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Biosorption of Rare Earth Elements by Different Microorganisms in Acidic Solutions

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Abstract: Acidic solutions from metal bioleaching processes usually contain mixtures of metals in different concentrations which need to be separated and concentrated in downstream processing. Aim of this study was to explore and compare biosorption of rare earth elements (REE) by different microorganisms in acidic solutions. Biosorption of REE by bacteria and fungi showed element selective biosorption. The gram-positive bacterium *Bacillus subtilis* showed a higher selectivity to ytterbium (Yb) and lutetium (Lu) than the gram-negative bacteria *Leisingera methylohalidivorans* and *Phaeobacter inhibens*. In contrast, the tested fungi (*Catenulostroma chromoblastomyces*, *Pichia* sp.) showed a preference for the middle rare earth elements. Algae exhibited a low biosorption performance. Additionally, for *B. subtilis* and one yeast (*Pichia* sp.), better results were achieved with living than dead biomass. This study compares for the first time biosorption of different microorganisms at standardized conditions at low pH and application related conditions.

Keywords: biosorption; rare earth elements; acidic solution; *B. subtilis*; *Pichia* sp.

1. Introduction

The ability of microbial cells to sequester solutes selectively from aquatic solutions, via non-metabolically mediated pathways, has been termed biosorption [1]. With other words, biosorption can be defined as the removal of substances from solution by biological material [2,3]. Biosorption can be performed in a wide range of pH values 3–9 [4]. Nevertheless, the major part of the studies under acidic conditions were carried out for one or two elements in solution and mild acidic conditions (pH > 3) [5,6]. The pH is possibly the most important physico-chemical parameter for biosorption and competition between cations and protons for binding sites means that biosorption of metals like Cu, Cd, Ni, Co and Zn is often reduced at low pH values [3].

Bioleaching usually takes place in acidic solutions with low pH (<2–3). These solutions contain mixtures of heavy metals in different concentrations which need to be separated and concentrated in downstream processing. Here biosorption might be an option.

Due to high economy delivery risks, special attention has been paid to rare earth elements (REE) and their recovery from primary and secondary resources over the last years [7].

Biosorption of REE at low pH has been studied in some cases [8–16]. Comparing these studies, it is obvious that the conditions under which biosorption experiments took place are often not comparable due to different sorbent/metal ratios even if isotherm studies with increasing metal concentrations were carried out. Therefore, the results in the literature for various microorganisms often rather reflect the experimental conditions than comparable differences between the microorganisms. Aim of this study was to elucidate biosorption of REEs with different microorganisms at comparable acidic conditions. Biosorption experiments were carried out with a mixed solution containing eight REEs (lanthanum, cerium, neodymium, gadolinium, dysprosium, erbium, ytterbium and lutetium).

Additionally, to test the applicability, an experiment with a gadolinium-containing wastewater was performed.

2. Material and Methods

2.1. Microorganisms and Cultivation Conditions

Different Bacteria and Eukarya were selected and grown at specific cultivation conditions (Table 1) to obtain enough biomass for biosorption experiments. The organisms were mainly selected because REE biosorption was described for them in the literature, partly at low pH.

Two eukaryotic microorganisms from the BGR culture collection were selected because of their high biosorption performance at neutral pH studied in previous work.

Their phylogeny was investigated as described below.

The composition of the media is described as follows:

FP medium: meat peptone (Roth #2366.1) 8 g/L, casein peptone (Roth, #8986.1) 2 g/L, yeast extract 0.5 g/L, NaCl 2 g/L adjusted to pH 7.0, after autoclaving 0.2 µm filtered glucose (400 g/L) was added to a final concentration of 4 g/L.

NB medium: Difco™ Nutrient Broth.

Malt medium: biomalt 40 g/L, casein peptone 5 g/L, (NH₄)₂HPO₄ 1 g/L, adjusted to pH 6.

Cyanidium medium: (NH₄)₂SO₄ 1 g/L; K₂HPO₄ 0.02 g/L; MgSO₄·7H₂O 0.02 g/L; soil extract 30 mL/L, adjusted to pH 4.0.

