higher concentrations. Addition of the isolated compounds significantly reduced the precipitation of Eu at pH-values between 6.7 and 9.5, indicating that the interaction between the isolated chelators and Rare Earth Metals is stable even at high pH-values.

Keywords: biosorption, cyanobacteria, rare earth elements, mechanism, calothrix, complexation

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INTRODUCTION

Many industries, such as metallurgy or mining, discharge enormous quantities of aqueous effluents with relatively high concentrations of heavy metals into the environment. This poses a significant threat to ecosystems and public health. Physical and chemical methods for the removal of metal ions from aqueous effluents have been proposed and applied but these methods are often commercially impractical, either because of high operating costs or the generation of toxic solid wastes (Gavrilescu 2004). A promising environmentally friendly approach for the removal or recovery of metal contaminants from aqueous wastes is biosorption (Abbas et al., 2014). Different living and non-living bio-materials, such as fungal- or bacterial biomass and agricultural waste, have been reported as suitable biosorbents (Kumar et al., 2014; Atiba-Oyewo et al., 2019). So far, various autotrophic microorganisms, including cyanobacteria, green- and brown algae, have also been used and investigated for the removal of metals from aqueous solutions (Romera et al., 2006; Mustapha and Halimoon 2015; Singh 2020). Biosorption of metals is a passive process, that involves the attachment of different elements to the surface of biomass. Various mechanisms have been reported for the binding of metals from aqueous solutions, such as chelation/complexation, surface precipitation or ion exchange (see **Figure 1**) (Volesky and Holan 1995; Li and Yu 2014; Salam 2019).

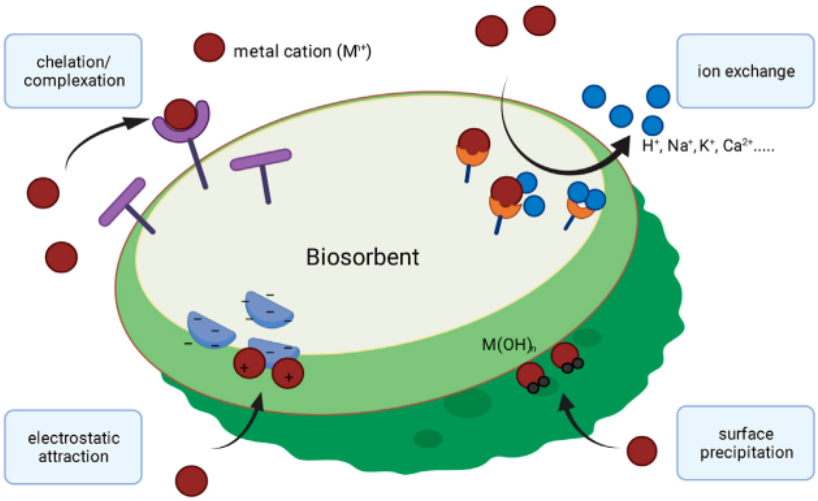

FIGURE 1 | Schematic overview of proposed mechanisms for biosorption of metal-ions.

Metal biosorption primarily depends on the components of the involved biomass, in particular the cell surface and the spatial structure of the cell wall (González et al., 2011). Several chemical groups on the surface of cells have been proposed to have an influence on the biosorption of metals, such as acetamido, amino, amido, hydroxyl, sulfhydryl, sulfate, phosphate and carboxyl-groups (Gardea-Torresdey et al., 1990; Schiewer and Volesky 2000; Lesmana et al., 2009; Heilmann et al., 2015). Variations in the presence of functional groups on the cell wall surface can be responsible for different biosorption mechanisms which, in turn, can influence the binding affinity or selectivity for certain elements (Gadd 2009). Extracellular polysaccharides (EPS) for instance are known to have a high binding affinity for metals (Liu et al., 2001; Yue et al., 2015). However, other structures like extracellular proteins can also contribute to metal-adsorption (Yang et al., 2020). In this context, metal-accumulation on the cell surface depends on various factors, such as number of functional groups, accessibility of binding sites or the chemical environment (Bilal et al., 2018). The interplay of metal-binding cell wall components and the mechanisms involved in the adsorption process are not yet fully understood. Interest in understanding these underlying mechanisms of biosorption has grown during the past years as the application of biomass can be an effective and sustainable method to recover and recycle valuable metals from industrial waste streams or remove pollutants from waste water (Vieira and Volesky 2000). In order to better understand the mechanisms involved in the adsorption of metals, this study focuses on chemical components in the cell wall of the cyanobacterium *Calothrix brevissima,* that are responsible for the metal-adsorption. *C. brevissima* has already been investigated in the 1950's as one of the nitrogen fixing phototrophic microorganisms from rice fields of Japan (Watanabe et al., 1951). It belongs to the genus *Calothrix* representing an ubiquitous blue green algae with a complex life cycle and a high variability among the known species. Their characteristic features are the formation of partially branched, filaments of vegetative cells ending in long trichomes as well as a durable sheat (Livingstone and Whitton 1983; Bohuslav 2007). It has been demonstrated on the example of *Calothrix parietina*, that this water insoluble sheath can be extracted and binds more than 0.7% w/w of heavy metal (Weckesser et al., 1979). It is therefore not surprising, that *C. brevissima* was listed as a good biosorbent of lanthanides with a capacity for approximately 0.5 mmol Nd g^{-1} biomass (Heilmann et al., 2015). In this study, we describe the isolation of soluble metal chelating constituents from *C. brevissima* that show interaction with Rare Earth Elements (REE) and other metals. The molecular structure of these compounds and their metal binding mechanism was further investigated by FT-IR spectroscopy and luminescence spectrometric methods. As the adsorption of metals takes place at the cell-surface it can be assumed, that the investigated structures are part of the cell wall. This study provides new insight in the underlying mechanisms for metal biosorption and is significant step towards the molecular understanding of this complex process.

MATERIALS AND METHODS

Cultivation and Harvesting of Biomass

The cyanobacterium *C. brevissima* (SAG Strain Number: 34.79) was maintained in BG-11 medium (Stanier et al., 1971) at 25°C in shaking flasks. Recurring recultivation was done after 4 weeks. The biomass of *C. brevissima* used throughout this experiments was obtained by cultivation in a 2 L glass CSTR photobioreactor (INFORS HT, Switzerland). The gasflow (1 VVM) has been finely dispersed through the sparger mounted below the stirrer turning at 500 rpm. Additional CO_2 (from 0.5 to 2% v/v adjusted daily) has been supplemented to keep the pH below 7.2 using a gas mixer. The medium used was BG-11 (Stanier et al., 1971), and the temperature was set to 25°C. After a cultivation period of one week, the biomass was harvested by filtration on a porcelain sieve

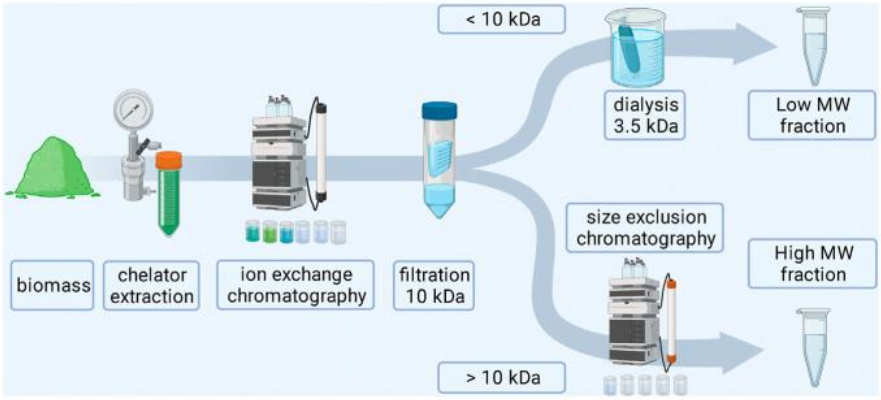

FIGURE 2 | Schematic workflow for the extraction of biomass derived chelators.

with 3 mm holes. Colonies adhering to the walls of the bioreactor were removed with a spatula. Subsequently the sample was washed with excess of deionized water to remove residues of growth medium, lyophilized and stored in a freezer at -20°C.

Extraction of Biomass Derived Chelators

The extraction began with cell disruption using a high pressure homogenizer (Avestin Emulsiflex B15, Aventis Europe, Germany) set to 2000 bar. Biomass (250 mg) suspended in 15 ml of deionized water (dH_2O) was passed through the device three times. The disrupted cells were incubated in a shaker at 50°C for 1 h to dissolve water soluble compounds. The undissolved residue was separated by centrifugation (Eppendorf 5810R centrifuge, Eppendorf, Germany) at 10,000 rcf for 10 min. Prior to the subsequent chromatographic run the supernatant was filtered with a 0.45 µm syringe filter (Filtropur S from Sarstedt Inc., USA) and a 0.2 µm membrane filter in a vacuum vial (Whatman, GE Healthcare, USA). Aliquots of 5 ml extract were injected into the system (NGC Chromatography System, Bio-Rad) consisting of a 5 ml sample loop, two pumps, mixer, UV/Vis detector, conductivity detector and a fraction collector. The separation was performed on a glass column filled with 5 ml anion exchange resin Q-Sepharose fast flow (GE Healthcare, USA). Initially 12 ml of dH_2O were pumped in order to pass the sample through the column and allow negatively charged molecules to bind to the resin. Subsequently a gradient of NaCl (0–1 M) was run for 15 min and 1 M NaCl was run for another 15 min to ensure full regeneration of the resin. The flow rate of 0.6 ml min^{-1} was constant for all steps. The collected fractions of 2.5 ml were probed for Tb luminescence sensitization according to a previously published protocol (Jurkowski et al., 2020). Further purification of the extracts was initiated by using a 10 kDa centrifugal filter concentrator (Centriprep, Merck, Germany). Both fractions were kept for further analysis. The fraction that was below the cut off limit of the filter was desalted using a dialysis membrane with a molecular weight cut off at 3.5 kDa (Spectra/Pro, Spectrum Laboratories Inc., USA) against H_2O

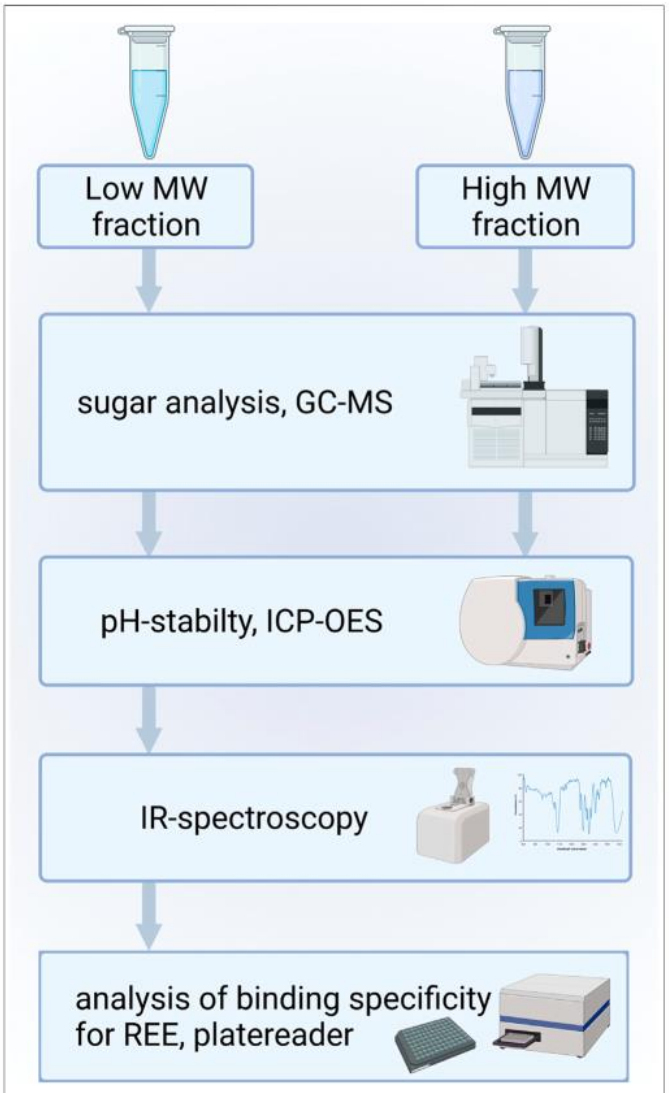

FIGURE 3 | Schematic workflow for the molecular characterization of isolated metal chelators.

until a conductivity of 0.01 mS cm^{-1} was obtained. The fraction that was retained by the 10 kDa filter was further purified using size exclusion chromatography with the same chromatographic system as before. For this purpose, a Tricorn Superdex 75 (GE Healthcare, USA) column was used. The flow rate was set at 0.5 ml min^{-1}. Fractions of 2.5 ml were collected and metal binding molecules were detected with the Tb sensitization method reported previously (Jurkowski et al., 2020). Thereafter, all samples were dried with a vacuum concentrator (HT-4 Atlas Evaporator) linked to a VC 3000 Vapour Condensator (both from GeneVac, United Kingdom) for storage and further analysis. A schematic overview for the isolation of biomass derived chelators is shown in **Figure 2**.

Molecular Characterization

The isolated biomass derived metal chelators were further characterized by analyzing their chemical composition and mode of interaction with metals. A schematic overview for the isolation of biomass derived chelators is shown in **Figure 3**.

