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Screening and selection of technologically applicable microorganisms for recovery of rare earth elements from fluorescent powder

Abbreviated running headline: Bioleaching of REE from FP

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BAM - Barium Magnesium Aluminate
CAT - Cerium Magnesium Aluminate
CBT - Cer-Gadolinium Magnesium Pentaborate
FP - fluorescent phosphor
HP - Halophosphate
HPLC – High Pressure Liquid Chromatography
K. xylinus- Komagataeibacter xylinus
L. casei - Lactobacillus casei
LAP - Lanthanum Phosphate
REE - Rare Earth Elements
XRD - X-ray diffraction analysis
XRF - X-ray fluorescence analysis
Y. lipolytica – Yarrowia lipolytica
YOE - Yttrium-Europium-Oxid

Abstract

Rare Earth Elements (REE) are essential elements in many new technology products. Up to now, recycling is poorly established and no environmentally friendly strategies are applied. Modern biotechnologies like bioleaching can contribute to overcome the current limitations. In this study, we investigated bioleaching approaches exemplary for fluorescent phosphor (FP), which is accumulated during the recycling of fluorescent tubes and energy saving bulbs. A broad spectrum of different microorganisms were tested regarding their potential to leach REE from FP. Among them were classical acidophilic microorganisms, as well as various heterotrophic ones, producing organic acids or metal complexing metabolites, or having a high metal tolerance. Larger amounts of REE were leached with the strains *Komatogateibacter xylinus*, *Lactobacillus casei,* and *Yarrowia lipolytica*. Besides the COOH-functionality, also other biotic processes contribute to metal leaching, as comparison with indirect leaching approaches showed. Among the different REE components of the FP preferably the oxidic red dye yttrium europium oxide (YOE) was leached that contain the critical REE yttrium and europium. The results provide the basis for the development of an environmentally friendly recycling process for REE from waste materials.

Keywords: Rare Earth Elements, Fluorescent phosphor, bioleaching, heterotrophic microorganisms, recycling

1 Introduction

Rare Earth Elements (REE) are assigned to the group of critical raw materials (European 2014). They are a part of nearly all new technologies (e. g. computer flat screens and lasers, as well as highly effective magnets for wind mills, and electric cars,) (Schüler, Buchert et al. 2011). Nevertheless, the end-of-life recycling-rates for REE are still less than one percent (Reck and Graedel 2012). Currently, about 175 tons of REE containing fluorescent phosphor (FP) from fluorescent bulbs and energy saving bulbs are yearly accumulated in Germany (Gallenkemper and Breer 2012, Riemann 2014). Keeping in mind that FP contains about ten percent of REE-oxides (Haucke, Huckenbeck et al. 2011), it can be estimated that these compounds account for one percent of the REE imports to Germany (Schüler, Buchert et al. 2011). Despite the increasing application of LEDs, there are still considerable amounts of compact fluorescent lamps in circulation, and moreover, during the last years huge amounts were stored. Besides, also LEDs contain fluorescent phosphors, although the amounts are smaller (Lim, Kang et al. 2013). To our knowledge, there is no existing industrial recycling process for waste FP, even though there are many studies about possible strategies. These approaches use strong inorganic acids or toxic chemicals (Tanaka, Oki et al. 2013).

Bioleaching methods are environmentally friendly alternatives to classical approaches. In contrast to conventional leaching methods that require a constant influx of reagents, in case of bioleaching the agents are directly produced in the system (Beolchini, Fonti et al. 2012). For these processes there are basically two strategies: At first, the classical bioleaching with acidophilic microorganisms as it is industrially used for copper leaching. These processes require an acidic pH-value as well as iron or sulfuric compounds that are not part of REE-waste. The other option is to use chemoorgano-heterotrophic microorganisms that mobilize metals mainly by the produced metabolites. Possible metabolites are metal-binding molecules like siderophores (Fe) and chalkophores (Cu), which can unspecifically bind also other metal ions. Furthermore, organic acids have a high potential to leach REE (Goyne, Brantley et al. 2010). Besides the effect of acids, which mobilize the REE-containing phosphor dyes, the leaching process is also influenced by complexation by removing the REE from the chemical equilibrium. An advantage of this process is the tolerance of a broader pH-range by the used microorganisms, as well as the possibility to use cheap nutrients like molasses or glycerin (Bosecker 1997, Krebs, Brombacher et al. 1997). Further strategies applying oxidative or reductive processes are precluded in case of FP, because the REE in FP are already at highest oxidation state, therefore further oxidation is not possible. The redox potential of REE is compared to other elements strongly negative, thus the REE cannot serve as electron acceptor (Morss 1985).

The recovery of metals from anthropogenic wastes by bioleaching was investigated in several publications during the last years. In an early publication Krebs, Brombacher et al. (1997) summarized leaching experiments from different metal containing material with various microorganisms and their metabolic products. Several studies using chemolitho-autotrophic bacteria like *Acidithiobacillus ferrooxidans* and *A. thiooxidans* as well as *Leptospirillum ferrooxidans* for leaching of waste materials, for example electronic scrap or fluorescent powders (Brandl, Bosshard et al. 2001, Zhu, Xiang et al. 2011, Beolchini, Fonti et al. 2012). However, there are also many examples for the application of chemoorgano-heterotrophic microorganisms such as *Aspergillus niger*, *Penicillium simplicissimum*, or *Yarrowia lipolytica*, always connected with a production of organic acids (Talasova, Khavski et al. 1995, Bosshard, Bachofen et al. 1996, Brandl, Bosshard et al. 2001). These organisms were successfully used for the extraction of metals from waste materials such as red mud, fly as, or electronic scrap.