Preparation of soil extract for cyanidium medium: Fill a 6-L flask one third with garden or leaf soil of medium, but not too great humus content which does not contain fertilizers or plant protective agents. Success of soil extract depends on selection of suitable soils. Those with high clay content are usually less satisfactory. Add de-ionized water until it stands 5 cm above the soil and sterilize by heating in a steamer for one hour twice in a 24 h interval. Separate the decanted extract from particles by centrifugation. Fill into small containers of stock solution each of a size appropriate to making a batch of media, autoclave for 20 min at 121 °C and store in the refrigerator. (http://sagdb.uni-goettingen.de/culture_media/17%20Cyanidium%20Medium.pdf)

WC medium: Prepared as described by Guillard and Lorenzen 1972 [22].

2× Allens medium (1:1 diluted): (NH₄)₂SO₄ 2.62 g/L; KH₂PO₄ 0.54 g/L; MgSO₄·7H₂O 0.5 g/L; Ca Cl₂·2H₂O 0.14 g/L; FeCl₃·6H₂O 0.016 g/L

Aron's A6 trace element solution for Allens medium: MnCl₂·4H₂O 1.8 g/L; ZnCl₂ 0.105 g/L; Na₂MoO₄·2H₂O 0.39 g/L; CoCl₂·6H₂O 0.05 g/L; CuCl₂·2H₂O 0.06452 g/L; add 2% to medium.

Table 1. Cultivation conditions for microorganisms used for biosorption experiments.

Organism	Taxonomy	Medium	Tempe-Rature	Type of Flasks	rpm on the Shaker	Incuba-Tion Time (Days)	Reference
<i>Leisingeria methylo-halidivorans</i> DSM 14336	Bacteria (gram-negative, <i>Roseobacter</i> clade)	Marine Broth medium (Difco, MB2216)	20 °C	Bullying flasks	80	2	[17]
<i>Phaeobacter inhibens</i> DSM 17395	Bacteria (gram-negative, <i>Roseobacter</i> clade)	Marine Broth medium (Difco, MB2216)	20 °C	Bullying flasks	80	2	[17]
<i>Bacillus subtilis</i> ATCC 7003	Bacteria (gram-positive)	FP medium	30 °C	Erlen-meyer flasks	120	2–4	[18–20]
<i>Pezizomy-cotina</i> sp.	Eukarya (fungus)	FP medium	30 °C	Erlen-meyer flasks	120	~10	BGR culture M8
<i>Fusarium</i> sp.	Eukarya (fungus)	malt medium	30 °C	Erlen-meyer flasks	120	2–4	BGR culture
<i>Catenulostro-ma chromo-blastomyces</i> CBS 597.97	Eukarya (fungus)	malt medium	30 °C,	Erlen-meyer flasks	120	2–4	[16]
<i>Pichia</i> sp.	Eukarya (fungus, yeast)	malt medium	30 °C,	Erlen-meyer flasks	120	2–4	BGR culture
<i>Pichia naganishii</i> [MB#320511]	Eukarya (fungus, yeast)	NB medium	30 °C,	Erlen-meyer flasks	120	2–4	BGR culture
<i>Saccharo-myces cerevisiae</i>	Eukarya (fungus, yeast)	Sabouraud medium	30 °C,	Erlen-meyer flasks	120	2–4	[21]
<i>Galderia sulphuraria</i>	Eukarya, Rhodo-phyta (algae)	Cyanidium medium and 2× Allens medium	20 °C, 30 °C sunlight and dark	Baffled shake flasks	200	~14	[15]
<i>Messastrum gracilis</i>	Eukarya, Chloro-phyta (algae)	WC medium	20 °C, 30 °C, sunlight and dark	Baffled shake flasks	200	~14	[12]

2.2. Biosorption Experiments with Suspended Biomass

Cell biomass (BM) was harvested by centrifugation with the High Speed Centrifuge Sorvall RC6 plus (7000 g, 10–20 min; Thermo Electron, Langenselbold, Germany) of grown cultures, and washed twice with autoclaved Milli-Q® water at pH 2.5 (HCl). BM dry weight was measured with the HC 103 device (Mettler Toledo, Albstadt, Germany). BM was suspended in pH adjusted and autoclaved Milli-Q® water in measuring pistons at room temperature.

REE solutions (pH 2.5, HCl) were manually prepared from chloride salts of REEs (La, Ce, Nd, Gd, Dy, Er, Yb and Lu) and added to BM to the required final concentrations (BM 5 g/L, REEs 15 µmol/L each). REE/zirconium/iron solutions for competition experiments were prepared in the same way with addition of iron (FeCl₂) and zirconium (ZrOCl₂) to a final concentration of 264 µmol/L and 16 µmol/L, respectively. For tests with dead vs. living BM, the collected and centrifuged samples were autoclaved with a small amount of medium before experiments.