Sugar Analysis

As cell wall components in cyanobacteria are composed of chemically complex polymeric carbohydrates, the monomeric sugar composition of extracted Tb chelator samples were additionally analyzed with a GC-MS based method. For the analysis of sugar monomers each Tb interacting sample has been hydrolyzed with 1% H_2SO_4 in an autoclave for 1 h at 121°C at 1 bar in order to release monomeric carbohydrate building blocks from the sample constituting polymeric carbohydrates. Each sample was then centrifuged for 10 min at 10,000 rcf. Following hydrolysis, the solution has been neutralized with calcium carbonate to a pH of 7. Precipitated calcium salt has been removed by centrifugation (30 min, 10,000 rcf) immediately after the neutralization. The supernatant was frozen at −40 °C for 48 h. After the sample was heated to 5°C and then the sample was centrifuged (30 min, 10,000 rcf) to remove any residual precipitate. The first step after centrifugation was to obtain an estimate of possible carbohydrate building blocks that constitute each sample to facilitate the GC-MS evaluation. This was achieved by using a HPLC Agilent Infinity II LC 1260 system (Agilent technologies, Waldbronn, Germany), equipped with an autosampler, quaternary pump, column oven, DAD and a Shodex RI detector (Showa Denko Europe GmbH, Munich, Germany). Prior to injection each sample was filtered with Modified PES 500 µL Centrifugal Filters (VWR, Ismaning, Germany) with a cut-off of 10 kDa. In a subsequent step, the monomeric sugar mixture resulting from chemical hydrolysis was analyzed using *via* HPLC. A Rezex ROA-Organic Acid H+ (8%) ion-exclusion column (300 mm, 7.8 mm internal diameter; Phenomenex LTD, Aschaffenburg, Germany) was used for the isocratic separation with 5 mM sulfuric acid at a flow rate of 0.5 ml/min at 70°C. After HPLC data evaluation, the remaining sample material was processed with a trimethylsilyl (TMS) derivatization for subsequent GC-MS based identification of the carbohydrate constituents. For this, a modified version of a previously described protocol was used (Gorner et al., 2016). 50 µL of pyridine were added to each sample. Thereafter, 50 µL of MSTFA (N-Methyl-N-(trimethylsilyl)trifluoroacetamide) with 1% TCMS (Trimethylchlorosilane) was added and the samples were incubated in a water bath at 50°C for 1 h. GC-MS analysis was carried out using a modified version of a previously described protocol (Ringel et al., 2020). The samples were analyzed using a Trace GC-MS Ultra system with DSQII (Thermo Scientific, USA). One microliter (1/10 split ratio) of the respective sample was injected by a TriPlus auto sampler onto a SGE BPX5 column (30 m, I.D. 0.25 mm, film 0.25 µm) with an injector temperature of 280°C. Helium gas was used as carrier with a flow rate of 0.8 ml min^{-1}. The initial oven temperature was set to 70°C for 2 min. The temperature was subsequently ramped to 290°C with a rate of 5°C min^{-1} and then held for 4 min. MS data was recorded at 70 eV (EI). Masses were recorded in positive mode in a range between 50 and 650 m/z.

pH-Stability of Chelates

In preparation of these measurements various lanthanide metals (Ce^{3+}, Tb^{3+}, Eu^{3+}) were incubated with the biomass derived chelator samples in order to identify the metal-chelator interaction, which provided the best spectrophotometric signal to noise ratio. In that regard the interaction of all biomass derived chelators with Eu^{3+} was superior to all other combinations. To evaluate the pH stability of the resulting biomass derived lanthanide chelates, Eu^{3+}-solutions were mixed with buffer solutions at pH values between 5.9 and 9.5.5 µL of a 10 mM europium(III)nitrate-solution were added to 50 µL of each fraction. The samples were filled up to 1 ml with 10 mM PIPES (1,4-piperazinediethanesulfonic acid) buffer set to pH-values of 5.9, 6.7, 7.5 and 8.0 as well as a 10 mM CAPS (N-cyclohexyl-3-aminopropanesulfonic acid) buffer at pH 9.5. To remove precipitated europium, all samples were centrifuged for 20 min at 10,000 rcf. For the determination of the remaining europium concentration 500 µL of supernatant were used for measurement *via* ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) (Agilent 725 Series ICP Optical Emission Spectrometer, Agilent Technologies Inc., USA).

IR-Spectroscopy

Attenuated total reflection infra-red (ATIR) spectra were obtained from each dry sample to determine the functional groups in the absence of rare earth metal binding (Coates 2000). In a similar experiment, samples were incubated with 1 µmol terbium(III)nitrate and again measured *via* ATIR to identify changes in the spectral properties of respective functional groups. Specifically, samples were mixed with the metal solution by iterative pipetting steps and subsequently dried in the vacuum concentrator. Finally, spectra were recorded with an FT-IR spectrometer equipped with a diamond ZnSe UATR (Universal Attenuated Total Reflectance) polarization accessory (Frontier, PerkinElmer, USA).

Binding Capacity

In order to determine the maximum binding capacity on the example of Tb, a derivation of the previously developed luminescence excitation method (Jurkowski et al., 2020) was

applied. When using the biomass specific excitation wavelength, unbound Tb-ions exhibits negligible luminescence at sub-millimolar concentrations in comparison to Tb chelates with the sample. Therefore, any increase of measured Tb emission intensity can only be attributed to more Tb-ions bound to the biomass (concentration dependent quenching can be excluded in highly diluted solutions). 50 µL of the isolated REE-interacting fraction at a gravimetrically determined concentration of 4.0 mg/ml were transferred into a quartz glass 96 multiwell plate (Hellma Analytics, Müllheim, Germany) and spiked with 5–20 µL of a 10 mM terbium(III) nitrate solution. The sensitized luminescence of Tb was measured for every sample at an excitation wavelength of 230 nm and an emission wavelength of 544 nm using a EnSpire multiplate reader (PerkinElmer, USA).

Binding Specificity

50 µL of each fraction was subsequently spiked with 6 µL of a terbium(III)nitrate solution with a concentration of 10 mM to reach a final concentration of 0.2 mM, giving a saturated sample. Different metal-solutions were added for final concentrations of 0.1, 0.2, 0.7 and 2.0 mM. The pH-value of all metal-solutions was prior set to 5 ± 0.2. Each well was filled to a final volume of 300 µL with demineralized water. As potential metal compounds that could interfere with the binding of terbium, sodium (NaCl), magnesium ($MgCl_2$), potassium (KCl), calcium ($CaCl_2$), cobalt ($Co(NO_3)_2$), lead ($Pb(NO_3)_2$) and lanthanum ($La(NO_3)_3$) were tested. Metal salts were bought from Carl Roth GmbH & Co. KG, Karlsruhe, with a purity ≥99%. All samples were transferred into a quartz glass 96 multiwell plate (Hellma Analytics, Müllheim, Germany) and excited with a wavelength of 230 nm and the emission spectrum from 460 to 570 nm was measured using an EnSpire multiplate reader (PerkinElmer, USA). Displacement of Tb-ions by other metal ions was observed by comparing the emission signal intensities at 544 nm.

RESULTS AND DISCUSSION

Cultivation and Extraction Method

During cultivation, *C. brevissima* has formed macroscopic colonies encapsulated in a remarkably stable biofilm, which adhered to the glass walls of the reactor. This fact has simplified harvesting as well as dewatering thus reducing the cost of necessary equipment and energy which makes it a suitable industrial strain. Dried samples were mixed with water and homogenized under high pressure. The lysed cells resulted in a faint yellowish coloration of the supernatant. Insoluble fractions remained as a pellet after centrifugation. After the removal of supernatant, the pellets have been resuspended in deionized water and incubated in a shaker to allow the weakly soluble compounds to dissolve. While this procedure has been repeated three times, each supernatant was tested for its potential to sensitize Tb luminescence. There was no difference between the first and second wash of the pellet, while the third and fourth repeat yielded decreasing amounts of active molecules. It can be therefore concluded, that about 60 ml of water are necessary to dissolve most of the chelators contained in 250 mg of biomass.

The use of buffers has been investigated by performing the procedure with sodium acetate (0.1 M) and TRIS-HCl (0.1 M), both at pH seven instead of deionized water. No difference in solubility could be detected. Therefore, the final protocol included deionized water only. The chelators could be separated from the bulk of other soluble compounds using anion exchange chromatography and were present in fractions 9 to 12 (see **Figure 4**), which were pooled for further processing. Already this step has yielded a good purity judging by the low UV absorption as compared to the first three fractions which did not interact with Q-Sepharose and were eluted immediately. After the centrifugal ultrafiltration significant precipitation has been observed in the fraction containing lanthanide chelator compounds indicating, that saturation has been reached as a consequence of volume reduction. By transferring this sample to a larger volume of deionized water most of the molecules could be resolubilized. Nevertheless, centrifugation prior to size exclusion chromatography produced a small pellet containing the metal binding molecules still attached to bulkier, insoluble cell fragments. Ensuring that only soluble molecules were injected was not only a measure against column clogging, but more importantly a route to exclude carry-over of non-metal binding compounds prior to characterization. As shown in **Figure 5** a high and a low molecular weight fraction that showed interaction with Tb could be separated. The purified sample (fraction 1-2) shows a much higher sensitization effect when compared to fractions collected after anion exchange. Due to the harsh purification conditions it is feasible, that the compounds with a lower molecular weight (faction 6-7) consist of severed building blocks of the larger compound.

Molecular Characterization

Sugar Analysis

A chemical hydrolysis with 1% H_2SO_4 and GC-MS analysis confirmed that both compounds have a similar chemical composition. The evaluation of GC-MS data showed that both the high molecular weight and <10 kDa fraction of the biomass derived lanthanide chelators are composed of arabinose, xylose, mannose, galactose and glucose respectively (see **Figure 6**). Rhamnose and fructose were not detected. This further supports the assumption that the chelators in the <10 kDa fraction originate from the larger isolated compound.

pH-Stability of Chelates

Most heavy metal ions, including lanthanides form insoluble hydroxides and carbonates, which precipitate from the solution when the pH rises above a certain threshold (Plancque et al., 2003). They can be however kept in the solution when a stable chelate is formed. Thus measuring the chelate dissociation as a function of rising pH provides an empirical guideline for the applicable pH range of the chelator. In our experimental setup Eu^{3+} was chosen as a model lanthanide due to the lowest detection limit in optical emission spectroscopy ensuring highest possible resolution. The Eu-concentration was expected to drop significantly above a pH of 6.7. This was confirmed in a negative-control test in which Eu^{3+}, solubilized in purified water, was mixed with the different buffer solutions.

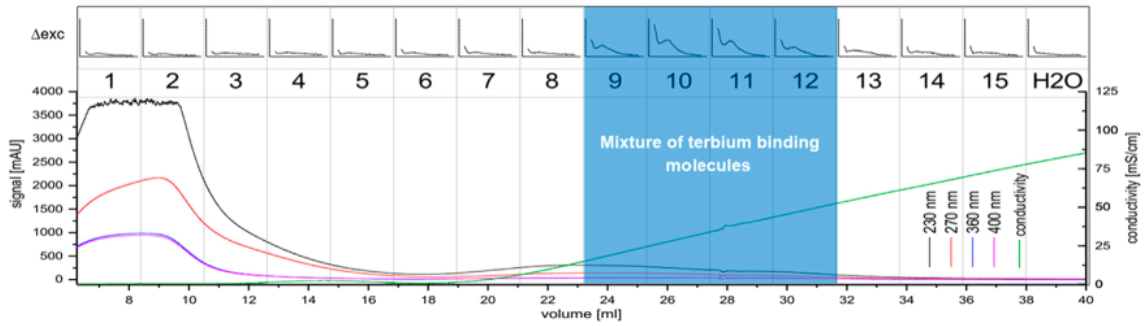

FIGURE 4 | Anion exchange chromatography of the *C. brevissima* biomass extract; The UV-signal in mAU is plotted against the elution volume from the wavelengths 230 nm (black), 270 nm (red), 360 nm (blue) and 400 nm (pink). The conductivity is displayed in green. 2.5 ml fractions were collected (numbered 1-15). Each fraction was tested for interaction with Tb *via* luminescence spectrometry. The ΔRFU-values (before and after addition of Tb) measured at 545 nm (200–5000, if not noted otherwise) are plotted against the excitation wavelength (230–402 nm). Also, the H_2O-background measurement is displayed. Fractions 9-12 were pooled.

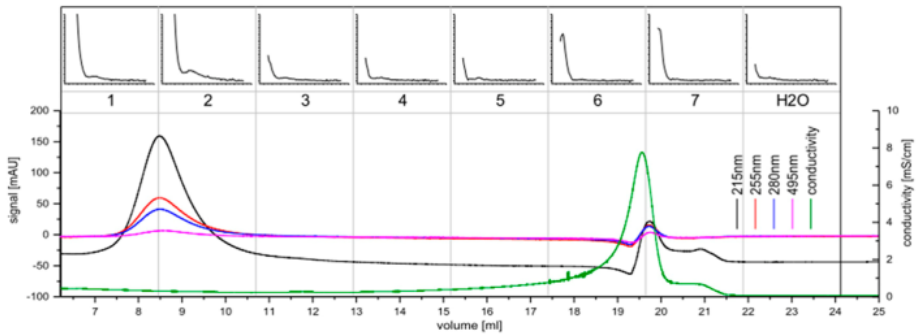

FIGURE 5 | Size exclusion chromatography of the concentrate of *C. brevissima*; The UV-signal in mAU is plotted against the elution volume for the wavelengths 215 nm (black), 255 nm (red), 280 nm (blue) and 495 nm (pink). The conductivity is displayed in green. 2.5 ml fractions were collected (numbered 1-7). Each fraction was measured via luminescence spectrometry. The ΔRFU-values (before and after addition of Tb) measured at 545 nm (200–5000, if not noted otherwise) are plotted against the excitation wavelength (230–402 nm). Also, the H_2O-background measurement is displayed. Fractions one and two were pooled for further analysis.

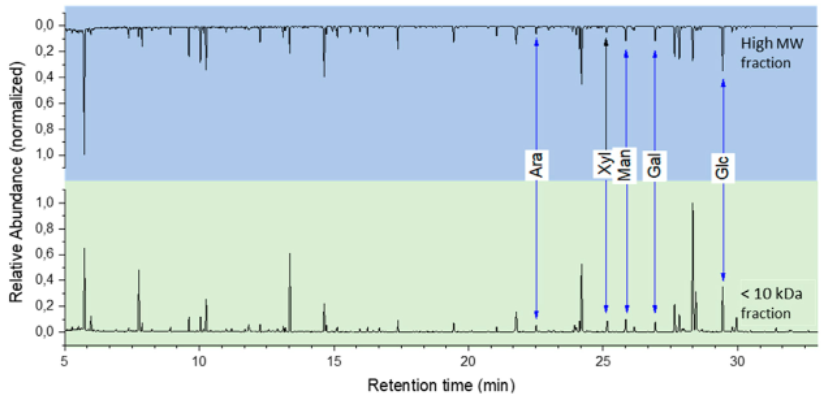

FIGURE 6 | GC-MS sugar analysis of the isolated fractions.

Here, about 95% (w/v) of the originally dissolved metal ions have precipitated at a pH between 7.5 and 8.0. In contrast to this observation, over 60% (w/v) of Eu^{3+} -ions remained in solution up to a pH of 9.5 (see **Figure 7**) when the isolated fractions were added. This indicates that the chelates are stable even at high pH values. Moreover, the applied Eu^{3+} concentration was above the

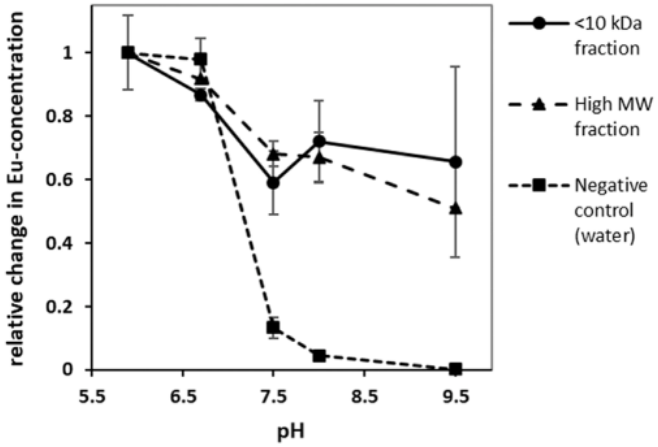

FIGURE 7 | Change in europium-concentration at different pH-values; without the addition of REE-interacting chelators europium precipitated at high pH values leading to a decrease in Eu-concentration. In presence of REE-interacting chelators however a significant proportion of europium remained in solution. (error bars indicate $\pm\sigma$, $n = 3$).

determined binding capacity of approx. 0.13–0.25 µmol mg^{-1} for Tb for both fractions and free metal ions were present in the solution. Those would precipitate as quickly as in the negative control between pH of 6.5 and 7.5 thus explaining the steep fall of the curve in this region.