Regarding REE, many publications investigate minerals. Most studies concentrated on monazite, which is a REE containing phosphate mineral. In most of these studies different organic acids that were produced by various microorganisms were used as leaching agents (Hassanien, Desouky et al. 2013, Shin, Kim et al. 2015, Maes, Zhuang et al. 2017). Other researchers proved that a mobilization of REE can be mediated by siderophores (Bau, Tepe et al. 2013). All these studies indicate the significance of microbial metabolites such as organic acids and siderophores for the biogeochemistry of REE.

Only few studies describe the microbial mobilization of REE from secondary resources. Most recently we used the “tea fungus” Kombucha, a symbiotic microbial consortium that is usually used for fermentation of tea and well known for the production of many different organic acids, for the extraction of REE from FP (Hopfe, Flemming et al. 2017). In this study, the FP was incorporated into the cellulosic pellicle during the leaching approach. The accessibility of the FP for the produced cellular metabolites and consequently their application is limited. Furthermore, leaching efficiency was too low for a technical application. Another recent study used *Gluconobacter oxydans* that produced gluconic acid for the extraction of REE from spent fluid cracking catalysts (FCC) (Reed, Fujita et al. 2016).

In summary, various articles demonstrate the principal ability of different microorganisms to leach anthropogenic waste products, but only few studies consider REE containing wastes. On the other hand, many studies describe microbial mobilization of REE from ores suggesting that it should be possible to leach REE also from secondary resources. In these studies, mainly organic acids, but also metal chelating molecules such as siderophores were identified as responsible agents. Therefore, in the present study several microorganisms producing various organic acids were selected and investigated regarding their ability to leach REE from spent FP.

2 Material and methods

2.1 Fluorescent Phosphor

Spent FP was provided by Larec Lampen-Recycling Gesellschaft mbH (Germany). FP of the same batch as described in Hopfe, Flemming et al. (2017) was splitted in amounts of 0.85 g and treated as previously described. The elemental composition of the FP was determined in detail in Hopfe, Flemming et al. (2017) by x-ray fluorescence analysis (XRF). These data were used in the present study as reference values. Furthermore, x-ray diffraction analysis (XRD) was used for determination of the single compounds of FP. A PANalytical EMPYREAN θ-θ diffractometer in a continuous step mode from 5 to 80°2θ with a step width of 0.016°2θ and a total time of 2 h and 4 min was used. The device was equipped with a Co tube (operating at 35 kV and 35 mA) and a Fe filter, a X'Celerator solid state strip detector (using 64 of 128 channels), an automatic divergence slit, and a 15 mm beam mask, for a constant irradiated area of 10 to 15 mm^2. The amount of glass in the FP was estimated by visualizing with phase-contrast microscope.

2.2 Microorganisms and cultivation

Microorganisms were selected based on leaching data given in literature e. g. Krebs, Brombacher et al. (1997) and Shin, Kim et al. (2015). Depending on the microbial strain and the envisaged metabolites, the microorganisms were cultured on different media. Detailed information are listed in Table S1 of supplementary material. *Acidithiobacillus ferrooxidans* DSM-No: 14882 und *Acidithiobacillus thiooxidans* DSM-No: 14887 were chosen as representatives for chemolitho-autotrophic acidophilic bacteria. All other selected microorganisms belong to the group of chemoorgano-heterotrophs: *Bacillus licheniformis* DSM-No: 8785 (production of polyglutamic acid), *Burkholderia glumae* DSM-No: 9512 (production of oxalic acid), *Corynebacterium callunae* DSM-No: 20147 and *C. stationis* DSM-No: 20305 (production of glutamic acid), *Komatogateibacter xylinus* DSM-No: 2325 (strain of the mixed culture Kombucha), *Lactobacillus casei* DSM-No: 20011 (production of lactic acid), the yeasts *Priceomyces haplophilus* DSM-No: 70365 and *Yarrowia lipolytica* DSM-No: 3286 (production of citric acid), *Pseudomonas fluorescens* DSM-No: 50090 and a strain of our own lab collection (formation of the siderophore pyoverdine), *Streptomyces acidiscabies* (production of siderophores of hydroxamate type (Dimkpa, Svatos et al. 2008)). *Lysinibacillus sphaericus* JG-A13, JG-B37 Iso 3, JG-B58, JG-B5T, and JG-C34 (isolates of a uranium mining waste pile, heavy metal tolerant, production of different organic acids and siderophores) (Selenska-Pobell, Panak et al. 1999).

2.3 Leaching experiments

The leaching experiments were performed as previously described (Hopfe, Flemming et al. 2017). Accordingly, the microbial strains were precultured in the respective medium at room temperature on a rotary shaker at 300 rpm. 30 ml of the same medium containing 0.85 g of FP was inoculated with 1 ml of the preculture and cultured at the same conditions. As control, cultivation experiments without FP or without microorganisms were performed. Approaches demonstrating a significant release of REE were investigated in more detail and the influence of microbial metabolites was studied in indirect leaching approaches. For these experiments, 30 ml of cell free supernatant were mixed with FP. In case of the controls, sterile medium was used instead of the supernatant. In addition Y_2O_3 (Aldrich) and $LaPO_4$ (Alfa Aesar), corresponding to the red dye yttrium-europium oxide (YOE) and green dye lanthanum phosphate (LAP) of the FP, were used as model substances in leaching experiments. Furthermore, leaching experiments with single lamp phosphors were done. For these leaching approaches, 0.3g of each YOE, LAP, CBT, CAT, or halophosphate (Leuchtstoffwerk Breitungen GmbH, Germany) were incubated with a 3 ml *Y. lipolytica* culture in a 6-well-plate. Samples were taken at the end of the experiments. Furthermore, as a control, each of 0.1 g FP was incubated with solutions of commercial organic acids and the siderophore deferoxamine E. In all cases, applied amounts of the leaching agents provided the same number of binding sites (0.83 mol/l).