Experiments were done in duplicates for BM assays and controls (assay without BM) if not shown differently. REE concentrations were measured in samples of supernatants taken after different time intervals by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700, Agilent Technologies, Waldbron, Germany) after centrifugation and filtration of samples (0.2 µm nylon membrane). The pH was measured with the pH-Meter 766 Calimatic device (Knick, Berlin, Germany, for medium) and the Five Easy Plus pH/mV device (Mettler Toledo, Albstadt, Germany, for samples). Calculations were carried out by subtraction of REE concentrations (c_e) from controls (c_i) taken at the same time to exclude possible precipitations. Relative biosorption was calculated as follows: $((c_i) - (c_e)) / (c_i) * 100$. Amount of REE uptake was calculated by dividing the difference of the mean values between control and sample assays through biomass.

2.3. Biosorption Conditions Experiments with Immobilized Biomass

For immobilization and recovery rate experiments with selected microorganisms, highly purified agarose was diluted in water and autoclaved (solution A). After autoclaving, solution A was acidified to pH 2.5 (HCl) and stored at 45 °C to prevent solidification. Suspended biomass was adjusted to pH 2.5 after washing twice with acidified autoclaved Milli-Q® water (pH 2.5, HCl), for the control samples acidified autoclaved Milli-Q® water was used (pH 2.5, HCl), (solution B). Solutions A and B were mixed and quickly poured into four forms and let solidify to get platelets. These platelets were put into Erlenmeyer flasks which were previously filled with the REE solution. Solid/solution ratio was 1:4, the experiments were done in duplicate with four 2.5 mL platelets (total 10 mL with acidified Milli-Q® water or acidified biomass) and 40 mL acidified REE solution. After completion of the experiment, the platelets were mixed with 238 µL concentrated nitric acid and incubated in a sandbath over night at 80 °C. The dry remnants were diluted in 2% nitric acid and analyzed by ICP-MS.

2.4. Phylogenetic Analysis

High-molecular-weight DNA of the yeast strain (BGR culture collection) and the fungus M8 (BGR culture collection) was extracted from pure cultures following the FastDNA Spin Kit for Soil (Bio101) protocol [23].

Cloning and sequencing of the ITS (internal transcribed spacer) sequence and the EF1α (translation elongation factor 1α) gene was done by the Microsynth AG (Switzerland). Sequences were manually edited and curated with BioEdit 7.2.5. Phylogenetic mapping was done at NCBI (www.ncbi.nlm.nih.gov) with BLAST [24]. Sequences were submitted to NCBI (accession numbers MN567305.1 and MN644911). For the fungus M8, similarly cloning and sequencing of the ITS sequence and 28 s Sequence was done by the Microsynth AG (Switzerland) and sequences were manually edited and curated with BioEdit 7.2.5 [25]. Phylogenetic mapping was done at NCBI (www.ncbi.nlm.nih.gov) with BLAST [24]. Sequences were submitted to NCBI (Accession numbers MN575689.1

and MN575688.1). The yeast strain was identified as *Pichia* sp., the fungal strain M8 was identified as *Pezizomycotina* sp.

3. Results and Discussion

3.1. Biosorption of REE by Bacteria

Initially biosorption tests with *B. subtilis* and four elements (lanthanum, neodymium, dysprosium and ytterbium, each 15 $\mu\text{mol/L}$, no parallels) showed a selective preference of *B. subtilis* for ytterbium at different amounts of biomass, and a reduction of uptake of REEs per biomass with increasing biomass (Figure S1, Table S1). Biosorption at pH 4 and pH 5 was also tested and resulted in more than 96% REE removal and did not show any difference concerning different amounts of biomass (data not shown). Uptake for lanthanum was 5.6, 5.5 and 5.8 $\mu\text{mol/g}$ for two parallel assays at pH 4 and a biomass of 2.5 g/L, these data are in accordance with a previous study with a REE/BM ratio of 2.9 with lanthanum as single metal in solution [26].

Increasing the REE concentration with increasing REE/BM ratio decreased the relative biosorption with small influence on the preference for the heavy rare earth elements (HREEs) (Figure 1).

Figure 1. Biosorption of REEs by *B. subtilis* with increasing REE/BM ratio at pH 2.5, BM 4 g/L, (a–c): REE/BM ratio 3.75, 7.5 and 15, respectively; REE concentration of each element was 15, 30 and 60 $\mu\text{mol/L}$, respectively. Error bars show standard deviation of two parallels.