FT-IR Spectroscopy

FT-IR spectroscopy is a useful tool for the qualitative measurement of organic functional groups. In this study, IR spectroscopy was used to identify and characterize functional groups in the isolated <10 kDa fraction that interact with Tb. The IR-spectra obtained from this fraction showed many similarities with other polysaccharide IR-spectra (Qian et al., 2009). The strong broad band in the region of 3348 and 3373 cm^{-1} in the IR-spectrum can be assigned to the stretching vibrations of hydroxyl groups (Qian et al., 2018), whereas the absorption bands at about 2932 and 2933 cm^{-1} can be related to the C-H stretching vibrations of CH_2 groups (Bhattacharya et al., 2014). The signals at 1595 and 1598 cm^{-1} could be antisymmetric stretching peaks of carboxylate ions (He 2015). Literature data (Kacurakova et al., 2000) suggests, that the signals around 1076 and 1047 cm^{-1} as well as a characteristic peak close to 1000 cm^{-1} may be assigned to ß-linked arabinans and ß-galactans respectively. The absorbance at 859 and 853 cm^{-1} could be caused by the presence of α-type glycosidic linkages (Ruperez and Leal 1987). As can be seen in **Figure 8**, the most pronounced change of the spectrum upon metal binding appeared at 1409 and 1365 cm^{-1}. This shift could be assigned to asymmetric stretching vibrations of organosulfate groups (Coates 2000). The coordination of these sulfate groups with Tb^{3+}-ions might change their IR-signals towards resembling sulfonates which can typically be found between 1365 and 1340 cm^{-1} (Coates 2000). Sulfated sugars in polysaccharide chains that are part of the cell wall have been reported for various cyanobacteria in the past (Hayashi et al., 1996; Ngatu et al., 2012; El-Baky et al., 2014).

Besides this indication for Tb-interaction, the weak signal shift of the hydroxyl stretching vibration from at 3348 cm^{-1} to 3373 cm^{-1} could indicate that these functional groups are also involved in the binding mechanism to a small extent.

Binding Capacity and Specificity

The binding of terbium of the isolated chelators was analyzed *via* luminescence spectroscopy. Due to their weak molar absorptivity lanthanides have low luminescence quantum yield. However, the quantum yields can be highly enhanced by chelating with suitable organic ligands that transfer energy intramolecularly to the emitting lanthanide ion (sensitization) (Al-Kindy et al., 2019) or provide shielding from solvent-induced non-radiative relaxation (Bunzli and Piguet 2005). The isolated chelators from *C. brevissima* biomass form a complex with Tb^{3+}-ions, which is more sensitive to excitation at a wavelength of 230 nm resulting in a higher emission intensity. Typically, Tb exhibits a strong emission signal at a wavelength of ca. 544 nm (Li and Korshin 2002). The replacement of Tb-ions by other metal ions results in a decreased signal intensity at this wavelength because of the reduced amount of luminescent Tb-complexes. In our experimental setup with a low Tb-concentration of 0.2 mM only the sensitized ions emit a detectable signal. Consecutive addition of Tb to the isolated metal chelators leads to an increased signal intensity until all available binding sites are saturated. Thus, the binding capacity for Tb of the compounds in the <10 kDa fraction could be roughly estimated to be between 20–40 mg/g. In order to analyze the binding specificity for terbium different metal solutions were added to samples in which all binding sites were saturated with Tb. A decrease in signal intensity at 544 nm indicates a replacement of Tb in the formed complexed by these added metals. **Figure 9** shows changes emission spectra of 0.2 mM Tb-solutions after the addition of different metal solutions with concentrations between 0.1 and 2.0 mM. In the conducted experiments the addition of alkali and alkaline earth metals (Na, K, Mg, Ca) did not result in a decreased signal intensity at 544 nm, indicating that these elements do not interfere with complex formation. Increasing the concentration of Co, La and Pb however reduced the intensity of the characteristic emission band for Tb. These elements seem to compete with Tb during complex formation. A *t*-test analysis comparing the

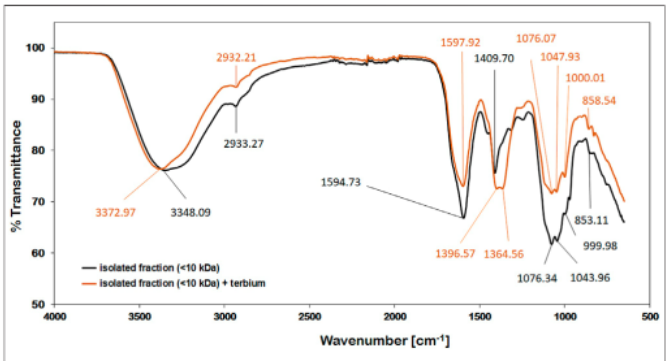

FIGURE 8 | IR-spectra of the isolated (<10 kDa) compounds; before (black) and after adding 1 µmol of Tb^{3+} (orange).

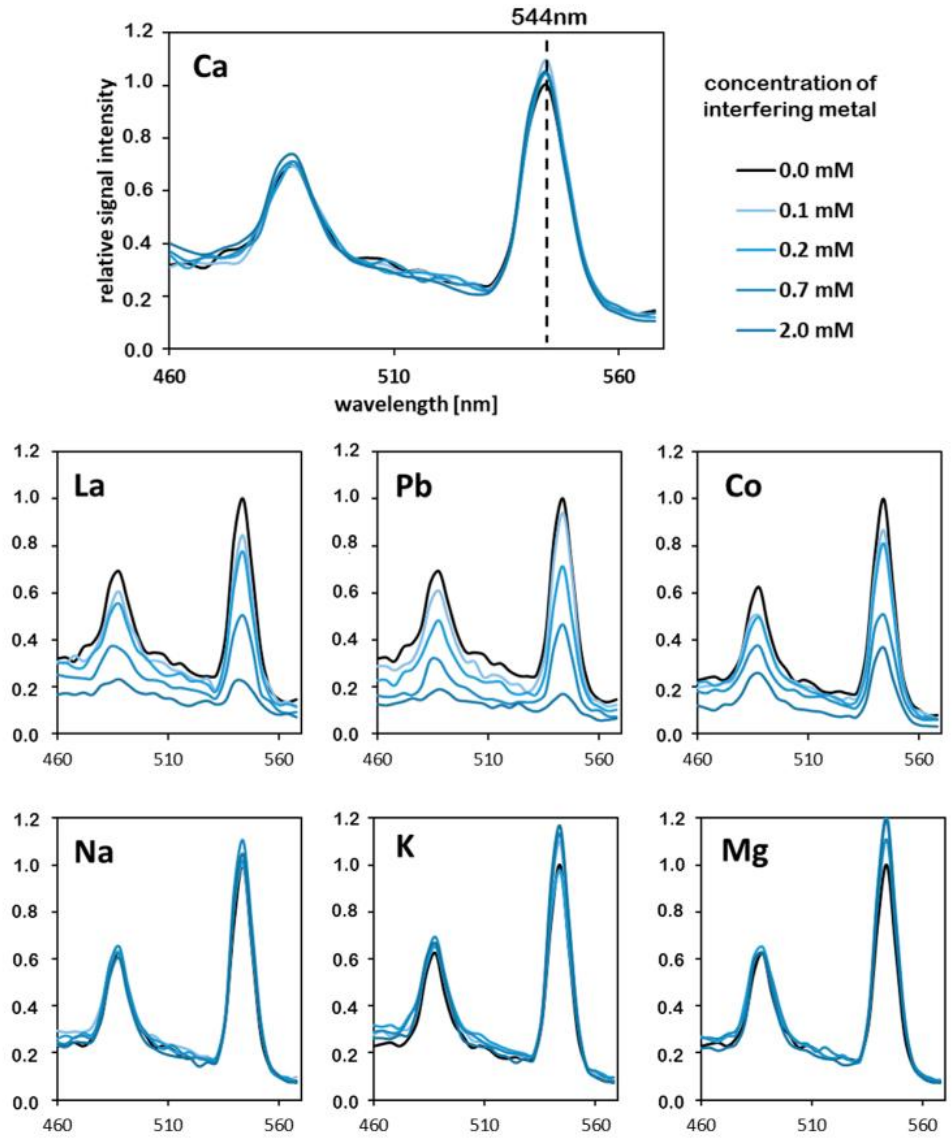

FIGURE 9 | Competition of different metals for complex formation with the isolated chelators (<10 kDa), tracked by analyzing the emission intensity of terbium; Tb concentration in all samples is 0.2 mM. Interfering metals were added in concentrations between 0.1 and 2.0 mM, excitation wavelength was set to 230 nm.

changes in emission intensity at 544 nm indicated a significant difference ($p < 0.05$) between the non-interfering metals (Na, K, Mg, Ca) and the metals Co, La and Pb at all tested concentrations between 0.1 and 2.0 mM.

CONCLUSION AND OUTLOOK

Biosorption is a promissing eco-friendly method for wastewater treatment and metal recovery. Yet, most research focused on the laboratory scale. Low mechanical resistance and stability of biosorbents remain challenging for industrial applications. Nevertheless, biosorption has many advantages, for instance, easy recuperation of adsorbed material, high efficiency at low metal concentrations and cost efficient biomass production (González et al., 2017). Fungal and bacterial biomass as well as agricultural waste have been investigated as promising biosorbent materials (Abbas et al., 2014; Atiba-Oyewo et al., 2019; Gallardo et al., 2020). Biomass derived from algae and cyanobacteria is of special interest for the development of biosorbtion materials because of the presence of many functional cellular compounds that promote metal adsorption, which lead to high adsorption capacities (Kanchana et al., 2014). In this study, negatively charged polysaccharide-based cell wall components derived from biomass of the cyanobacterium *C. brevissima* were sequentially purified *via* chromatographic methods and

subsequently characterized by differential spectroscopic methods. Interestingly, these cell wall polysaccharides showed a strong interaction with lanthanides particularly with Tb. These saccharide-based compounds were separated in a high molecular weight and a low molecular weight fraction. The former presumably is a subunit of the larger compound as analysis indicated that both the small and the large components are made up of identical monomeric sugars. Molecular characterization of the small subunit showed the metal-chelating compounds are sulfated polysaccharides. These sulfate groups could be the major contributing factor in the complexation of metal-ions. Interaction between the isolated polysaccharides and REE was demonstrated to be present over a broad neutral to alkaline pH-range (pH 6-9). Interestingly, competitive binding experiments revealed that alkali and alkaline earth metals do not compete with REE in formed complexes, whereas the metals Co and Pb can replace them at high concentrations. Previous studies by Crist et al. suggested that Ca or Mg ions can be replaced by other elements during biosorption as part of an ion-exchange mechanism (Crist et al., 1992; Crist et al., 1994). This was confirmed in a study by Sulaymon et al., in 2013 (Sulaymon et al., 2013). In this context, the binding of competing metals can depend on the functional groups of the biosorbent and ionic properties of the metals, such as electronegativity, ionic radius or redox potential (Naja et al., 2009). Further studies are required to fully understand this phenomenon, as in our experiments neither metal ion charge nor radius were decisive factors for REE binding selectivity to the functionalized polymeric sugar constituents of the *C. brevissima* cell wall. Further, fast kinetic studies should be carried out to investigate the mechanism of competitive binding in more detail.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Rare earths stick to rare cyanobacteria: Future potential for bioremediation and recovery of rare earth elements

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Rare earths stick to rare cyanobacteria: Future potential for bioremediation and recovery of rare earth elements

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Biosorption of metal ions by phototrophic microorganisms is regarded as a sustainable and alternative method for bioremediation and metal recovery. In this study, 12 cyanobacterial strains, including 7 terrestrial and 5 aquatic cyanobacteria, covering a broad phylogenetic diversity were investigated for their potential application in the enrichment of rare earth elements through biosorption. A screening for the maximum adsorption capacity of cerium, neodymium, terbium, and lanthanum was conducted in which *Nostoc* sp. 20.02 showed the highest adsorption capacity with 84.2–91.5 mg g^{-1}. Additionally, *Synechococcus elongatus* UTEX 2973, *Calothrix brevissima* SAG 34.79, *Desmonostoc muscorum* 90.03, and *Komarekiella* sp. 89.12 were promising candidate strains, with maximum adsorption capacities of 69.5–83.4 mg g^{-1}, 68.6–83.5 mg g^{-1}, 44.7–70.6 mg g^{-1}, and 47.2–67.1 mg g^{-1} respectively. Experiments with cerium on adsorption properties of the five highest metal adsorbing strains displayed fast adsorption kinetics and a strong influence of the pH value on metal uptake, with an optimum at pH 5 to 6. Studies on binding specificity with mixed-metal solutions strongly indicated an ion-exchange mechanism in which Na^+, K^+, Mg^{2+}, and Ca^{2+} ions are replaced by other metal cations during the biosorption process. Depending on the cyanobacterial strain, FT-IR analysis indicated the involvement different functional groups like hydroxyl and carboxyl groups during the adsorption process. Overall, the application of cyanobacteria as biosorbent in bioremediation and recovery of rare earth elements is a promising method for the development of an industrial process and has to be further optimized and adjusted regarding metal-containing wastewater and adsorption efficiency by cyanobacterial biomass.