2.4 Analytical methods

Liquid samples of 1 ml were taken from bioleaching approaches during incubation. In controls without FP, growth of microorganisms was monitored by determining the OD at 600 nm. Particles were removed by centrifugation for 10 min at

15000 rpm (Mirko 12-24, Hettich-Zentrifugen,) and pH was measured in the supernatants. The concentration of different elements (Mg, Al, Si, P, Ca, Y, Ba, La, Ce, Eu, Gd and Tb) was determined by ICP-MS in 3 replicates at normal resolution, using an internal standard of 5 µg/l Rh at a rf-power of 1100 W with a quadrupole mass filter (Elan 9000). It was not possible to separate the cells from the FP grains, thus hindering an analysis of the solid residues. Organic acids in relevant supernatants were analyzed with High Pressure Liquid Chromatography (HPLC) using an Agilent 2000 device equipped with a DAD detector at 210 nm (column: Nucleogel® ION 300 OA, conditions: 70 °C, 90 min, 5 mmol/l H_2SO_4 isocratic, 0.4 ml/min). Citric acid and isocitric acid were not separated, therefore the concentration of both isomers were determined in total.

Amount of each dye dissolved in each experiment was inferred indirectly by finding the combination of amounts of dissolved dyes best explaining the elemental composition of the supernatant measured by ICP-MS using a constraint nonlinear least squares algorithm. To infer the composition of each dye in the FP we calculated all possible solutions explaining the observed mineral and chemical composition (XRD and XRF analysis) within the literature doping range for each dye. The range of the non unique solutions was negligible compared to the statistical variation.

3 Results

3.1 Composition of Fluorescent Phosphor

A detailed knowledge of the material composition is necessary to interpret bioleaching results. A general description of spent FPs is given in Hopfe, Flemming et al. (2017). The present study used material of the same batch as in Hopfe, Flemming et al. (2017). Based on the previous results and further more detailed XRD-measurements, the quantitative composition of the compounds was calculated. Standard deviations for all calculations were below 0.02%. The results are depicted in figure 1. According to the

XRF-measurements, the applied FP contains 19.05% REE, which consisted mainly of yttrium (12.1%), lanthanum (3.4%), and cerium (1.4%). The REE are only part of the so called triband dyes, which represent more than the half of the whole FP. Yttrium is totally bound in the red dye YOE that occurs in an amount of nearly 10% of FP. In opposite, lanthanum and cerium are distributed across the green dyes LAP, cerium magnesium aluminate (CAT), and cerium magnesium pentaborate (CBT). LAP represents with 5.9% of FP the largest fraction in this group, whereas CAT and CBT occur only in minor amounts. As blue dyes, barium magnesium aluminate (BAM, 5.6%) and the exceptional blue-green $BaSi_2O_5$:Eu_2 comprising 27.1%, were detected (Nakanishi and Tanabe 2008). The doping values of the dyes are almost always lower than 0.1%, except for CAT with 6.7% terbium, BAM with 2.0% europium, and YOE with 0.5% europium. The other half of the FP consisted mainly of the old white dye halophosphate as well as alumina and glass residues.

Figure 1

3.2. Abiotic leaching

Organic acids and other metal complexing metabolites have been described to mediate metal release in several bioleaching studies. As a control, the leaching performances of some commercial organic acids and the siderophore deferoxamine E were tested. In all cases, applied amounts of the leaching agents provided the same number of binding sites (0.83 mol/l). After one week, REE-concentrations in the supernatants were measured (figure 2). In contrast to the tested organic acids release of REE was only low in case of deferoxamine E. Further, the leaching values differed between the different organic acids. For example, although both possessing one COOH-group, lactic acid (0.27%) mobilized only about one tenth of the REE compared to acetic acid (2.60%). With an amount of 2.90% REE, malonic acid was the most efficient leaching reagent. Furthermore, experiments with mixed organic acids and the siderophore deferoxamine E were performed. The addition of deferoxamine increased the REE only in case of gluconic and acetic acid (0.19% resp. 4.27% in after one week). In another leaching approach 0.83 mol/l citric acid (corresponding to 2.49 mol/l binding sites) was used resulting in a leaching amount of 4.0 % that was significantly higher compared to a concentration of 0.28 mol/l citric acid (corresponding 0.83 mol/l binding sites) with 2.33 % of REE release.

Figure 2

3.3 Leaching-tests with different microorganisms

For the leaching experiments various microorganisms producing organic acids or siderophores were used to screen their REE leaching performance. The approaches were categorized into groups according to the leaching success: no bioleaching without or with microbial growth; low bioleaching rate (mobilization of up to 0.2% of REE after two weeks) as well as high bioleaching rate (mobilization of 5% and more REE), see also table S2 of supplementary material.

Acidithiobacillus ferrooxidans and *Acidithiobacillus thiooxidans* were selected as example for chemolitho-autotrophic bacteria (Bosecker 1997, Krebs, Brombacher et al. 1997). In our case, growth of both strains was inhibited in presence of FP and subsequently no bioleaching could be monitored. A possible reason is an increase of the pH-value caused by the FP. REE are mobilized by acidification after adjustment of pH by the addition of huge amounts of sulfuric acid. Accordingly, chemical leaching occurred instead of bioleaching. Therefore, further leaching experiments concentrated on the use of chemoorgano-heterotrophic microorganisms that could grow also at neutral pH-values and produce organic acids or complexing reagents that contribute to bioleaching (Bosecker 1997).

The growth of some heterotrophic strains like the glutamic acid producing bacteria *Corynebacterium stationis* and *Corynebacterium callunae* as well as the citric acid producing yeast *P. haplophilus* (Krebs, Brombacher et al. 1997) was inhibited in the presence of FP. Other strains like the siderophore producer *S. acidiscabies* (Dimkpa, Svatos et al. 2008) grew in the presence of FP but without REE release. In case of some strains leaching rates were slightly enhanced by cultivation on alternative media (table S2 in supplementary material). In other cases addition of 2% glycerin slightly increased the bioleaching activity.