This aspect together with the result, that biosorption did not increase very much especially for ytterbium with a biomass of more than 5 g/L (Figure S1b,d,f,h) is essential for application of biosorption technologies because producing biomass is an energy and resource consuming process. In conclusion, for comparing different microorganisms in terms of possible applications, REE/BM ratio, pH, time and absolute REE and BM concentrations must be taken into consideration.

Therefore, to evaluate the biosorption pattern of the eight REEs in solution a biomass of 5 g/L for the gram-positive bacterium *B. subtilis* as well as for the gram-negative bacteria *L. methylohalidivorans* and *P. inhibens* was chosen for further experiments. The preference for the HREEs with different selectivity at pH 2.5 was confirmed (Figure 2). Additionally, for *B. subtilis*, a slight reduction of biosorption after four hours was found (Figure S1), for *L. methylohalidivorans* and *P. inhibens* a reduction of biosorption after 20 h was observed (Figure S2).

Figure 2. Biosorption of REEs by *B. subtilis* ((a) gram-positive), *L. methylolalidivorans* ((b) gram-negative) and *P. inhibens* ((c) gram-negative). BM 5 g/L, initial REE concentration was 15 $\mu\text{mol/L}$ for each element, sampling took place after 2 h. Bars show mean of two parallel assays, error bars show standard deviation.

Takahashi et al. found that heavy REE (HREE, especially Tm, Yb, and Lu) are enriched on the cell surface of the gram-positive bacterium *B. subtilis* and the gram-negative bacterium *E. coli* compared to other REEs and concluded that the REE patterns suggest that there are at least two binding sites on the bacterial cell surface, i.e., carboxylate and phosphate groups [18]. Our results are in accordance with a later study of Takahashi et al. who furthermore found that HREEs form complexes with multiple phosphate sites (including phosphoester sites) with a larger coordination number (CN) at lower REE-bacteria ratios (REE/BM) [19]. In contrast, light and middle REEs form complexes with the phosphate sites at a lower CN. However, the fraction coordinated to carboxylate increased for all REEs with increasing REE/BM ratio, but the enrichment of HREE in the REE distribution patterns of the bacteria was less marked with an increasing REE/BM ratio [19]. Another previous study, which combined surface complexation modelling of macroscopic adsorption data with X-ray spectroscopic measurements to identify lanthanide sorption sites on the bacterial surface by the gram-negative *Pantoea agglomerans* suggested that there may be variations in the dominant sorption sites across the lanthanide series. The adsorption of both LREEs used (La and Nd) was best modelled assuming adsorption to phosphate sites, whereas Gd, Er and Yb could be modelled equally well with carboxyl or phosphate sites; samarium was better modelled with carboxyl relative to phosphate sites. However, one restriction of this study was that the observed differences in modelling were small [14] and it has to be mentioned, that the used REE/BM ratio was high, this could also contribute to a higher impact of the carboxyl sites. In contrast, concerning the europium/*B. subtilis* system it was found that carboxyl, phosphate, and hydroxyl (and/or amine) groups were present at the bacterial surface of *B. subtilis* [20]. At pH 5, the adsorption data could be well described by mainly carboxyl groups and with increasing pH, an increasingly deprotonated phosphate environment should be considered [20]. Concerning the data of the literature provided by Takashi et al. [18,19] the REE/BM ratios are similar to the here presented data.

Considering the obvious difference between gram-positive and gram-negative bacteria in cell wall structures, it was shown, that powder of a wild type vs. a lipoteichoic acid defective strain of *B. subtilis* showed a better biosorption performance, indicating that lipoteichoic acids contribute to the absorption of REEs and followed the order of effectiveness $\text{La} < \text{EU} < \text{TM}$ [27].