KEYWORDS

cyanobacteria, biosorption, mechanism, rare earth elements, ion exchange

1 Introduction

Rare Earth Elements (REE) consist of scandium, yttrium, and 15 elements of the lanthanide series. These elements have exceptional electromagnetic, catalytic, and optical properties making them crucial for the production and development of modern high-technology products. Due to their similar chemical properties, separating REE demands sophisticated industrial processes that are energy-intensive and use environmentally toxic chemicals (Haque et al., 2014). Standard methods, for example, apply metal leaching with acids or bases and extraction methods to purify REE (Opare et al., 2021). Moreover, REE production is focused on a few countries, resulting in an oligopoly that can dictate supply and price regimes. REE are crucial for technology transition towards a renewable energy-driven society. For instance, cerium or lanthanum have applications in catalysts for air purification or chemical processing. Other metals like neodymium or terbium are crucial for producing permanent magnets or modern LEDs (Charalampides et al., 2015; Shan et al., 2020). Hence, industrialized countries increasingly focus on alternative supply routes and the development of cost and ecologically compatible recycling routes. In this context, REE recovered from dilute mining or industrial wastewater, as well as, electronic waste streams are opening new, regional supply routes. Establishing new biotechnologically based REE recovery methods therefore leads to enhanced market stability and supply chain independence for industrialized regions, such as the EU. Hence, there is a growing interest in the recovery and recycling of REE from industrial wastewater streams (Li et al., 2013; Barros et al., 2019). Over the past decades, biosorption has been regarded as a relatively simple and cost-efficient method for wastewater treatment (Volesky 2001). It is a physicochemical process that involves a solid phase (biosorbent) consisting of organic biomass and a liquid phase containing the dissolved or suspended chemical compounds to be sorbed (sorbate) (Fomina and Gadd 2014). Biosorption has a wide range of potential applications in wastewater remediation, including the removal of organic substances like dyes, pharmaceuticals, or pesticides (Bell and Tsezos 1987; Aksu 2005; Crini and Badot 2008; Menk et al., 2019). However, most research on biosorption in conjunction with the removal of pollutants has been conducted on metals, including heavy metals, actinides, and lanthanides (Dhankhar and Hooda 2011; Abbas et al., 2014; Giese 2020; Mattocks and Cotruvo 2020). Yet, developed processes based on biosorption have not achieved a commercial breakthrough. For example, it has been shown that environmental factors, such as changes in the pH value, can alter the affinity of biomass towards different elements (Zinicovscaia et al., 2019). A low technology readiness level, including a poor understanding of the underlying mechanisms, kinetics, and thermodynamics of the process are areas that require more research (Fomina and Gadd 2014; Elgarahy et al., 2021). It is widely accepted that the chemical structure, in particular the composition of functional groups on the cell surface, profoundly influences the adsorption properties of biomass (Eccles 1999; Volesky 2007). These active moieties may include hydroxyl-, carboxyl-, carbonyl-, phosphate-, sulfonate-, amine-, amide-, and imide-groups, among many others. Studies on biological, physical, or chemical modification of biomass by adding functional groups have shown that it is possible to improve binding specificity and capacity for target sorbates (Wang and Chen 2006; Park et al., 2010; Abdolali et al., 2015; Ciopec et al., 2020). Especially the recovery of REE with chemically modified organic polymers has been the focus of recent studies (Gabor et al., 2017; Negrea et al., 2018; Negrea et al., 2020). Nevertheless, these resulting biosorbents are still inferior in target selectivity to chemically synthesized ion-exchange resins with a defined structure and composition (Gadd 2009). Due to the heterogeneity of functional groups on the cell surface of microbial biomass, binding specificity for elements remains a challenging factor for industrial applicability. The adsorption of heavy metals by eukaryotic algae and cyanobacteria is well documented (Al-Amin et al., 2021; Ankit et al., 2022). At present, the screening of new species regarding biosorption and potential novel applications in metal recovery remains of great interest due to high variability in cell wall composition and structure, resulting in differences in adsorption properties [e.g. (Micheletti et al., 2008)]. Cyanobacteria have shown promising adsorption properties for heavy metals, which could be used in the sequestration of metals from water on a technical scale. If similar adsorption properties exist for the bioremediation of REE has not been studied extensively yet. Moreover, the adsorption properties of terrestrial cyanobacteria were seldom investigated. Therefore, we taxonomically and biotechnologically identified new and promising cyanobacterial strains and evaluated their properties for REE adsorption. In this context, we also aimed to correlate taxonomic identity and adsorption characteristics. In this study, 12 cyanobacterial strains with broad phylogenetic origin and inhabiting different ecological habitats such as terrestrial, freshwater, and saltwater habitats were investigated for their potential applicability in an adsorption process for the enrichment of REE. Their phylogenetic relationship was determined using 16S rRNA sequences. The screening for maximum adsorption capacity with four different REE (i.e. lanthanum, cerium neodymium, and terbium), as well as the effect of several parameters on biosorption, including initial pH value, incubation time, and metal concentration for cerium, were evaluated. Additionally, binding specificity for cerium in the presence of other metal cations was investigated.

2 Materials and methods

2.1 Cultivation of cyanobacteria

12 cyanobacterial strains with broad phylogenetic origins and from different habitats were used for the study (Table 1). The cultures were inoculated with approximately 0.1–0.3 g of wet biomass from a stock culture (stored at 17°C and light, dark rhythm 16:8 h at 30 µmol photons m^{-2} s^{-1}) in 1 L bubble columns containing BG11 cultivation medium (Stanier et al., 1971) (or Spirulina-medium for *Limnospira*) (Andersen 2005) and cultivated at 23°C and light, dark rhythm 16:8 h at 300 µmol photons m^{-2} s^{-1} photosynthetic photon flux density). All cultivated cells were harvested using filters (two sieves of 0.5 mm and 0.1 mm and finally paper filters of 40 µm openings), and wet biomass was dried by lyophilization. *Synechococcus elongatus* UTEX 2973 was cultivated in a 3.7 L Labfors 5 Photobioreactor (Infors GmbH, Sulzemoos, DE) in BG11 medium at 37°C and constant

TABLE 1 Overview of all investigated cyanobacteria strains in this study.

Strain	Strain number	Origin	Isolator, year	Order
Nostoc. (sp.)	20.02	Epiphytic on lichen *Peltigera* sp.; Germany	B. Büdel, 2000	Nostocales
Synechococcus elongatus	UTEX 2973	Freshwater, United States	J.Yu, 2011	Synechococcales
Desmonostoc muscorum	90.03, PCC 7906	Soil, United States	F. E. Allison, before 1930	Nostocales
Calothrix brevissima	SAG 34.79	Freshwater, Asia	Watanabe, before 1969	Nostocales
Komarekiella sp	89.12	Hypolithic on quartz, Namibia, South Africa	B. Büdel 1989	Nostocales
Limnospira maxima	SAG 49.88	Marine, Italy, Europe	Unknown, before 1988	Oscillatoriales
Limnospira platensis	SAG 85.79	Natron lake, Chad, Africa	G. Laporte, 1963	Oscillatoriales
Phormidium autumnale	97.20	small brook, polluted with sewage, Versasca Valley, Swiss, Europe	A. Zehnder, 1964	Oscillatoriales
Komarekiella sp	90.01	Chasmolithic in stone, South Africa	B. Büdel, 1990	Nostocales
Reptodigitus sp	92.01	Endophilic, South Africa	B. Büdel, 1992	Nostocales
Symphyonema bifilamentata	97.28	Soil, Switzerland	A. Zehnder, 1965	Nostocales
Scytonema hyalinum	02.01	Ephedaphic on arid soil, Namibia, Africa	S. Dojani, 2002	Nostocales

illumination at 300 µmol photons m^{-2} s^{-1}. During cultivation, the pH was kept at eight by adding CO_2 gas. Biomass was harvested by centrifugation after reaching the stationary phase.

2.2 Phylogenetic characterization of strains

About 50 mg of biomass from all cultures were collected during stationary growth phase and used for gDNA extraction with the DNeasy PowerSoil Pro Kit (Quiagen, Hildesheim, Germany) following the manufacturer's instructions. The 16S–23S ITS gene region was amplified by PCR in a 50 µL reaction using the primers Wil1 and Wil18 (Wilmotte et al., 1993) and ready-to-go PCR mini beads (GE Healthcare, Chicago, United Sates) in a MiniAmp Plus Thermal Cycler (Thermo Fisher Scientific, Waltham, United Sates). PCR products were checked by gel electrophoresis using 1% (w, v) agarose and the E-Gel Power Snap Electrophoresis System (Invitrogen, Waltham, United Sates). Subsequently, PCR products of the expected length were purified with NucleSpin Gel and PCR Clean-up Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the DNA and PCR cleanup protocol and sent for Sanger sequencing to Genewiz, Azenta (Germany GmbH, Leipzig, Germany) using the primers Wil1, Wil4, Wil5, Wil10, Wil11, Wil16, and Wil18 (Wilmotte et al., 1993). The generated sequences were assembled with Geneious Prime (v2021.0.1) software package (Biomatters Limited, New Zealand) and compared to already submitted sequences of those strains from public culture collections in terms of authenticity using the BLAST tool of the National Center for Biotechnology Information (NCBI) GenBank. Sequences of strains that are novel were submitted to GenBank, and their accession numbers are given in the phylogenetic tree. The assembled 16S rRNA gene sequences and related sequences of cyanobacterial strains cited from GenBank were used for phylogenetic analyses, including *Gloeobacter violacaeus* as outgroup for the 16S rRNA gene alignment, applying the Muscle algorithm in Mega X (Kumar et al., 2018). The evolutionary model that was best suited for the database used was selected based on the lowest Akaike information criterion value and calculated in Mega X which was the RGT G + I model of nucleotide substitutions. The maximum likelihood method (ML) with 1,000 bootstrap replications was calculated with Mega X and Bayesian inference (BI) phylogenetic analyses, with two runs of eight Markov chains executed for one million generations with default parameters with MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). Each analysis reached stationarity (average standard deviation of split frequencies between runs <0.01) before the end of the run.

2.3 Metal analysis

The metal concentration in the analyzed solutions was determined via ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) (Agilent 725 Series ICP Optical Emission Spectrometer, Agilent Technologies Inc., United Sates). A TraceCERT® Rare earth element mix for ICP with 16 elements from Sigma-Aldrich (Sigma-Aldrich, Taufkirchen, Germany) and a Certipur® ICP multi-element standard solution IV from Merck (Merck KGaA, Darmstadt, Germany) with 23 elements, were used as standards for calibration. Data analysis was done with ICP Expert II Agilent 725-ES Instrument Software Version 2.0 (Agilent Technologies Inc., United Sates).

2.4 Biosorption

Before the experiments, all biomass samples were washed three times with demineralized water to remove residual media components that could falsify the measurement results. The washed biomass was frozen at −80°C and lyophilized. Sorption experiments were carried out by incubating lyophilized biomass in metal solutions with a defined concentration. Each experiment was performed in triplicates. Metal uptake was determined by

comparing the metal concentrations before and after incubation. Prior to measuring the metal concentration, each sample was centrifuged at 10,000 rcf for 5 min at room temperature. The supernatant was subsequently used for analysis. The adsorption experiments in this study predominantly focused on cerium, as it is the most prevalent REE.

2.5 Adsorption capacity

Adsorption experiments were performed based on a methodology described in previous studies (Heilmann et al., 2015; Heilmann et al., 2021). To determine the metal adsorption capacity (Q) of the different strains, 10–20 mg of dry biomass of individual species were weighed into centrifuge tubes and incubated in 2 mL metal solutions for 3 h under constant shaking at room temperature. Subsequently, the adsorption of the metals to the biomass was determined by dividing the changes in metal concentration by the amount of incubated biomass (see Eq. 1).

$$Q = \frac{n_i - n_f}{m} = \frac{(c_i - c_f) \times V}{m} \quad (1)$$

with Q = adsorption capacity, n_i = initial amount of substance, c_f = final amount of substance after incubation, c_i = initial metal concentration, c_f = final metal concentration after incubation, V = volume, and m = weight of biomass.

For the determination of the maximal adsorption capacity during the screening, metal solutions with a concentration of 10 mM and an initial pH value of 5 ± 0.2 were used.

2.6 Adsorption kinetics

Experiments on adsorption kinetic were carried out by varying the incubation time of the biomass in cerium (III) nitrate solutions with a concentration of 10 mM and a pH-value of 5 ± 0.2. Samples for analysis were taken after an incubation time of 2 min, 5 min, 15 min, 30 min, and 60 min.

2.7 Effect of initial pH value

The influence of the pH value in metal biosorption was investigated similarly to the method previously described. The pH value of the applied metal solution was adjusted using hydrochloric acid and sodium hydroxide. Due to the formation of insoluble REE hydroxides at pH values above 7 (Plancque et al., 2003; Heilmann et al., 2021) the experiments were carried out ranging from pH 1 to 6.

2.8 Adsorption isotherms

Adsorption isotherms were studied by varying the metal concentration of solutions applied to the biomass samples between 0.5 and 10 mM. Samples were incubated for 1 h at room temperature under constant shaking and analyzed as previously stated. The adsorption isotherms were described using the Langmuir and Freundlich model (see Eqs. 2, 3). The Langmuir model is often used for the description of metal adsorption as it assumes adsorption in the form of a monolayer onto a surface containing a finite number of identical binding sites (Dada et al., 2012). By contrast, the Freundlich model assumes metal adsorption on non-identical bindings sites over a heterogeneous surface (Koong et al., 2013).

$$\text{Langmuir model: } Q_{eq} = Q_{max}\frac{K \times C_{eq}}{1 + K \times C_{eq}} \quad (2)$$

$$\text{Freundlich model: } Q_{eq} = K_f C_{eq}^{\ bf} \quad (3)$$

with Q_{eq} = adsorption capacity at equilibrium, Q_{max} = maximum adsorption capacity, K = Langmuir adsorption coefficient, K_f = Freundlich adsorption capacity constant, C_{eq} = metal concentration at equilibrium, and b_f = Freundlich isotherm constant.

Calculations for data analysis and model fitting were done using OriginPro 2020.

2.9 Adsorption specificity

Wastewater usually contains a mixture of different metal cations. In addition to examining the capacity for a single element of interest, it is therefore important to investigate whether some metal cations are adsorbed preferentially over others by the cyanobacterial biomass. Experiments were carried out with equimolar mixed-metal solutions with concentrations of 0.5–4 mM to investigate the binding specificity of the biomass for different metals. Following previous experiments on green algae and cyanobacteria by Klimmek (Klimmek 2003), the adsorption of cerium in the presence of aluminum, lead, nickel, and zinc was investigated. The uptake of metals by the biomass was measured using ICP-OES measurements, analogous to determining the adsorption capacity with single metal solutions.

2.10 FT-IR analysis

IR spectroscopy is a useful tool for the qualitative measurement of organic functional groups. In this study, IR spectroscopy was used to identify functional groups in cyanobacteria biomass samples and to detect possible interactions with metal cations. Samples were incubated in a cerium (III) nitrate solution (1 µmol 1 mg^{-1} biomass) for 2 h and subsequently lyophilized. IR spectra were recorded using a Nicolet iS50R FT-IR spectrometer from Thermo Fisher Scientific (Thermo Fisher Scientific, Waltham, United Sates) equipped with an iS50 ATR (Attenuated total reflection) multi-range, diamond sampling station. For each sample, IR spectra were obtained in a range from 400–4,000 cm^{-1}.