The highest leaching values after two weeks were obtained with the bacteria *Komatogateibacter xylinus* and *Lactobacillus casei* as well as with the yeast *Yarrowia lipolytica* comprising a total release of REE of 12.6 %, 10.6 %, and 6.1 %, respectively. In case of *K. xylinus*, cell growth could not be monitored by OD600 measurements due to the formation of bacterial cellulose. Growth of *L. casei* and *Y. lipolytica* was comparable to control experiments as demonstrated by control experiments, reaching an OD600 of 0.4 and 24.8, respectively. All three strains were selected in order to investigate cell growth, development of pH, as well as formation of organic acids in more detail.

Figures 3 and 4

3.4 Investigation of the bioleaching activities of K. xylinus, L. casei, *and* Y. lipolytica

The bioleaching results are visualized in figures 3 and 4, as well as summarized in table S3 of supplementary material. The leaching of the single dyes was calculated by mathematical methods using the obtained ICP-MS data for REE. Samples were taken at different time points and the overall REE-concentrations, pH as well formation of organic acids were determined. Furthermore, the leaching of the single dyes was calculated by mathematical methods using the obtained analytical data. In order to investigate the influence of microorganisms, besides direct bioleaching of FP in presence of microorganisms also indirect leaching using spent culture broth was done.

Figures 3a, b, and c show the results of the direct leaching approach using *K. xylinus*, *L. casei*, and *Y. lipolytica*, respectively. For all three strains, the concentration of dissolved REE increased during the whole leaching time (figure 3a). The highest release of REE was monitored for *K. xylinus* with 12.6%, followed by *Y. lipolytica* (10.6%), and *L. casei* (6.1%). Comparison with control experiments prove that this release is mainly caused by microbial activity whereas the media has only little effect on mobilization (up to 1.1% in case of *L. casei* medium). In case of *K. xylinus* and *Y. lipolytica* only small amounts of REE were mobilized during the first three days. In contrast, in case of *L. casei* the REE were dissolved over the whole period of time. In case of *Y. lipolytica*, the leaching rate decreased after 7 days. In case of *K. xylinus* and *L. casei* no saturation was visible after 14 days.

Phosphate of the growth medium might interact and precipitate with dissolved REE (Maes, Zhuang et al. 2017). Therefore phosphate was removed from the medium of *Y. lipolytica* as described by Brisson, Zhuang et al. (2016) and Shin, Kim et al. (2015) for bioleaching of monazite. In this case, the yeasts have to cover their phosphorous needs from FP (Shin, Kim et al. 2015). In our experiments the REE release after two weeks was much lower than in the approaches with added phosphate (7.7% or 9.7 % in case of potassium phosphate substituted by potassium or potassium chloride compared to 10.6% in case of potassium phosphate containing medium). Therefore, all further experiments were done with phosphate containing medium.

In case of all three strains, REE release was accompanied by a decrease of pH in the media. In the first three or seven days the pH-value decreased in case of all three strains, afterwards it was nearly constant. After two weeks, approaches with *Y. lipolytica* showed the lowest pH (with pH 2.5), followed by *K. xylinus* (with pH 2.7), and *L. casei* (with pH 4.1), although REE release was the highest in case of *K. xylinus*. Apparently, leaching effect depended also from other factors than just the H^+-concentration.

All strains were selected due to their ability to produce organic acids. It is obvious that the decrease of pH is related to the production of organic acids affecting also the mobilization of REE. Therefore the production of organic acids was investigated in more detail and the production of organic acids was confirmed in all three cases (figure 3b). The production rates corresponded the growth rates of the respective microorganisms. The type of produced organic acid was strain dependent. *K. xylinus* produced (iso)citric, gluconic, and in smaller amounts, also tartaric acid, whereas *L. casei* and *Y. lipolytica* produced only lactic or (iso)citric acid, respectively. The overall COOH-concentration was calculated as the number of COOH-groups per mole organic acid. The highest COOH-amount was monitored in case of *Y. lipolytica* (437.0 mmol/l), showing also the lowest pH after two weeks of incubation. Interestingly, in case of *K. xylinus* and *Y. lipolytica*, the amount of produced organic acids was much higher in case of the samples containing FP than the amount in case of the controls without FP (*K. xylinus*: 240.4 mmol/l resp. 85.1 mmol/l, *Y. lipolytica*: 437.0 mmol/l resp. 94.1 mmol/l). In contrast, the organic acid production in the approaches with *L. casei* was nearly equal in FP samples and control.

Figure 3c depict the results of the mathematical calculation. Obviously, mainly the red dye YOE was leached in case of all three strains. The leaching rates were consistent with the overall REE-leaching rates. Accordingly, YOE was leached by *K. xylinus* with the highest amount (27.5%), followed by *Y. lipolytica* (23.5%), and *L. casei* (11.5%). In the approaches with *K. xylinus* and *Y. lipolytica* also $BaSi_2O_5:Eu^{2+}$ was leached. The leaching rate was low during the first days and increased up to the end of observation period. The overall leaching amount of $BaSi_2O_5:Eu^{2+}$ after two weeks was with 10.7% resp. 11.7% much smaller than that of YOE. In case of *Y. lipolytica*, minor amounts of BAM were leached as well. All other dyes were nearly not dissolved. Experiments with pure $LaPO_4$ and pure Y_2O_3 confirm these results. After two weeks, less than 0,015% of La were released from $LaPO_4$ in all cases. In contrast much larger amounts of Y were released from Y_2O_3 (*K. xylinus*: 2.8%, L. casei: 1.6%, *Y. lipolytica*: 3.5%).

Y. lipolytica was used for additional leaching experiments of single fluorescent dyes. After two weeks 7.0% of YOE and 1.3% of HP were mobilized, but only 0.2% of LAP and less than 0.01% of CBT and CAT (figure 5). Accordingly, the calculation results seem to be reliable.

Figure 5

K. xylinus produces bacterial cellulose during growth resulting in the incorporation of FP particles in the cellulosic pellicle. Therefore, FP particles are more difficult to access by the microbial metabolites.