Regarding the relevant chemical groups for lanthanide binding, Gadd et al. stated that peptidoglycan carboxyl sites are the main binding sites for metal cations in the gram-positive bacterial cell and that phosphate groups contribute significantly to biosorption in gram-negative species. Furthermore, other bacterial metal-binding components are proteinaceous S-layers, and sheaths largely composed of polymeric materials with proteins and polysaccharides [3]. Interestingly, a genetically engineered gram-negative bacterium *Caulobacter crescentus* with a high-density cell surface display of lanthanide binding tags (composed of small proteins on its S-layer) exhibited a preference for HREEs [28]. Considering literature results

and the observed higher selectivity for ytterbium and lutetium of the gram-positive bacterium *B. subtilis* than of the tested gram-negative bacteria (at the same REE/BM ratio), the question remains open what kind of binding mainly contributes to the biosorption of REEs at acidic pH. A relative higher amount of phosphate groups contributing to binding of HREEs in gram-positive bacteria or a better accessibility and amount of carboxyl groups in the proteinaceous S-layer in gram-negative bacteria at low REE/BM ratios could explain the observed difference. This assumption together with results from Takahashi et al. [18] that HREEs form complexes with multiple phosphate sites (including phosphoester sites) with a larger coordination number at lower REE/BM ratios would also explain the diminishing relative difference between the absorption of LREEs and HREEs with increasing biomass (2.5 g/L vs. 5 g/L) and decreasing REE/BM ratio (6 $\mu\text{mol/g}$ vs. 3 $\mu\text{mol/g}$). This also supports the results from Takahashi et al. [19] that, at low REE/BM ratios, HREEs are bound to phosphate sites with a higher coordination number (Figure 3).

Figure 3. Biosorption of REEs by *L. methylohalidivorans* (a) and *P. inhibens* (b). BM 2.5 g/L (grey) and 5 g/L (black), initial REE concentration was 15 $\mu\text{mol/L}$ for each element, sampling took place after 2 h. Bars show mean of two parallel assays, error bars show standard deviation.

Competition of metal cations for metal binding tags has been described in some studies [29]. For example, it has been shown that cations like Al^{3+} are strong inhibitors for biosorption of REEs by *Pseudomonas aeruginosa*. In the same study, lanthanum biosorption was strongly affected by the presence of europium and ytterbium, whereas the extent of removal of ytterbium did not decrease to the same extent than with the addition of lanthanum ions [11]. Comparison of the results of pretests with four elements (La, Nd, Dy, Yb) with those experiments with eight REEs (biomass 5 g/L, pH 2.5, REEs 15 μmol each) confirmed this observation (Table S1). To investigate the question, whether at acidic pH other cations like Fe(II) and Zr(IV) compete with the REEs for biosorption binding sites, the relative biosorption of the eight REEs in presence of iron (FeCl_2) and zirconium (ZrOCl_2) was tested, however, although biosorption and uptake of zirconium was high, competition was not found. The mean decrease of biosorption of all eight REEs after two hours was only 0.5% compared to the experiment without zirconium and iron (Figure 4). These data are in accordance with a previous study which pointed out that Tm was selectively removed by *B. subtilis* powders in the presence of Fe(II) [27].

Figure 4. REE biosorption (a,c) and uptake (b,d) by *B. subtilis* in absence (a,b) and presence (c,d) of iron and zirconium (Fe(II): 264 $\mu\text{mol/L}$, Zr(IV) 16 $\mu\text{mol/L}$, BM 5 g/L, pH 2.5). Bars show mean of two parallel assays, error bars show standard deviation.

In conclusion, at low pH, for selective removal of HREEs, biosorption by *B. subtilis* is a promising approach; for removal of all REEs, the marine bacterium *L. methylhalidivorans* seems suitable.

3.2. Biosorption of REE by Eukaryotes

Concerning the eukaryotes, several fungi and two algae were tested at low pH. Results are summarized in Table 2.

Table 2. Overview about biosorption capabilities of eukaryotes.

Organism	Result
<i>Saccharomyces cerevisiae</i>	Tested with BM of 5, 10 and 20 g/L, at a BM of 5 g/L biosorption was <50%, biosorption was 100% at a BM of 10 g/L, preference for Gd, Dy, Yb, Lu
<i>Pichia naganishii</i> [MB#320511]	Tested for La, Nd, Dy, Yb at a BM of 2.5, 5 and 10 g/L. At a BM >5 g/L, biosorption >80%.
<i>Pichia</i> sp.	Preference for Ce, Nd, Gd, Dy
<i>Catenulostroma chromoblastomyces</i> CBS 597.97	Preference for Ce, Nd, Gd, Dy, at a BM of 7.5 g/L ~30% biosorption
<i>Pezizomycotina</i> sp.	Tested with BM of 2, 5 and 10 g/L, biosorption <20%, preference for Gd, Yb, Lu
<i>Fusarium</i> sp.	Preference for Nd, Gd, Gy, Lu, tested for 4 elements (La, Nd, Dy, Yb), BM 4 g/L ~40%, BM 5 g/L biosorption ~100%
<i>Messastrum gracilis</i>	Literature data not confirmed [12]
<i>Galdieria sulphuraria</i>	Literature data not confirmed [15]