3 Results

3.1 Phylogenetic analysis

Twelve different cyanobacteria, including four strains from public culture collections and eight environmental isolates were investigated. The identity of all strains from public culture collections was confirmed

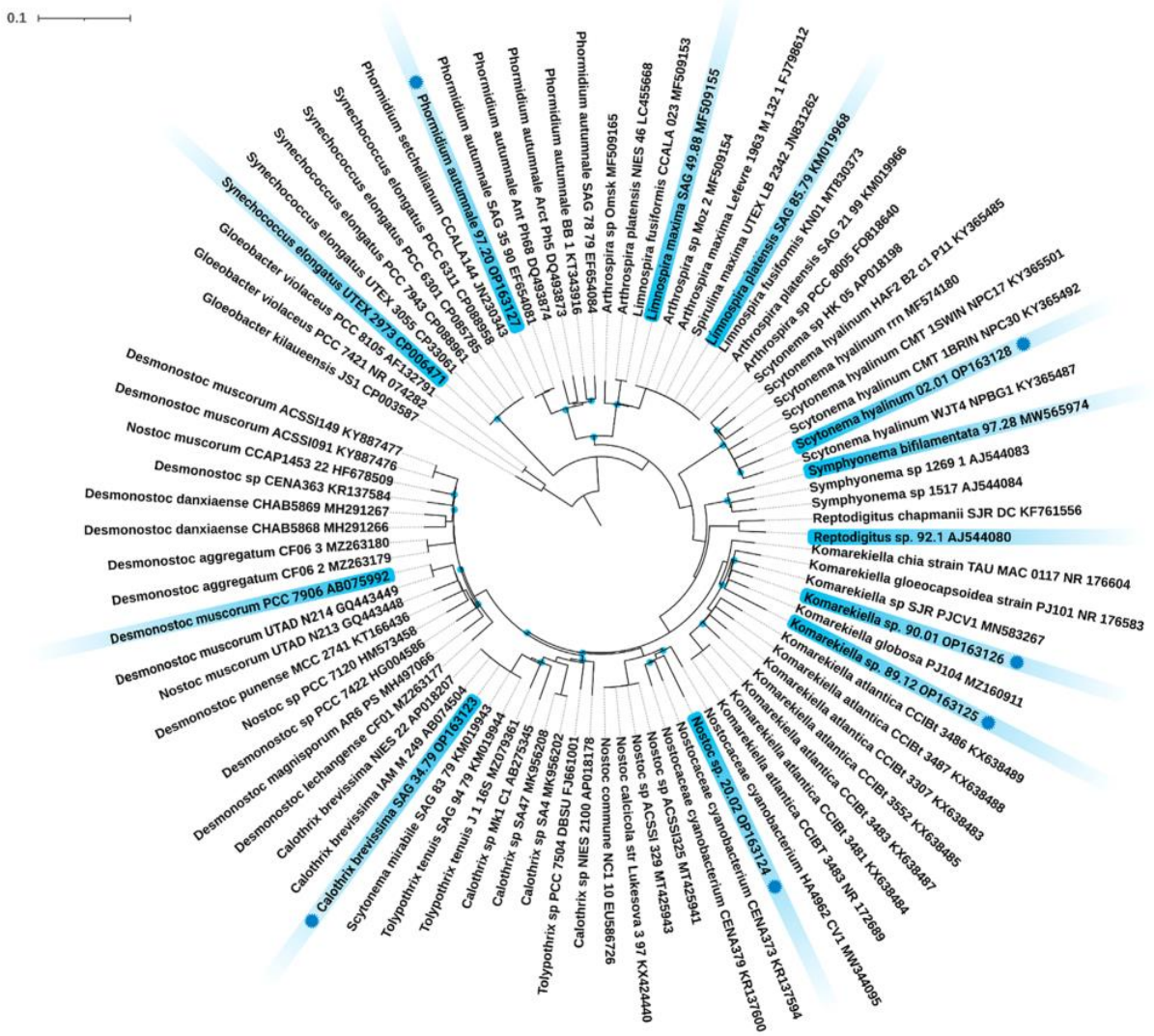

FIGURE 1
Maximum Likelihood (ML) phylogenetic tree based on the 16S rRNA gene region. Marked in blue are the twelve investigated strains including their strain number and NCBI accession number. Strains with a blue dot indicate novel 16S rRNA sequences generated during this study. Since the resulting Bayesian Inference (BI) and ML phylogenetic trees mostly showed the same topology, a single tree with both BI and ML bootstrap values is shown. Supports at the nodes greater than 90% statistical support from BI and ML represent posterior probabilities, and bootstrap values indicated as blue circles. The scale bar specifies 0.1 expected changes per site.

based on their 16S rRNA sequence using the BLAST tool of GenBank. The 16S rRNA sequences of the strains 97.20, 02.01, 90.01, 89.12, 20.02, and SAG 34.79 were originally recovered, and their phylogenetic position was analyzed (Figure 1). In detail, strains 90.01 and 89.12 clustered well supported within the filamentous, heterocyte-forming genus *Komarekiella* while strain 20.02 clustered within the genus *Nostoc sens. lat*. The strain *Calothrix brevissima* SAG 34.79 joined a cluster of other *C. brevissima* strains and strains assigned to the genera *Tolypothrix* and *Scytonema* with 100% identity. Strain 02.01 fell well supported in the large cluster of *Scytonema hyalinum,* whereas strain 97.20 could be assigned to *Phormidium autumnale* based on its high similarity with 16S rRNA sequences from other filamentous, non-heterocyte forming strains representing this species. Thus, the cyanobacterial strains represent a broad phylogenetic origin out of the three orders Synechococcales, Oscillatoriales and Nostocales, inhabiting different ecological habitats such as terrestrial, freshwater, and saltwater habitats and most are new for biotechnological applications, particularly for their adsorption process of REE.

3.2 Screening for maximum adsorption capacity

In this study, the maximum adsorption capacity for REE (cerium, neodymium, terbium, and lanthanum) of 12 different cyanobacteria was investigated. The results of this screening are shown in Figure 2,

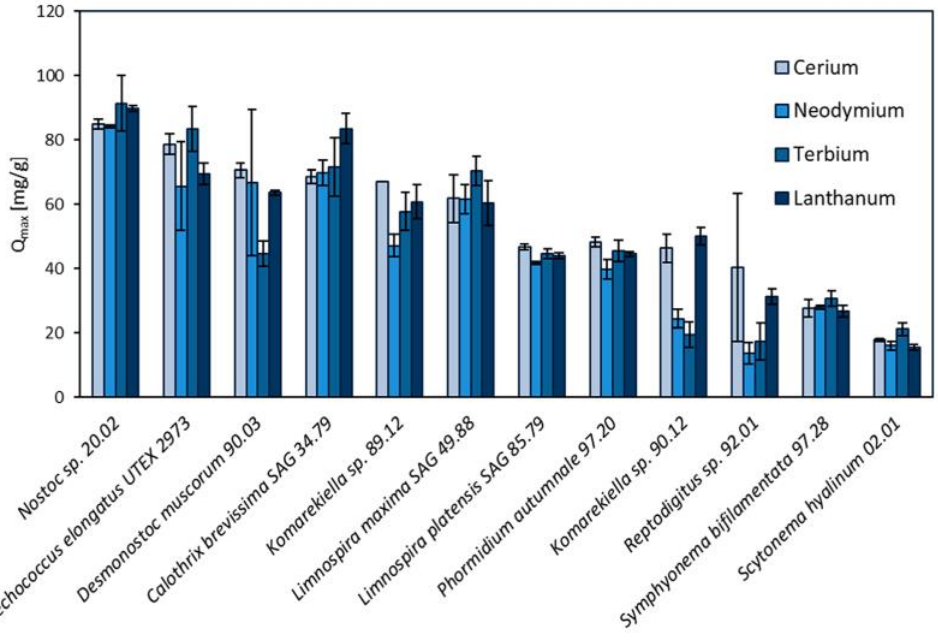

FIGURE 2
Screening of 12 different cyanobacteria for their maximum adsorption capacity (Q_{max}, mg REE g^{-1} dry mass) of cerium, neodymium, terbium, and lanthanum (pH: 5 ± 0.2, n = 3).

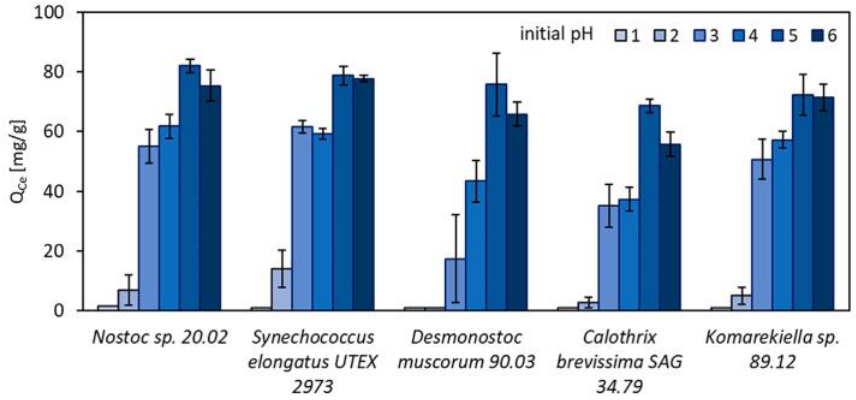

FIGURE 3
Adsorption capacity for Ce^{3+} (Q_{Ce}, mg Ce^{3+} g^{-1} dry mass) of different cyanobacteria in solutions with initial pH values between 1 and 6 (n = 3).

depicting distinct differences in total metal uptake depending on the species. There was no apparent correlation between the capacity for REE adsorption for the phylogenetic relationship and the ecological habitat. The highest overall metal uptake of the four tested REE was observed for *Nostoc* sp. 20.02 adsorption capacities between 84.2 and 91.5 mg g^{-1}, while *S. hyalinum* 02.01 exhibited the lowest maximum adsorption capacity with 15.5–21.2 mg g^{-1}. Based on these results, the biosorption properties of the five most efficient cyanobacteria (*Nostoc* sp. 20.02, *Synechococcus elongates* UTEX 2973, *Desmonostoc muscorum* 90.03, *C. brevissima* SAG 34.79, and *Komarekiella* sp. 89.12) were investigated in more detail.

3.3 Effect of different initial pH values on metal adsorption

The effect of the initial pH value of metal-solutions on biosorption of REE was examined in a pH range between 1 and 6. Experiments

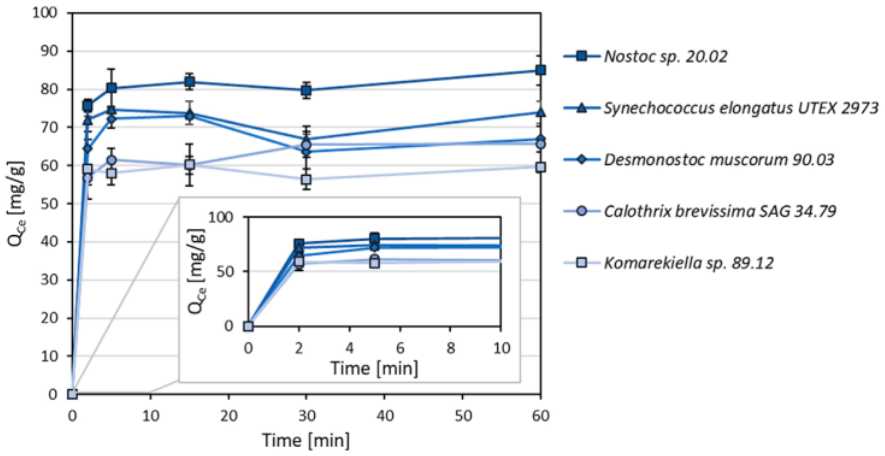

FIGURE 4
Adsorption kinetics of different cyanobacterial biomasses for Ce^{3+} (Q_{ce}, mg Ce^{3+} g^{-1} dry mass) with incubations times between 2–60 min (n = 3).

with 10 mM cerium (III) nitrate showed a strong influence of the pH value on metal adsorption (Figure 3). The highest metal uptake was observed at pH 5, with a minor decrease at pH 6. With increasing acidity, metal adsorption rapidly decreased. At pH 1, no notable metal adsorption was measured for all tested biomasses. These results are in accordance with previous studies on cyanobacteria, bacteria, and green algae regarding metal adsorption (Kuyucak and Volesky 1988; Gong et al., 2005; Lupea et al., 2012; Liang and Shen 2022).

3.4 Adsorption kinetics

As shown in Figure 4, metal adsorption for cerium (Ce^{3+}) to all tested cyanobacterial biomasses occurred rapidly. The adsorption capacity equilibrium was reached within an incubation time of 5 minutes. After this time, there was no significant change in adsorption capacity within 60 min.

3.5 Adsorption isotherms

For the intended application of cyanobacterial biomass for the removal of metals from wastewater, high sorption capacities at relatively low metal concentrations are beneficial. Sorption capacities for the biomass of five selected cyanobacteria species were investigated at concentrations between 0.5–10 mM. The resulting data points were fitted according to the Langmuir and Freundlich model. The best correlation was achieved using the Langmuir model (Figure 5). Although the overall correlation with the model was weak, maximum adsorption capacities predicted by the model are in accordance with the values determined during the screening experiments (Supplementary Tables S1, S2). Adsorption capacities for all tested cyanobacteria exhibited a steep increase at lower equilibrium metal concentrations, showing that sequestration of metals is possible even at low concentrations.

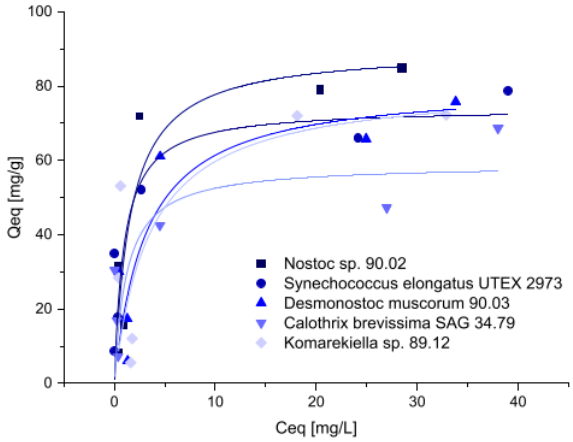

FIGURE 5
Isotherms for the adsorption of Ce^{3+} (adsorption capacity Q_{eq}, mg Ce^{3+} g^{-1} dry mass *versus* Ce^{3+}-concentration C_{eq}, mg L^{-1}) with biomass of five different Cyanobacteria, data points were fitted according to the Langmuir-model.