In order to investigate the biotic effects of bioleaching on bioleaching efficiency, the FP was incubated with the cell-free supernatants of spent broth cultures (indirect bioleaching). The results are depicted in figure 4a and 4c. Generally, the amount of mobilized REE after two weeks was considerable lower than that in the direct leaching process. Especially in case of *K. xylinus* this effect is conspicuous (12.6% resp. 2.9%), whereas in case of *L. casei* the difference is much smaller (6.1% resp. 5.8%). Furthermore, the kinetics of the two leaching variants differed. In case of the supernatants, the REE were mainly released at the beginning of the leaching process. The maximum was reached after 3 (*K. xylinus*) or 7 (*L. casei*, *Y. lipolytica*) days. The development of the pH values correspond to these results.

Table S3 of supplementary material presents the amounts of the different organic acids that were observed in the bioleaching approaches after two weeks as well as in the spent culture broth cultures that were used for the indirect leaching approaches. It is conspicuous that the amount of organic acids in the spent culture broth was considerable smaller (overall COOH: *K. xylinus*: 240.4 mmol/l resp. 182.8 mmol/l, *Y. lipolytica*: 437.0 mmol/l resp. 102.8 mmol/l), which corresponds to the lower leaching efficiency. Analogous to the direct leaching approaches, mainly the red dye YOE as well as in smaller amounts the blue-green dye $BaSi_2O_5:Eu^{2+}$ were leached. In case of *K. xylinus* and

Y. lipolytica also minor amounts of BAM were dissolved. The dissolution of the dyes varies in accordance to the overall REE-leaching rate. The highest leaching amount was monitored for *Y. lipolytica* with 15.7% of YOE.

4 Discussion

4.1 Abiotic leaching

FP was used for different leaching experiments with and without microorganisms. In a first test, FP was incubated with different commercial organic acids and the siderophore deferoxamine E as representative for metal complexing metabolites. Although the concentrations of organic acids were adjusted that the amount of COOH was similar, the leaching values varied strongly in dependence of the type of the acid. The REE release did not correlate with the number of COOH-functionalities or the stability constants. For example, among the organic acids possessing one COOH-functionality, the highest chemical leaching value was measured in case of acetic acid. Lactic acid, although possessing also one COOH-group, leached only about 1/10 of REE in comparison to acetic acid. According to Moeller, Martin et al. (1965) and Byrne and Li (1995) the stability constant of acetic acid accounts for 1.8 and that of lactic acid for 2.6. Therefore a correlation to the stability constant is not visible. The good performance of citric acid corresponds to several other bioleaching studies using citric acid producing bacteria (Brandl, Bosshard et al. 2001, Hassanien, Desouky et al. 2013, Zhuang, Fitts et al. 2015). Besides organic acids, the siderophore deferoxamine E was used as leaching agent and REE release was monitored to a minor extent. Some studies describe the usage of siderophore producing microorganisms for bioleaching (Desouky, El-Mougith et al. 2011, Bau, Tepe et al. 2013). It was possible to further enhance the leaching by a combination of deferoxamine and acetic acid. For all other tested acids such an effect was not observed. Similarly, Reichard, Kretzschmar et al. (2007) describes the synergistic effect of low-molecular-mass organic acids and the siderophore deferoxamine B for iron-leaching from the mineral goethite. It can be assumed that it is possible to further enhance leaching by other combinations of organic acids and metal complexing compounds.

4.2 Test of different microorganisms

For the bioleaching experiments, different microorganism strains were selected on the basis of literature and the abiotic leaching results. In a first step bioleaching of FP with the classical acidophilic microorganisms *A. thiooxidans* and *A. ferrooxidans* was tested. In contrast to other studies investigating the bioleaching of electronic scrap (Brandl et al. 2001, Beolchini et al. 2012) the FP increased the pH-value in the medium, probably due to an amphoteric effect of metal oxide compounds in FP. As a result, the microbial growth was inhibited and no bioleaching could be monitored. Furthermore, leaching approaches using chemolitho-autotrophic organisms require iron or sulfur sources. An extensive addition of these elements to the FP seemed not to be appropriate for the design of simplified recycling processes.

Therefore, further leaching experiments concentrated on the use of chemoorgano-heterotrophic microorganisms that can grow also at neutral pH-values and produce metal mobilizing metabolites. Growth of some of the selected strains was inhibited in presence of FP, too. Inhibition of microbial growth by pulp density has been described in many cases, e.g. Brandl, Bosshard et al. (2001). Other strains grew in presence of FP, but leaching rates were rather low or negligible. However, some preliminary experiments indicated an influence of the growth substrate that could have a positive effect on REE release assuming that leaching efficiencies can be significantly enhanced by the growth conditions. These observations should be studied in more detail in future. Especially the addition of glycerin had an influence on the growth and the leaching. It is known that glycerin can be metabolized to different organic acids (Silva, Mack et al. 2009) probably enhancing the mobilization of REE in our study.

Among the tested strains, reasonable bioleaching rates were obtained only with the bacteria *K. xylinus* and *L. casei* and the yeast *Y. lipolytica*. The leaching process with these strains was investigated in detail in further experiments.

4.3 Leaching with K. xylinus, L. casei, *and* Y. lipolytica

4.3.1 General considerations

In our first study investigating the bioleaching of FP with the mixed culture Kombucha (Hopfe, Flemming et al. 2017), high leaching values were monitored with the acetic and gluconic acid producing isolate *Komatogateibacter hansenii*. Based on these results, we used *K. xylinus* in the present study, which is a close relative of *K. hansenii* and which is more typical for Kombucha cultures (Chen and Liu 2000). In Shin, Kim et al. (2015) *Acetobacter acetii*, another related bacterium, was the most efficient microorganism for leaching of REE phosphates.