Concerning biosorption capacities of fungi, a preference for the MREEs (middle rare elements), partly together with a preference for ytterbium and lutetium was observed (Table 2). Interestingly, strain *Pezizomycotina* sp. which was isolated from an abandoned acid copper mine (Marsberg, Germany) showed the lowest biosorption (<20% at a BM of 10 g/L). This might be due to adaptation

to the metal-rich environment inhibiting absorption of cations to the cell wall. Because the *Pichia* sp. strain was most promising for biosorption at low pH, the total biosorption at different pH values with two different amounts of biomass (2 and 4 g/L) was tested in order to estimate a suitable biomass for further work. Biosorption increased still after four hours, therefore it was tested up to 24 h. In a pretest, a compensation of pH effect on biosorption with increasing biomass but a decreasing biosorption per g/dry weight was observed (Figure S3). To discriminate a possible preference for elements, 2 g/L BM was chosen for further tests. Pretests with 1.5% agarose and 2% agarose as immobilization matrix carried out with *B. subtilis* showed a better biosorption with 1.5% agarose (Figure S4), therefore a recovery experiment with immobilized *Pichia* sp. in 1.5% agarose was conducted. The preference of certain REEs, specifically cerium, neodymium, gadolinium and dysprosium was confirmed by the data (Figure 5). For application, the metals could be recovered from the ashes.

Figure 5. Recovery experiment: biosorption of *Pichia* sp. immobilized in agarose, biomass 2 g/L, pH 2.5, samples were taken after 24 h. Bars show mean of two parallel assays, error bars show standard deviation, (a–d) pH 1.5, 2.5, 3.5 and 4.5, respectively.

The reason for the reduction of biosorption at pH 3.5 remained open, due to the fact, that the biomass was diluted after centrifugation, experimental bias (e.g., clumping of biomass) cannot be completely excluded. Nevertheless, preference for the middle rare elements was confirmed.

To test the REE biosorption capability under application related conditions wastewater from a sewage plant was used. Concerning the middle rare elements, the annual emission of gadolinium (used as contrast agent) by medical hospitals and ambulances was estimated to be 1160 kg in Germany for 1996/1997, resulting in an average gadolinium concentration in surface water of 0.026 µg/L [30]. Gadolinium load resulting from magnetic resonance imaging is well investigated in Germany and Switzerland [31–33]. Recently, the first evidence that anthropogenic gadolinium enters human food chain was found [34]. Therefore, to elucidate whether biosorption is applicable here, an experiment with two samples (pH 8.1) from pre- and post-clarification wastewater from a sewage treatment plant

in Germany was carried out. The amount of REEs was determined with ICP-MS. The gadolinium concentration was 3.8 and 3.9 nmol/L for the two water samples, respectively (0.6 µg/L). The amount of all other REEs together was below 0.6 nmol. For experiments, two approaches were selected: first, the samples were used without any modification, second, the samples were acidified to pH 2.5. Additionally, it was tested whether filtration (0.2 µm) of the samples had an influence.

The results did not show any biosorption using the not acidified samples. It is known that rare earth ions form complexes with hydroxide or carbonate at pH higher than approximately 5.5 [35]. Biosorption of the acidified samples was strongly depended on the REE/BM ratio, increasing the biomass resulted in biosorption of ~75% for both (pre- and post-clarification) samples. Post-clarification samples exhibited a better biosorption, this might be due to the lack of competing cations or complexing organic molecules. At the higher REE/BM ratio, a better biosorption within the unfiltered samples was found (Figure 6a,c). In conclusion, *Pichia* sp. seems suitable to recover gadolinium from wastewater at appropriate conditions.

Figure 6. Biosorption and amount of uptake of gadolinium by *Pichia* sp. from acidified wastewater. (a,c) REE BM/ratio: 3.9 µmol/g; (b,d) REE/BM ratio: 0.13 µmol/g; samples 1, 3: post clarification wastewater; samples 2, 4: pre clarification wastewater; samples 1, 2: 0.2 µm filtered; samples 3, 4: not filtered. Error bars show standard deviation of two parallels.