3.6 Binding specificity

The binding specificity of cyanobacterial biomass towards cerium was determined in adsorption experiments with other metals (Al, Pb, Ni, and Zn) in equimolar solutions. Starting with a concentration of 0.5 mM, the adsorption capacities were investigated for increasing metal concentrations up to 4 mM. The experiments showed that all elements could be adsorbed by the tested biomass. However, the metal uptake for some elements varied strongly amongst the tested metals (Figure 6). The adsorption capacity for zink and nickel was the lowest, whereas for cerium, the tested biomass showed the highest adsorption capacity in solution with a concentration of 0.5–2.0 mM. Nevertheless, the adsorption capacity for cerium in mixed metal solutions was

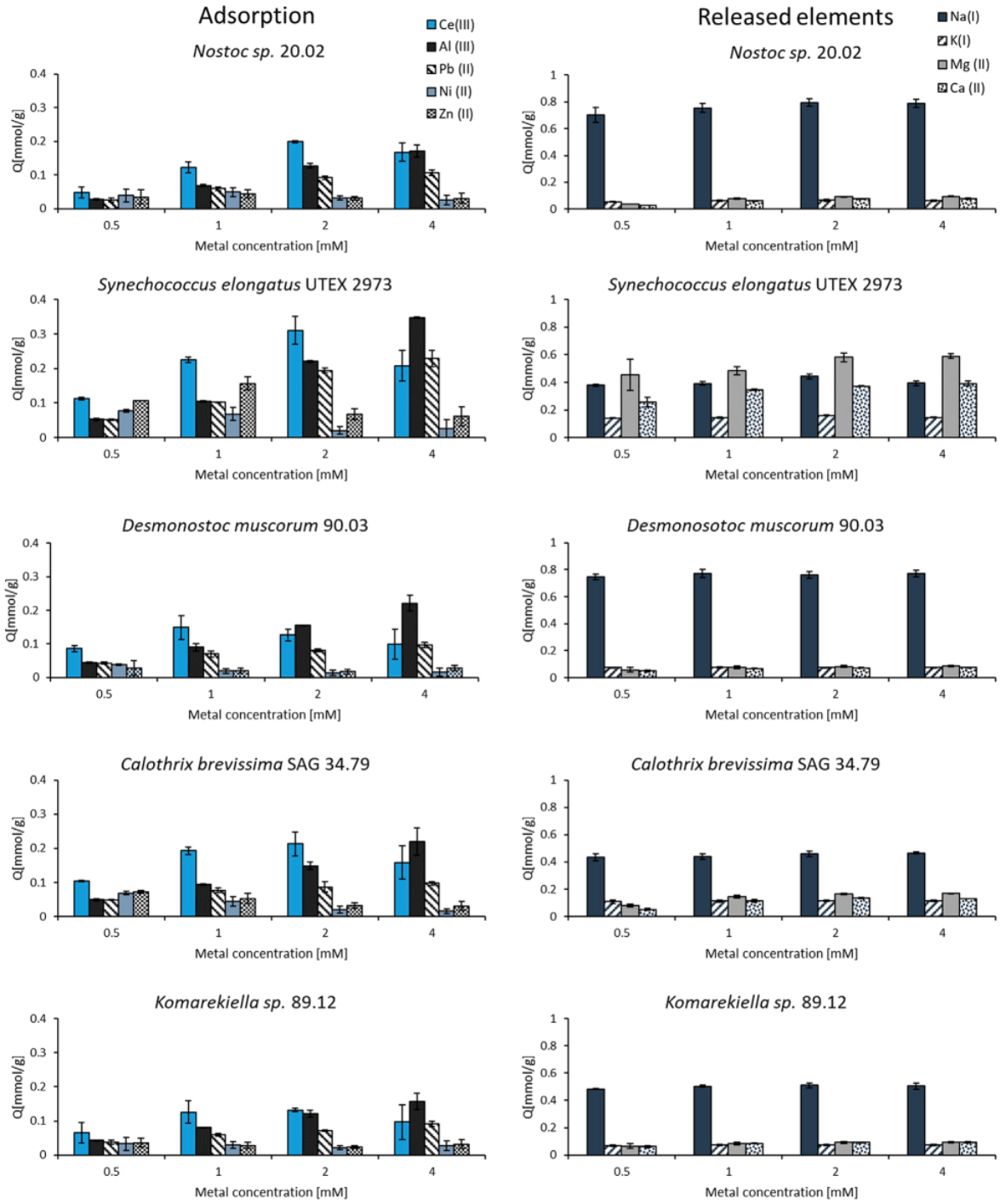

FIGURE 6
Adsorption and release of elements from biomass incubated in equimolar mixed-metal (Ce, Al, Pb, Ni, and Zn) solutions with concentrations between 0.5–4.0 mM (n = 3).

significantly lower compared to experiments with single-element solutions in previous experiments. This indicates a competition of different elements for the same, limited binding sites on the biomass. The metal uptake of aluminum and lead steadily increased with rising metal concentrations. At a metal concentration of 4 mM, these elements even seemed to replace cerium as the binding capacity for this element dropped for all tested cyanobacteria biomasses. The analysis of metal concentrations via ICP-OES revealed a release of

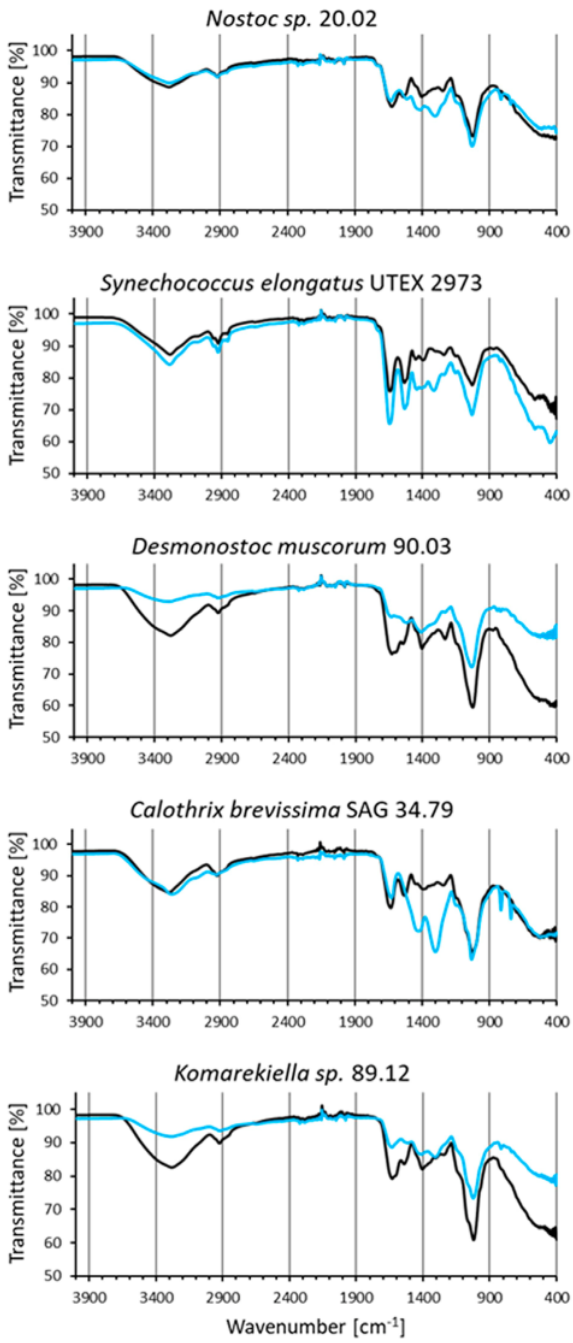

FIGURE 7
FT-IR spectra of biomass samples before (black) and after incubation in cerium (III) nitrate solution (1 µmol mg^{-1} dry mass) (blue).

alkaline and alkaline earth metals (Na, K, Mg, and Ca) during the adsorption process. The concentration of these elements increased after incubating the biomass in the equimolar metal solutions containing Ce, Al, Pb, Ni, and Zn. By contrast, mixing the biomass with pure demineralized water did not lead to a notable increase in the concentration of alkaline and alkaline earth metals. This indicates an ion-exchange mechanism in which positively charged metal ions bind to the biomass and replace other ions that exhibit a weaker interaction. For all tested cyanobacteria, Na^+ ions were the most prevalent ions being released. The biomass of *S. elongatus* was an exception, as Mg^{2+} and Ca^{2+} played a more important role in this case.

3.7 FT-IR analysis

IR spectra of all analyzed biomass samples displayed signals that can be assigned to different functional groups (Figure 7). The broad band in the region around 3,350 cm^{-1} in the spectra are linked to the stretching vibrations of hydroxyl groups (Qian et al., 2018), whereas the signal at 2,920 cm^{-1} can be related to the C-H stretching vibrations of CH_2 groups (Bhattacharya et al., 2014). Signals around 1,630 cm^{-1}, which can be assigned to C=O stretching vibrations, indicate the presence of carboxyl groups (Qian et al., 2009). The strong signals around 1,040 cm^{-1} can be assigned to C-O stretching vibration in polysaccharides (Nakamoto 2009). FT-IR spectra of biomasses after interaction with cerium (III) nitrate (Figure 7 blue lines) are characterized by changes in intensity and shifts in position of certain bands due to the interaction with the adsorbed metal ions. The first observed change was the attenuation of intensity in the region between 3,600–3,000 cm^{-1}, indicating a decrease of free hydroxyl groups in the biomass (Mitic-Stojanovic et al., 2011). This was most prominent in biomass samples from *D. muscorum* 90.03 and *Komarekiella sp.* 89.12. Likewise, changes in intensities around 1,630 cm^{-1} and 1,040 cm^{-1} indicate an interaction with carboxyl groups (Qian et al., 2009). These changes were more profound for *S. elongates* UTEX 2973, *D. muscorum* 90.03, and *Komarekiella sp.* 89.12. Distinct changes in signal intensities around 1,410 cm^{-1} and 1,290 cm^{-1}, which can be observed in samples of *Nostoc sp.* 20.02, *S. elongates* UTEX 2973, and *C. brevissima* SAG 34.79, might be linked to an interaction with aromatic C-C groups and C-O or C-N groups respectivly (Theivandran et al., 2015).

4 Discussion

4.1 Phylogenetic and taxonomical remarks

In the broad context of biotechnology, cyanobacterial strains are often used without respecting their ecological niche. This is a problem, because some taxa e.g. from aquatic habitats, often cannot be used during biotechnological processes that involve heat or desiccation, while others, such as terrestrial strains, are better candidates and *vice versa*. In addition, it happens quite often that results are not linked to strain identifiers or to wrongly identified taxa what can lead to an incorrect comparison and interpretation of data—a mistake that can remain uncorrected over decades (e.g., Jung et al., 2021b). For these reasons we respected the ecology of the strains used in this study and depicted the phylogenetic placement of the strains. This creates a transparent background for the cyanobacterial strains that we used and allows others to better compare their results. Besides publicly available cyanobacterial strains with a clarified identity, several new

isolates were phylogenetically analyzed during this work based on their 16S rRNA gene region (Figure 1). Among these were, for example, the heterocytous, false-branching strain *S. hyalinum* 02.01 that joined the large *S. hyalinum* cluster as outlined by Johansen et al., (Johansen et al., 2017). In addition, the two true-branching, heterocytous strains *Symphyonema bifilamentata* 97.28 and *Reptodigitus sp.* 92.1 were included in the study in order to complement the setup of heterocytous, branching cyanobacteria. The strain 97.28 was treated as *Fisherella ambigua* for the last 50 years of biotechnological research on secondary metabolites but was recently re-assigned as the type strain of the genus *Symphyonema* (Jung et al., 2021b). This strain has great biotechnological potential, because it grows fast and produces a diverse set of secondary metabolites, such as various ambigols (summarized in (Jung et al., 2021b)). The strain 92.1 was formerly treated as *Nostochopsis lobatus,* but doubts about this assignment arose because *N. lobatus* is only known from aquatic habitats. Recently, the new genus *Reptodigitus* was emerged, and the authors pointed out that strain 92.1 needs to be correctly described as a novel *Reptodigitus* species (Casamatta et al., 2020) which the authors of this study will carry out in a follow up study. In contrast to the above named strains, which are low producers of EPS (extracellular polymeric substances), the genus *Komarekiella* and related genera are well known to produce cells and filaments covered by thick EPS sheaths (Scotta Hentschke et al., 2017; Soares et al., 2021). EPS might play a role in metal adsorption (e.g. Al Amin et al., 2021). However, the two strains investigated here are the first strains of this genus described from a desert environment, while the other species of the genus have multiple origins, including lichen symbioses (Jung et al., 2021a; Soares et al., 2021; Panou and Gkelis 2022). All of them have a very complex life cycle in common that can hamper biotechnological applications due to different metabolic activity depending on the developmental stage of the culture. Also, the two strains 90.01 and 89.12 will be described as new species in the future. More challenging to interpret are the phylogenetic and taxonomical positions of *Nostoc* sp. 20.02 and *C. brevissima* SAG 34.79 (Figure 1). The strain 20.02 was isolated as an epiphyte on a cyanolichens and can be considered as a *Nostoc* strain not involved in the symbiosis because most true *Nostoc* lichen photobionts usually join distinct *Nostoc* 'photobiont clusters' based on their 16S rRNA (O'Brien et al., 2005). The overall taxonomic position of this strain remains unsure as it also does not cluster within the *Nostoc sensu stricto* clade. Similar uncertainties affect strain SAG 34.79 that could be assigned to *C. brevissima* based on its morphology and phylogenetic position, although there is no cohesive cluster formed and no type strain for the genus deposited. Closely related strains such as *Tolypothrix tenuis* SAG 94.79, *Scytonema mirabile* SAG 83.79, and *T. tenuis* J1 (Figure 1) need further investigation to clarify the state of the genus. *Calothrix* exhibits a notorious morphological heterogeneity and extreme polyphyly, which is evident from various independent clades in the phylogenetic trees of past research [reviewed in (Nowruzi and Shalygin 2021)]. However, even if no phylogenetic or habitat correlation with adsorption capacity could be found, biotechnological studies of cyanobacterial strains should be more often accompanied with phylogenetical studies applying the current standard for taxonomical classification by the so called polyphasic approach (Komárek et al., 2014) to identify taxonomic rearrangements and to avoid confusion regarding species names and strain names from culture collections for biotechnology.