Y. lipolytica is besides the fungus *Aspergillus niger* the typical producer of citric and isocitric acid (Förster 2006). Citric acid was identified as the most effective low molecular weight organic acid for leaching of REE from phosphate minerals in the study of Goyne, Brantley et al. (2010). In addition, Talasova, Khavski et al. (1995) used spent culture broth of *Y. lipolytica* for the extraction of aluminum, scandium, and yttrium from red mud. The bacterium *L. casei* is used in cheese production and produces mainly lactic acid (Grigoriev, Nordberg et al. 2012).

In case of *K. xylinus* and *Y. lipolytica*, the leaching rates increased after an initial delay of one day probably due to an adaption of the microorganisms to the FP, as described in other studies for other materials (Brandl, Bosshard et al. 2001, Hassanien, Desouky et al. 2013). In the study of Zhu, Xiang et al. (2011) the inoculation concentration of microorganisms did not influence the bioleaching efficiency of printed circuit boards. In another study investigating the bioleaching of REE from red mud by fungi, Qu and Lian (2013) showed that in case of 2% pulp density, biomass concentration and leaching rate were higher than for lower pulp densities, indicating that the leaching material can promote growth of microorganisms in some cases. In case of leaching with *L. casei*, the release of REE started immediately after addition of medium and microorganisms to FP, most likely due to an abiotic leaching by the medium.

In contrast to *K. xylinus* and *L. casei,* the maximum metal release seems to be reached after two weeks in case of leaching with *Y. lipolytica*. The bioleaching experiments were stopped after two weeks in order to obtain comparable results. Although it can be expected that higher leaching values can be obtained by prolongation of the incubation time, probably leaching rate would decrease soon, because the pH-values and organic acid concentrations already reached their minimum resp. maximum.

It is possible, that some of the REE adsorbed to the microbial cells or formed secondary precipitates, thus lowering the leaching efficiency, as indicated by studies of (Delvasto, Ballester et al. 2009, Emmanuel, Vignesh et al. 2011). On the other hand, the study of Reed, Fujita et al. (2016) suggests that only small amounts of REE are adsorbing to the microorganisms. However, for an application the nature of immobilized REE is not important but the amount of dissolved REE as this is the only state that can be extracted in subsequent processes with further processes and reused in new products.

4.3.2 Organic acid production during bioleaching process

In further experiments the underlying reasons for the different leaching rates were investigated. According to Bosshard, Bachofen et al. (1996) and Goyne, Brantley et al. (2010) leaching by organic acids is mediated through acidolysis and complexolysis by forming of inner-sphere surface complexes and metal-ligand complexes. Based on these findings several microorganisms that have been described to produce different organic acids were selected and tested regarding their leaching activities. It can be suggested that organic acids that are produced by these microorganisms mediate the mobilization of REE.

Analogous to Reed, Fujita et al. (2016), increase of leaching rate was slower than the decrease of pH-value. Although the pH-value dropped already after one day in case of *K. xylinus* and *Y. lipolytica*, significant leaching activity started after three days. The earlier increase of leaching in case of *L. casei* could be caused by abiotic bioleaching by the culture medium, as it was also observed in our previous study with Kombucha (Hopfe, Flemming et al. 2017).

In general, REE release rose with increasing acid concentration, but without a direct correlation between pH-value, overall acid- or COOH-concentrations and FP dissolution. For example after two weeks, the lowest pH-value (2.5) and highest acid concentration (437 mmol/l COOH) were reached in approaches with *Y. lipolytica*, but *K. xylinus* showed highest leaching amount (12.6%) within the same time. *K. xylinus* produced (iso)citric, gluconic and tartaric acid, whereas *Y. lipolytica* produced only (iso)citric acid. The overall COOH-concentration was higher in case of *Y. lipolytica* with 437 mmol/l COOH compared to 240.0 mmol/l COOH in case of *K. xylinus*. In literature (Goyne, Brantley et al. 2010, Hassanien, Desouky et al. 2013, Zhuang, Fitts et al. 2015) as well as in our abiotic leaching experiments, REE mobilization strongly depended on the type of organic acid while citric acid showing the highest leaching rate. Similar results were obtained by Brandl, Bosshard et al. (2001) for leaching of electronic waste materials with fungi. *Aspergillus niger* produced higher amounts of organic acids, nevertheless, with *Penicillium simplicissimum* higher leaching rates were monitored. In Reed, Fujita et al. (2016) higher leaching rates of retorted catalysts were obtained with culture supernatant containing 12.5 mmol/l gluconic acid as with abiotic solutions containing up to 90 mmol/l gluconic acid. Possibly, also other mechanisms than organic acids contribute to leaching processes.

In case of *K. xylinus* and *Y. lipolytica*, concentrations of organic acids in samples (240.4mM resp. 437.0 mmol/l) were higher than in the corresponding controls

(85.1 mmol/l resp. 94.1mM). In case of *L. casei*, the organic acid production was nearly similar in samples and controls. A possible explanation for the enhanced organic acid production in samples is given by the study of Karasu-Yalcin, Bozdemir et al. (2010). For maximizing the citric acid production of *Y. lipolytica* 40 g/l $CaCO_3$ was added to the medium as at pH-values below 5 the citric acid production decreases and some polyalcohols like erythriol, arabitol and mannitol are accumulated. As the FP also increases the pH-value, buffering could enhance the production of organic acids. Also Bosshard, Bachofen et al. (1996) describes such an effect for bioleaching of fly ash from municipal waste incarnation with *A. niger*. Another reason could be the leached REE itself. In Emmanuel, Vignesh et al. (2011) it is reported, that REE induce and enhance the production of organic acids. Other bioleaching studies describe changes in the organic acid pattern, when microorganisms were incubated with or without material to be leached (Qu and Lian 2013).