Concerning *Fusarium* sp., *Catenulostroma chromoblastomyces* and *Pichia* sp., in various pH tests, decreased biosorption at a pH of 2.5 or 3.5, respectively, was seen (Figure 5, Figure S5, for *Fusarium* sp. not shown).

Because fungal cell walls are complex macromolecular structures predominantly consisting of chitins, glucans, mannans and proteins, but also containing other polysaccharides, lipids and pigments, e.g., melanin [3], it is conceivable, that at low pH the lanthanide binding tags of the eukaryotic cell wall integrity are destroyed. Complex interactions of protons with for example chitin [36] may mask lanthanide binding tags. The observed increasing biosorption at pH below 2.5–3.5 could also be explained by absorption on released intracellular matter (e.g., proteins or walls of cell organelles).

On the other hand, for the green algae *Galderia sulphuraria*, an active incorporation of REEs into the cell at pH 1.0–1.5 was suggested [15]. Further research to clarify this observed phenomenon is needed.

In addition to fungi, REE biosorption was tested for the red algae *G. sulphuraria* and the green algae *M. gracilis* for different cultivation conditions as described in the literature [12,15]. However, significant biosorption capabilities were not detected for both algae.

3.3. Tests with Dead vs. Living Biomass

The use of dead biomass instead of to living biomass has several advantages: storage of biomass is easier, versatility is better, the degree of uptake is higher, it might be possible to reuse the biomass, recovery with toxic substances is possible [29]. We tested this for *Bacillus subtilis* and one fungus (*Fusarium* sp.) with increasing REE concentrations and better results were achieved with living biomass vs. dead biomass (Figure 7).

Figure 7. Comparison of REE biosorption by *B. subtilis* with living (a–c) and dead (d–f) biomass, (a,d): concentration of REEs each 15 µmol/L, (b,e): concentration of REEs each 30 µmol/L, (c,f): concentration of REEs each 60 µmol/L, BM 4g/L, pH 2.5.

Higher biosorption with living biomass was also achieved with *Fusarium* sp. (Figure 8).

Figure 8. Comparison of REE biosorption by *Fusarium* sp. with living (a–e) and dead (f–k) biomass at different time intervals, (a,f): 0 h, (b,g): 2.5 h, (c,i): 5.5 h, (d,j): 8 h, (e,k): 25 h. Biomass was 5 g/L, pH was 2.5.

These results are in accordance with those of the red algae *Galderia sulphuraria* which also performed better biosorption with living vs. dead BM [15].

4. Conclusions

At low pH, a careful selection of microbial species for biosorption of REE is necessary. The importance of the REE/BM ratio for application strategies was shown. Biosorption preferences were shown for different REEs at comparable application conditions (pH, REE/BM ratio). Competing effects were found for *B. subtilis* for interaction of eight REEs but not for the addition of zirconium to the eight REEs at acidic pH. These results confirm and complement previous investigations with three elements [11]. For the chosen approach (to elucidate biosorption capabilities of microorganisms without further genetic or chemical modifications at low pH), the better biosorption results with live versus dead biomass confirmed the importance of cell integrity for biosorption. Due to discrimination between HREEs and LREEs, the gram-positive *B. subtilis* seems suitable for biosorption of HREEs. Due to the lower need of biomass to achieve a relevant biosorption of all REEs, eukaryotes—especially fungi and yeasts such as *Pichia* sp.—should be given priority. Nevertheless, gram-negative marine bacteria show a high potential for biosorption of all REEs (*L. methylohalidivorans*) and HREEs (*P. inhibens*). *Pichia* sp. could be used successfully to recover anthropogenic gadolinium from wastewater. For successful application strategies, attention should be paid on REE/BM ratios, competition experiments and enlightenment of biosorption mechanisms based on the biochemical and physical background.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2075-4701/10/7/954/s1>, Table S1: Comparison of the relative biosorption of four REEs by *B. subtilis* with the relative biosorption of eight REEs by *B. subtilis* at pH 2.5. Figure S1: Relative biosorption and amount of REE uptake by *B. subtilis*. Figure S2: Relative biosorption by *L. methylohalidivorans* and *P. inhibens* at different amounts of biomass. Figure S3: REE biosorption by *Pichia* sp. at two different amounts of biomass. Figure S4: Relative biosorption by *B. subtilis* immobilized in 1.5% and 2% agarose. Figure S5: Relative biosorption by *C. chromoblastomyces*.

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