4.2 Metal adsorption experiments

For microalgae, the bioremediation, bioaccumulation, or biosorption of common heavy metals such as Pb, Cd, Cr, As, Hg, Ni, etc. is often studied [e.g. (Ahuja et al., 1999; Ç etinkaya Dönmez et al., 1999; Mehta and Gaur 2005)]. The mechanisms behind these adsorption processes vary with species and environmental conditions (Kumar et al., 2015). However, different mechanisms are discussed, such as ion exchange, complexation, electrostatic attraction, and micro-precipitation (Kumar et al., 2015; Yadav et al., 2021). In contrast, the biosorption of REE is studied less. For the adsorption process of REE, the results in this study indicate an ion-exchange mechanism in which cations of alkaline and alkaline earth metals (Na, K, Mg, and Ca) are replaced by other metal cations during the biosorption process with cyanobacterial biomass (Figure 6). This is in agreement with previous experiments using biomass of different microorganisms (Crist et al., 1994; Matheickal et al., 1997; Sulaymon et al., 2013; Liang and Shen 2022). Ion exchange has been proposed as a dominant mechanism during biosorption (Chen et al., 2002; Iqbal et al., 2009). Apart from *Synechococcus elongates* UTEX 2973 biomass, sodium was the predominant element during the ion exchange process. This differs from previous reports in which cations of earth alkaline metals were released in higher percentages (Iqbal et al., 2009; Sulaymon et al., 2013). Additionally, studies reported the replacement of protons with metal cations leading to a decrease in pH during the sorption process (Mashitah et al., 1999; Vasudevan et al., 2002). However, this aspect was not focused on in the experimental setup of this study. The strong influence of pH value on metal uptake shown in this study further emphasizes the correlation between charges on the surface of the biosorbent and the adsorbed metal ions. In previous studies, the effect of pH value on biosorption has been confirmed (García-Rosales et al., 2012; Abdel-Aty et al., 2013). At low pH values, functional groups on the cell surface are either neutral or positively charged. Carboxyl groups for instance are protonated at pH values below 3, whereas amino groups are protonated at pH 4.1 (Eccles 1999). As similar charges create a repulsive force, positive charges on the biomass surface repel metal cations, leading to poor metal uptake at low pH values. Previous studies described a strong influence of hydroxyl and carboxyl groups on the adsorption process for different biomasses (Gupta and Rastogi 2008; Luo et al., 2010; Utomo et al., 2016). Experiments on adsorption kinetics showed a quick metal uptake for all tested biomasses, reaching equilibrium within only a few minutes. In general, the process of metal cations attaching to adsorbents with a mesoporous surface involves two stages (Zinicovscaia et al., 2021). Specifically, the steps involve the migration of ions from the main solution to the boundary layer surrounding the intermediate-pore matrices, and the attachment of the metal ions to the active sites of the adsorbent material *via* adsorption. Previous studies have reported fast kinetics for the adsorption of metals on biomass of other green algae and cyanobacteria (Klimmek et al., 2001). On the other hand, experiments in other studies resulted in incubation times of up to 60 min and more before reaching the maximum adsorption equilibrium (Ahuja et al., 1999; Zinicovscaia et al., 2017). Fast metal uptake is a beneficial factor for the process development beyond laboratory scale as long incubation periods can be avoided, and higher flow rates can be achieved. Adsorption

experiments with equimolar mixed-metal solutions were carried out, revealing a preference for certain elements influenced by the total metal concentration. The tested biomasses showed the highest overall adsorption capacity for Ce^{3+} at low metal concentrations. However, cations of these elements were replaced by Pb and Al at higher metal concentrations (2–4 mM) in this experimental setup. Zn and Ni showed to lowest affinity to the tested biomasses. Similar results have been reported for biomass of other microorganisms (Klimmek 2003; Wilke et al., 2006; Huang et al., 2018). At present, our ability to make predictions on binding specificity based on single-element adsorption experiments is limited (Wilke et al., 2006). Regarding a potential industrial application for the recovery of REE, these are promising results, as metal concentrations usually are lower than the highest concentrations in the experimental setup of this study. Furthermore, it should be considered that this study predominately focused on the adsorption of the element cerium. Due to high chemical similarities between REE, it is likely that the adsorption properties of the tested biomasses will be similar for other elements of this group. Nevertheless, additional experiments with other REE are advisable. Target elements could be extracted from the resulting metal-loaded biomass in follow-up processes. The destructive recovery by combustion, resulting in metal-enriched ash, is a simple method with the drawback of losing the initial biomass. An economically more desirable approach is the targeted desorption of elements from loaded biomass, enabling the recycling of the biosorbent. Previous studies have tested various approaches using different acids or complexing agents (Gong et al., 2005; Abdolali et al., 2015). Unfortunately, the adsorption properties of biosorbents are impaired over the curse of a few cycles (Hammaini et al., 2007). Future studies should address the binding specificity and durability of biosorbents to implement biosorption in industrial processes successfully. In competitive systems, the adsorption of different metal cations on biomass is influenced by functional groups on the cell surface. The interaction between metal cations and functional groups still requires more research. According to the current state of knowledge, various ionic properties of metal cations, such as electronegativity, redox potential, and ionic radius can influence the adsorption on biomass (Naja et al., 2010). Depending on the biomass and physico-chemical conditions, multiple mechanisms may be involved in metal sorption simultaneously (Gadd 2009). With respect to different cyanobacterial strains, FT-IR analysis indicated the involvement of various functional groups during like hydroxyl or carboxyl groups during metal adsorption. However, at present, there is no discrete chemical entity that has been identified as dominant cell wall feature that governs metal binding. In a previous study, for instance, it was shown that complex polymeric sugars are involved in the adsorption of terbium by *C. brevissima* (Jurkowski et al., 2022). Cell wall-derived binding entities most likely vary for every organism and metal presented.

5 Conclusion

In this study, a diverse group of 12 cyanobacteria was investigated for their potential in the enrichment of REE in a biosorption process. Metal uptake varied strongly among the tested strains, with *Nostoc* sp. 20.02 showing the highest maximum adsorption capacity of 84.2–91.5 mg g^{-1}. However, there was no apparent correlation between maximum adsorption capacity and phylogenetic relationship nor for the ecological habitat of the strains. This could be explained by variations in the composition of metal interacting functional groups located at the cell surface. Moreover, many cyanobacteria that showed high adsorption capacities for REE produce extracellular polymeric substances (EPS) that are known to facilitate metal adsorption (Pagliaccia et al., 2022). The composition of these EPS and their influence on the adsorption of REE should be further investigated in future studies. The determination of relevant parameters for improving the metal uptake revealed a pH optimum at 5 to 6 and fast adsorption kinetics reaching adsorption equilibrium within an incubation time of a few minutes. In addition, metal analysis strongly indicated an ion-exchange mechanism during the biosorption process in which Na^+, K^+, Mg^{2+}, and Ca^{2+} ions are replaced by metal cations that bind to the surface of the biomass. These observations are in accordance with previous studies that were conducted on algal, bacterial, and other biomasses (Acheampong et al., 2011; Sulaymon et al., 2013; Liang and Shen 2022). The isolation of single target elements in a technical biosorption process remains a challenging task due to the complex surface structure and the heterogeneity of functional groups. Nevertheless, based on the results of this study, the enrichment of metal elements from diluted solutions is possible. For the development of an industrial process, parameters need to be further optimized and adjusted depending on the metal composition in the wastewater and the biomass that is used as biosorbent.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author. The 16S rRNA gene sequences generated during this study were added to NCBI GenBank stated by their accession number in the phylogenetic tree (Figure 1).

Author contributions

MP, MK, and PJ contributed equally to this work. Conceptualization, MP, ML, and TB; Methodology, MP, MK, and PJ; Validation, all authors; Writing—original draft preparation, MP and PJ; Writing—review and editing, ML, TN, and TB; Visualization, MP and PJ; Supervision, ML, TN, and TB; Project administration, ML, TN, and TB; Funding acquisition, ML, TN, and TB All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2023.1130939/full#supplementary-material

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4. Discussion and Outlook

Biosorption is recognized as a feasible and effective low-cost approach for metal recovery. Despite substantial advances in understanding this complex phenomenon, commercial applications of biosorption-based technology have yet to be implemented.[7] To that end, major challenges must be overcome to reach actual practical relevancy on a large scale.

On the one hand, the binding specificity for target elements has to be improved, which can be supported by broadening our understanding of the underlying mechanisms of biosorption. On the other hand, biosorbent materials with low production costs, sufficient long-term stability, and availability in large quantities have to be established to enable the development of a cost-effective metal recovery process.

In that context, cyanobacterial biomass has huge potential for the utilization as biosorbent in future biosorption-based systems. The sorption properties for REE of numerous cyanobacterial strains presented in this work have been demonstrated and relevant parameters for a biosorption-based metal recovery process have been investigated.

Nevertheless, there are many aspects that can be improved and require further research and development in order to reach a technological readiness level for industrial applications.

Biomass modification to improve adsorption properties

Due to the complex and heterogeneous surface composition of biological materials, metal adsorption usually is governed by various functional groups simultaneously.[70,71] Over the past years, there have been different approaches to improve the adsorption properties of biosorbents by modifying their chemical features. Those concepts could be applied to the cyanobacterial strains described in the second publication “Rare earths stick to rare cyanobacteria: Future potential for bioremediation and recovery of rare earth elements” [156] to improve overall metal uptake and binding specificity towards REE. In the following paragraphs, different approaches for biomass modification are outlined in more detail.

Influence of cultivation parameters and environmental conditions

A promising approach to improve metal binding characteristics is the directed variation of specific growth parameters during biomass production. Variations in environmental conditions, such as temperature or nutrient supply, can influence the final composition of biomass in a production process.[157,158] This phenomenon can be utilized to increase the formation of functional groups that can interact with target metal ions, which in turn, often has a beneficial influence on the overall metal uptake.[159] Studies on *Saccharomyces cerevisiae*, for example, have shown that a

supplementation with L-cysteine in the growth medium can enhance the adsorption capacity of the yeast cells for chromium and silver because of an increased occurrence of sulfhydryl-groups at the cell surface.[160,161] Likewise, cultivation conditions can be modified to influence the metal adsorption properties of phototrophic microorganisms. A study published in 2019, for example, reported an enhanced display of phosphate groups on the cell surface of *Chlorella* sp. after increasing the phosphorus content in the cultivation medium, leading to improved adsorption of lead.[162] Similarly, adding excess sulfate into the medium increased the overall adsorption of mercury, mediated by a higher number of sulfhydryl sites.[163] Furthermore, for *Chlorella vulgaris,* the limitation of nitrogen sources has been reported to significantly improve adsorption capacities for several metals like cadmium, lead, and nickel.[164,165]

The approach of modifying cultivation parameters aiming to enhance metal adsorption predominantly has been pursued with regard to heavy metals. However, this concept is likely to be applicable to improving the metal adsorption of REE. In particular, the cultivation under nitrogen-limited conditions should be investigated for the cyanobacteria presented in this work as it can enhance the production of extracellular polymeric substances (EPS).[166]

Influence of extracellular polymeric substances on metal adsorption

Interestingly, the cyanobacteria demonstrating the highest adsorption capacity for REE in the presented studies are capable of producing EPS. This has been reported for the investigated cyanobacteria or closely related species in previous studies.[167–170] In general, the formation of EPS has been described for a multitude of different microorganisms, including bacteria, fungi, cyanobacteria, and microalgae.[171–174] Their composition is usually a complex mixture of high molecular weight organic polymers located at the cell surface or being released into the surrounding environment.[175,176] The main components of EPS can be carbohydrates, lipids, proteins, nucleic acids, and other bioactive substances.[177,178] In literature, EPS are also referred to as “exopolysaccharides” [168,179] or “extracellular polysaccharides” [169,180] putting a stronger emphasis on carbohydrates. Recently, EPS have gained increasing attention for potential applications in the recovery of various metals in biosorption-based processes.[54,181–183] Due to the presence of different functional groups, such as amide, carboxyl, phosphate, sulfhydryl, or hydroxyl groups, EPS can interact with metal ions by complex formation or electrostatic attraction.[184–186] Consequently, there usually is a direct positive correlation between EPS produced by cyanobacteria and the adsorption of metal ions.[187,188]

In addition, cyanobacterial EPS exhibit several unique compositional features compared to those produced by other microorganisms, such as a high content of uronic acids.[189] Furthermore, many cyanobacterial EPS contain sulfate groups, a feature that can be found in EPS produced by

eukaryotes and archaea but otherwise is unprecedented for bacteria.[190] The accumulation of functional groups typically found in cyanobacterial EPS lead to particular anionic attributes. These anionic properties are beneficial for the interaction with cations and especially enhance the affinity to metal ions. The formation and composition of cyanobacterial EPS can be influenced by environmental factors, such as water supply, light conditions, or the C:N ratio in the water.[191,192]

Due to the aforementioned characteristics of EPS, EPS-producing cyanobacteria have been investigated in the context of heavy metal sequestration in the past.[54,193,194] A study on *Microcystis sp*., for example, reported higher adsorption of iron and copper cations in capsulated biomass compared to decapsulated biomass.[195] Likewise, EPS from *Cyanospira capsulata* and *Nostoc* PCC7936 have shown beneficial effects on metal uptake and have been suggested for future applications in metal sequestration from wastewater.[196,197]

Cyanobacterial EPS can be categorized into polysaccharides that are released in the environment and polysaccharides that are attached to the cell surface.[190] Depending on their appearance, consistency, and thickness, EPS associated with the cell surface can be referred to as slimes, capsules, or sheaths.[198] The compositional analysis and determination of metal adsorption by EPS can be carried out following their separation from cyanobacterial cells. EPS that are released are usually water-soluble and can be directly collected by low-speed centrifugation or filtration.[199] Those soluble EPS have been reported to exhibit better metal adsorption properties resulting from their exceptionally high number of carboxyl and hydroxyl groups.[200,201] EPS that are attached to the cell surface can be separated using various methods, including a treatment with EDTA, formaldehyde, glutaraldehyde, sonication, or an incubation in hot water.[202–205] Thereafter, extracted EPS can be precipitated and purified using ethanol.[189]

In future studies, it would be interesting to investigate the EPS derived from the cyanobacteria strains described in the article of chapter II “Rare earths stick to rare cyanobacteria: Future potential for bioremediation and recovery of rare earth elements” [156] in regard to their composition and their role in REE adsorption.