4.3.3 Leaching of different components of FP

In a next step we analyzed the dissolution of single REE containing triband dyes of the FP by mathematical calculation out of the data from elemental analysis. It could be shown, that in case of all approaches, mainly the yttrium and europium containing red dye YOE was leached. Accordingly, mainly the strategically important REE were leached from FP (Golev, Scott et al. 2014). In smaller amounts, also the untypical dye $BaSi_2O_5:Eu^{2+}$, which contains only small amounts of europium in the doping (less than 0.1%), was leached. Finally, some BAM was solubilized, also containing only europium in the doping (about 2%). All other dyes were leached only in minor amounts. The results of BAM, an aluminate, are possibly a calculation artefact, since aluminates are poorly soluble (Binnemans, Jones et al. 2013). For comparison, bioleaching approaches with pure Y_2O_3 and pure $LaPO_4$ were performed. The results of these experiments approve the above results, as yttrium from Y_2O_3 could be mobilized by the microorganisms, but lanthanum from $LaPO_4$ nearly not. Also experiments using the single fluorescent dyes YOE, LAP, CBT CAT and HP confirm these results. YOE and HP were mobilized, LAP, CBT and CAT (nearly) not.

Similar results can be derived from data given in the literature. However, these studies concentrated on the release of REE and did not consider single components. In our previous study (Hopfe, Flemming et al. 2017) with Kombucha, more than 60% of Y_2O_3 were leached, but only 0.01% of $LaPO_4$ throughout the same time period. No calculation for leaching of single triband dyes from FP was done, instead, leaching of single REE was compared. A more than proportional amount of yttrium and europium were leached. Consequently, leaching of mainly YOE was postulated. As $BaSi_2O_5:Eu^{2+}$ and BAM contains only small amounts of REE, these compounds were not considered. In the study of Reed, Fujita et al. (2016), mainly yttrium and europium were mobilized from FP too, indicating that the same dyes as in our studies were mobilized. Also in Maes, Zhuang et al. (2017) REE-oxides from roasted REE-monazite-ore are much more soluble than untreated REE-phosphates. Similarly, chemical leaching experiments using strong acids or toxic chemicals proved higher solubility of YOE (Rabah 2008), whereas REE-phosphates and aluminates were less soluble (Binnemans, Jones et al. 2013). On the other hand, several studies about leaching of monazite, a REE-phosphate mineral with varying leaching rates between 0.1% and 75% exist (Hassanien, Desouky et al. 2013, Shin, Kim et al. 2015, Brisson, Zhuang et al. 2016). No leaching data were found in case of $BaSi_2O_5:Eu^{2+}$ (Nakanishi and Tanabe 2008).

4.3.4 Indirect bioleaching of FP

Apart from microbial metabolites secreted into the medium, also the direct contact of cells with the substrate may influence the solubility of the REE (direct bioleaching). Such benefits were described in several bioleaching studies for ores (Hassanien, Desouky et al. 2013, Brisson, Zhuang et al. 2016) and waste materials (Beolchini, Fonti et al. 2012).

In most cases, lower leaching rates were obtained with indirect approaches compared to direct approaches. Contrarily, in the study of Bosshard, Bachofen et al. (1996) it depended on the element whether the leaching was higher in a direct or indirect process. Reed, Fujita et al. (2016) obtained equal results for direct and indirect leaching approaches.

In this study, for all three strains, higher leaching amounts were measured in case of direct leaching approaches, including also the leaching of single triband dyes. In indirect leaching approaches all dyes were proportionally leached with lower amounts. These findings correlate to our previous study describing different abiotic leaching approaches using mixtures of different organic acids that led to lower bioleaching rates than biotic leaching approaches in the presence of microorganisms or spent culture broth (Hopfe, Flemming et al. 2017). This indicates that other processes or metabolites participate in leaching than just single organic acids. These results correlate to the studies of Maes, Zhuang et al. (2017) and Corbett, Eksteen et al. (2017), investigating the microbial mediated REE release from monazite. Apparently, the interaction of FP with the microorganisms has a conducive influence on the leaching process. A possible explanation is the increased organic acid concentration in samples of direct leaching approaches compared to the culture broth used for indirect leaching, with the reasons described above. In Qu and Lian (2013) and also in our previous study using Kombucha (Hopfe, Flemming et al. 2017), the leaching substrate had a positive influence on the cell growth, probably accompanied by the formation and secretion of a higher amount of metabolites. Therefore, potentially also more leaching metabolites were secreted. In the present study, cell growth was measured in controls by OD600 measurement and microscopically compared with the samples. Cell growth was similar in samples and controls, therefore possibly only the amount of leaching metabolites increased. It is also possible, that the FP induces the production of other metabolites like siderophores, which enhance leaching (Bau, Tepe et al. 2013). Apart from this, a direct interaction of FP with microbial metabolism could be possible.

Nevertheless, indirect approaches are according to Brandl, Bosshard et al. (2001), Qu and Lian (2013) and Maes, Zhuang et al. (2017) promising: Indirect leaching processes have several advantages, for example biomass is not in direct contact with the leaching material, enabling the continuous usage of microorganisms and repeated leaching of the waste material, as well as addition of supporting agents is possible. Besides, production of leaching metabolites can be optimized and higher waste concentrations as well as subsequent extractions steps can be applied, resulting in higher absolute leaching rates.

4.3.5 Overall bioleaching and outlook

The overall bioleaching amount of REE from FP ranged between 5.0% in case of *L. casei* and 12.6% in case of *K. xylinus*. The obtained bioleaching amounts are similar or slightly higher than in our previous study ranging between 5.2% and 7.9% when using the mixed culture Kombucha over the same period and FP from the same batch (Hopfe, Flemming et al. 2017). Several other studies investigated the bioleaching of different types of FP, too. Reed, Fujita et al. (2016) obtained a leaching amount of 2% of REE when applying gluconic acid producing microorganisms. Beolchini, Fonti et al. (2012) reached leaching amounts of up to 70% in case of yttrium at a solid concentration of 2% by using acidophilic bacteria. In this approach Fe^{2+} and Fe^{3+} were added in order to enhance the bioleaching activity. Metal release was also influenced by the ferric ions. Similar variations of leaching amounts were also monitored for monazite, a REE phosphate mineral. In Shin, Kim et al. (2015) only 0.1% of REE were leached, whereas in Hassanien, Desouky et al. (2013) leaching amounts of up to 75% were reported. Bioleaching depends on many factors like composition of substrates, microorganisms, cultivation conditions, medium, mixing, or particle size (Brisson, Zhuang et al. 2016), explaining the enormous differences in bioleaching efficiencies.