Physical or chemical modification of biomass

Besides indirectly influencing the occurrence and composition of functional groups in biomass by variations in cultivation parameters, biosorbent properties can also be directly augmented by controlled physical or chemical modifications. Physical methods usually involve heat treatment or a boiling process but also include mechanical disruption to increase the accessible surface area.[206,207] On the other hand, properties of existing functional groups at the biomass surface can be chemically altered by treatment with various acids or bases.[208–211] Alkali pretreatment of

biosorbents, for example, can lead to deprotonation of the cell surface, causing a stronger attraction of metal cations due to a higher density of negative charges.[212] In contrast to modifying already existing functional groups, new specific functional groups can be selectively added to the biomass surface, for example by using organic acids, such as citric, malonic, or tartaric acid, to introduce new carboxyl groups for improved metal adsorption.[213] A study from 2005, for instance, reported that the pretreatment of fungal biomass by *Neurospora crassa* with acetic acid increased its biosorption capacity for lead from 0.89 to 12.90 mg g^{-1} compared to untreated biomass.[212] In contrast, the elimination of carboxyl groups after methanol esterification can cause a decrease in the adsorption capacity for metals.[214]

The modification of biomass for improved adsorption of metals has been investigated for numerous agricultural waste products or plant residues in the past.[215,216] These biosorbents often occur in large quantities but only display average metal adsorption. Enhancing sorption properties can improve the applicability of these materials in practical bioremediation processes.[86,217,218] For microalgae and cyanobacteria, the directed modification of biomass has not been pursued to a large extent. However, there have been studies that proved this approach could be suitable for the improvement of microalgae or cyanobacteria-based biosorbents. The pretreatment of biomass from *Microcystis* sp. with 0.1 M HCl, for example, significantly improved the adsorption of antimony.[219] Similarly, an analogous pretreatment of *Chlorella vulgaris* biomass enhanced the adsorption of copper and nickel ions.[220] Moreover, an alkaline modification with Na_2CO_3 has been described for *Arthrospira platensis* biomass to improve the biosorption of chromium, iron, copper, and nickel.[221] The modification of biosorbents can be directed towards target elements by increasing the portion of specific functional groups in the treated biomass. Cadmium, for example, has a stronger interaction with sulfate groups, while zinc shows a higher affinity to functional groups containing oxygen or nitrogen.[222] However, with the current understanding of biosorption mechanisms and the commonly used methods, it is not possible to produce tailor-made biosorbents with a particularly high affinity to a single target metal element. Nevertheless, improving the general performance of biosorbents related to total adsorption capacities is possible.

Molecular biological approaches

A novel approach to achieving high adsorption specificity for a target element is biomass production with genetically modified organisms. The expression and display of specific structures, such as metal-binding proteins, at the cell surface can improve binding specificity and overall capacity for target elements. A study in 2013, for example, demonstrated enhanced and selective adsorption of lead by recombinant *Escherichia coli* cells displaying specific peptides.[223] A similar

concept has been applied for the adsorption of REE in a study in 2021.[224] Here, an elastin-like polypeptide and the REE-binding domain of lanmodulin, a peptide with remarkably high selectivity for lanthanides that was first isolated from *Methylobacterium extorquens*,[225] was fused and produced in *Escherichia coli.* Subsequently, the produced peptide was used to recover REE from steel slag leachate.

At present, genetic modification of phototrophic organisms in the context of metal recovery was predominantly applied to microalgae rather than cyanobacteria.[53] In that context, studies usually focused on heavy-metal removal by living cells using bioaccumulation. The two main strategies to improve metal uptake were gene-overexpression or the introduction of foreign DNA into the microalgae cells.[226,227] These modifications lead to enhanced active metal transport mechanisms or increased the cells´ resistance against toxic effects of the accumulated metals.[228–230] Although the approach of genetic modification bears great potential for bioremediation, so far, practical applications are limited.[228]

Analytical methods for the investigation of biosorption mechanisms

Due to the high compositional complexity of chemical structures involved in metal ion binding in biological materials, studying biosorption processes is a challenging endeavor. In addition to the analytical tools applied in the presented studies, other methods can be used to obtain a more complete picture of the underlying mechanisms. Apart from FT-IR spectroscopy, which was applied in the presented studies, the investigation of metal interactions with functional units can be supplemented, for example, by electron spin resonance (ESR) spectroscopy [231,232] or nuclear magnetic resonance (NMR) spectroscopy.[233–235] Additionally, functional groups in biomass samples that might be involved in metal adsorption can be identified by potentiometric titration.[78,154,155,236]

Scanning electron microscopy (SEM) [237–239] or transmission electron microscopy (TEM) [240] can be used to visualize the morphology of biosorbent materials. These methods are often combined with energy dispersive X-ray spectroscopy (EDX), which provides additional information about the local distribution of the adsorbed elements on the biomass.[241–243] Furthermore, X-ray absorption spectroscopy (XAS) or X-ray photoelectron spectroscopy (XPS) could be applied to determine changes in the oxidation state of metal elements during the adsorption process.[244–246] The changes in the oxidation state can be mediated by functional groups of biomass and usually take place at the cell surface.[247] This can alter the binding affinity of metal ions to the biomass or lead to reduced solubility, which can facilitate surface precipitation.[248] Additionally, X-ray adsorption

fine spectroscopy (XAFS) analysis has been used to identify chelate-complexation with functional groups on the cell surface.[214]

Binding specificity of biosorbents for metal ions

Ideally, the development of a biosorption-based metal recovery process would enable a targeted selective sequestration of a desired target element. However, due to the heterogeneity and the complex composition of functional groups in biological materials, directed adsorption has yet to be achieved.

Most studies on metal adsorption are conducted with single-metal solutions. However, in multi-element solutions, the interaction of metal cations with free binding sites is generally observed to be competitive.[249] Various selectivity series which reflect such competition have been published in that context, arranging different metal cations from highest to lowest affinity towards the investigated biosorbent material.[79,250,251] There are notable tendencies in binding strength for different metal ions, with some displaying a relatively strong affinity, while other elements usually have a lower affinity towards the biosorbent. Lead, for example, usually exhibits strong interactions with biomass, while the adsorption of elements like nickel or zinc often is lower due to weaker binding affinities.[79,238,252–254] Alkaline and alkaline earth metals generally have the weakest binding strength to the active sites and are often replaced by other metal cations, leading to an ion exchange process.[255] Nevertheless, the affinity of metal cations towards binding sites is influenced by both the functional groups involved in the adsorption process and the presence of other competing ions. For example, a study on the metal adsorption of *Exiguobacterium* sp. reported a selectivity series of “Cd ≥ Pb > Zn” based on the adsorption capacity in single-element experiments.[256] This, however, changed to “Pb > Cd > Zn” in equimolar mixed-element solutions. A similar study on *Saccharomyces cerevisiae* reported changes in adsorption selectivity from “Cu > Cd = Pb > Zn” to “Pb > Cu > Zn = Cd” for single and mixed-metal systems, respectively.[94] In this context, data presented in the second study of this dissertation [156] indicated that the overall metal concentration in a multi-element solution can influence binding specificity. More precisely, the adsorption of the element cerium in multi-element experiments was highest for equimolar metal concentrations between 0.5 mM and 2 mM. In 4 mM-solutions, however, cyanobacterial biomass displayed a higher affinity for aluminum ions. A selectivity series for *Synechococcus elongatus* UTEX 2973, for example, could be stated as “Ce > Zn > Al = Pb > Ni” and “Al > Pb = Ce > Zn = Ni” for equimolar metal concentrations of 1 mM and 4 mM, respectively.

The coexistence of ions in multi-element solutions can significantly decrease the total metal

adsorption capacity of biosorbents. This has been reported for *Nostoc sphaeroides,* for example.[257] However, it is important not to generalize this effect. For example, a deliberate preloading of biomass with cations of a specific element may enhance biosorption capacities for other metal cations that exhibit higher affinity towards the biosorbent. For example, the saturation of fungal biomass with calcium enhanced zinc biosorption, presumably because of pH buffering effects.[258]

The interaction between different metal ions and functional groups of the biomass is very complex and poorly understood. The diverse metal composition of industrial wastewater streams makes it very hard to predict the behavior of a biosorbent towards a specific metal element in terms of adsorption selectivity in advance. Differences in sorption selectivity have been explained with higher electronegativity (according to the Pauling scale), ion radii, or ionic charge.[82,259,260] However, in most cases, these chemical properties alone cannot account for the binding specificity observed in biosorption processes. The concept of hard and soft acids and bases ("HSAB")[261] also is insufficient to describe and predict metal adsorption on different biosorbents universally.

Although biosorbents can be modified to exhibit stronger adsorption for specific metal ions, the directed, selective adsorption of a single target element from a multi-element solution has not been achieved yet using naturally occurring biomaterials.[262]

Practical applicability of biosorption-based metal recovery

Although cyanobacterial biomass, in general, has displayed promising biosorption properties, so far, no industrial-scale process has been fully developed and implemented.[263] At present, the costs for process development and biomass production are too high to operate in a financially sustainable way, as most wastewaters contain predominantly metals with a relatively low market value. Nevertheless, these drawbacks may be offset in the future with the recovery of more valuable metals where conventional methods cannot be applied cost-effectively.[264] Less than 1% of REE are currently recovered from discarded products as their separation from other more abundant metals is too expensive and complex in regular recycling processes.[265]

A study in 2023 stated that recycling from industrial wastewater would become economically feasible for some metals in the coming years, especially as the demand for those metals continues to rise.[266] The elements that were considered high-priority metals are geologically scarce, critical for high-technology industries, energy-intensive to produce, and only have scarcely established recycling systems. Among metals like gallium, vanadium, or lithium, REE were included in that

list. Therefore, as cyanobacterial biomass is well-suited for metal recovery from industrial wastewater in biosorption processes, there is potential for future applications in this area.

Selective desorption and biosorbent regeneration

From an economic perspective, the usage of an adsorbent material depends not only on its adsorption characteristics but also on its regenerative capacity, reusability, and long-term stability. To reduce the operational cost of a biosorption-based process, it is preferable to regenerate the biosorbent while simultaneously facilitating a recurrent and continuous recovery of adsorbed metals. The regenerated biomaterial could then be used for numerous sorption and desorption cycles. In this context, it is critical that the desorption process not only removes the target elements from the biomass but also restores the biosorbent close to its original state without damaging or altering its physical and chemical characteristics.[267] Thus, allowing an effective reuse without a decrease in original metal binding properties. To that end, diluted mineral acids, such as hydrochloric, sulfuric, or nitric acid, and organic acids, like citric or acetic acid, have been applied for biosorbent regeneration.[268–270] In addition, the utilization of complexing agents like EDTA or thiosulfate has been reported.[26,271] However, due to the complex composition of functional groups involved in the adsorption process, it is very difficult to selectively desorb a single target element in a multi-element adsorption process.

A second drawback of biological materials in the context of reusability is their generally poor long-term stability. The sorption capacities for cadmium of *Oedogonium* sp., for example, decreased by 18% after five adsorption-desorption cycles with a 15-20% loss of biomass.[27] In a different study on *Cystoseria indica* and *Sargassum glaucescens*, a desorption process with 0.5 and 1.0 M NaOH and KOH reduced the sorption capacity of the tested biomass by 46-51% after nine cycles.[272] At present, this aspect is a crucial challenge for biosorbents in competing with chemically synthesized resin materials.

Concluding remarks

This work provides further insights into the metal adsorption by cyanobacterial biomass, thereby offering a better understanding of underlying mechanisms. Moreover, it was demonstrated that cyanobacterial biomass bears great potential for future applications in biosorption-based metal recovery from diluted solutions. With rising demand for Rare Earth Elements and the accompanying increase in market value, the implementation of bio-based processes might become economically feasible in the near future. Therefore, biosorption should be explored as an alternative biotechnological approach for the bioremediation of aquatic waste streams and the recovery of valuable metals to ensure supply security.

Ideally, a biosorption-based process would enable the cheap recovery of one specific target molecule from a mixture of many in a single step. However, at present, biosorption-based processes in industrial applications for wastewater treatment are very limited, even as an addition to conventional methods. Two significant challenges of biosorption-based processes are the insufficient durability of biosorbents and the overall poor binding selectivity.[8,71] Improving our understanding of underlying mechanisms during the sorption process is vital to establish biosorption as a viable alternative method. In the future, the isolation of specific functional moieties from biomass and their subsequent, deliberate enhancement of expression on the cell surface may lead to improved sorption specificity. This could be achieved through genetic modification of the cultivated organisms or by chemical or physical treatment of the produced biomass.

5. List of Publications

Isolation and Investigation of Natural Rare Earth Metal Chelating Agents from *Calothrix brevissima* – A Step Towards Unraveling the Mechanisms of Metal Biosorption

Wojciech Jurkowski, **Michael Paper,** and Thomas B. Brück.

Frontiers in Bioengineering and Biotechnology 10 (2022), 87.

https://doi.org/10.3389/fbioe.2022.833122

Efficient Green Light Acclimation of the Green Algae *Picochlorum sp.* Triggering Geranylgeranylated Chlorophylls

Michael Paper, Matthias Glemser, Martina Haack, Jan Lorenzen, Norbert Mehlmer, Tobias Fuchs, Gerhard Schenk, Daniel Garbe, Dirk Weuster-Botz, Wolfgang Eisenreich, Michael Lakatos, and Thomas B. Brück.

Frontiers in Bioengineering and Biotechnology 10 (2022), 689.

https://doi.org/10.3389/fbioe.2022.885977

Rare earths stick to rare cyanobacteria: Future potential for bioremediation and recovery of rare earth elements

Michael Paper, Max Koch, Patrick Jung, Michael Lakatos, Tom Nilges, and Thomas B. Brück.

Frontiers in Bioengineering and Biotechnology 11 (2023), 172

https://doi.org/10.3389/fbioe.2023.1130939

Book chapter: Photosynthetic conversion of CO_2 into bioenergy and materials using microalgae

Felix Melcher, **Michael Paper,** and Thomas B. Brück

De Gruyter, Photosynthesis – Biotechnological Applications with Microalgae (2021)

https://doi.org/10.1515/9783110716979-009

Publications in preparation

Stripped: contribution of cyanobacterial extracellular polymeric substances to the adsorption of rare earth elements from aqueous solutions

Michael Paper, Patrick Jung, Max Koch, Michael Lakatos, Tom Nilges, and Thomas B. Brück

Elution of rare earth elements from kaolin sands via mild acid treatment

Max Koch, **Michael Paper**, Thomas B. Brück, and Tom Nilges

Identification, characterization and application of a unique, ulvan-like exopolysaccharide secreted by the green algae *Chlorella sorokiniana*

Tobias Fuchs, Sandra Radziej, Paula Großmann, Moritz Kränzlein, Maria Bauer, Carolin Rickert, Ahmed Raouf Fahmy, Theresa Lutz, **Michael Paper**, Jan Lorenzen, Mahmoud Masri, Martina Haack, Claudia Huber, Daniel Garbe, Norbert Mehlmer, Bernhard Rieger, Oliver Lieleg, Thomas Becker, Mario Jekle, Wolfgang Eisenreich, and Thomas B. Brück

Nutrient profile comparison and sensory analysis of eight different types of chlorella biomass

Felix Melcher, Florian Utz, **Michael Paper**, Nikolaus Stellner, Diego Atoche, Tillmann Peest, Max Koch, Dr. Jennifer Schneiderbanger, Lisa Obermaier, Tom Nilges, Thomas Becker, Michael Rychlik, Wolfram Brück, Corinna Dawid, and Thomas B. Brück

Genome analysis of the thermophilic cyanobacterium *Thermostichus lividus* and its protein expression patterns during adaption to different temperatures

Nathanael Arnold, **Michael Paper**, Tobias Fuchs, Dirk Wortmann, Petrina Kapewangolo, Norbert Mehlmer, and Thomas B. Brück

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