The efficiency of the described leaching approaches is much lower than that of technical processes applying strong inorganic acids or toxic chemicals. However, in comparison with these processes, bioleaching is much more environmentally friendly. The presented study gives a proof of principle for the application of various heterotrophic microorganisms for REE extraction from technical waste products. Principally the approaches can be transferred to other REE containing waste materials, e.g. magnetic scrap. Several strategies can be applied to enhance leaching efficiencies and develop economic processes, such as the prolongation of leaching time, different cultivation media, repeated leaching of residues, variation of parameters such as stirring, temperature, or solid concentration, addition of chelating compounds, or others. Very recent an interesting approach was described by Maes, Zhuang et al. (2017) aiming the extraction of REE from monazite. The researchers obtained a relatively high leaching amount by combining different methods such as roasting of samples, reuse of leaching solution and electrochemical recovery of REE from leachates. Leaching of REE phosphates could be enhanced by other phosphate solubilizing microorganisms.

5 Conclusion

This study is the first study proving various microorganisms to extract REE from waste products like FP. Good leaching results were obtained with *K. xylinus*, *L. casei*, and *Y. lipolytica*. Higher leaching rates in case of direct leaching approaches indicated, that beneath organic acids, also the interaction with microbial cells influenced the leaching process. In all experiments, yttrium and europium were dissolved selectively from FP. The results form the basis for the development of environmentally friendly recycling strategies of REE containing waste materials. However, the described processes are inefficient and expensive in the current stage, thus requiring an optimization. E-supplementary data of this work can be found in online version of the paper.

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Compliance with Ethical Standards

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References

Bau, M., N. Tepe and D. Mohwinkel (2013). "Siderophore-promoted transfer of rare earth elements and iron from volcanic ash into glacial meltwater, river and ocean water." Earth Planet Sc Lett 364: 30-36.

Beolchini, F., V. Fonti, A. Dell’Anno, L. Rocchetti and F. Vegliò (2012). "Assessment of biotechnological strategies for the valorization of metal bearing wastes." Waste Manage 32(5).

Binnemans, K., P. Jones, B. Blanpain, T. Gerven, Y. Yang, A. Walton and M. Buchert (2013). "Recycling of rare earths: a critical review." J Clean Prod 51: 1-22.

Bosecker, K. (1997). "Bioleaching: metall solubilization by microorganisms." FEMS Microbiol Rev 20: 591-604.

Bosshard, P., R. Bachofen and H. Brandl (1996). "Metal leaching of fly ash from municipal waste incineration by *Aspergillus niger*." Environ Sci Technol 30(10): 3066-3070.

Brandl, H., R. Bosshard and M. Wegmann (2001). "Computer-munching microbes: metal leaching from electronic scrap by bacteria and fungi." Hydrometallurgy 59: 319–326.

Brisson, V., W.-Q. Zhuang and L. Alvarez-Cohen (2016). "Bioleaching of rare earth elements from monazite sand." Biotechnol Bioeng 113(2): 339-348.

Byrne, R. H. and B. Li (1995). "Comparative complexation behavior of the rare earths." Geochim Cosmochim Acta 59(22): 4575-4458.

Chen, C. and B. Liu (2000). "Changes in major components of tea fungus metabolites during prolonged fermentation." J Appl Microbiol 89: 834-839.

Corbett, M. K., J. J. Eksteen, X.-Z. Niu, J.-P. Croue and E. L. J. Watkin (2017). "Interactions of phosphate solubilising microorganisms with natural rare-earth phosphate minerals: a study utilizing Western Australian monazite." Bioprocess Biosyst Eng(40): 929–942.

Delvasto, P., A. Ballester, J. A. G. Muñoz, F., M. L. Blázquez, J. M. Igual, A. Valverde and C. García-Balboa (2009). "Mobilization of phosphorus from iron ore by the bacterium *Burkholderia caribensis* FeGL03." Miner Eng 22(1): 1-9.

Desouky, O. A., A. A. El-Mougith, H. W. A., G. S. Awadalla and S. S. Hussien (2011). "Extraction of some strategic elements from thorium–uranium concentrate using bioproducts of *Aspergillus ficuum* and *Pseudomonas aeruginosa*." Arab J Chem.

Dimkpa, C., A. Svatos, D. Merten, G. Büchel and E. Kothe (2008). "Hydroxamate siderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata L.*) under nickel stress." Can J Microbiol 54(3): 163-172.

Emmanuel, E. S. C., V. Vignesh, B. Anandkumar and S. Maruthamuthu (2011). "Bioaccumulation of cerium and neodymium by *Bacillus cereus* isolated from rare earth environments of Chavara and Manavalakurichi, India." Indian J Microbi 51(4): 488–495.

European, Commission (2014). "Communication from the Comission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions: On the review of the list of critical raw materials for the EU and the implementation of the Raw Materials Initiative." http://eur-lex.europa. eu/legal-content/EN/TXT/PDF/?uri=CELEX:52014DC0297&from=EN (accessed 8 January 2015).

Förster, A. (2006). Die Nutzung der Hefe *Yarrowia lipolytica* zur Produktion von Citronensäure aus nachwachsenden Rohstoffen. Dr. rer. nat., Technische Universität Dresden.

Gallenkemper, B. and J. Breer. (2012). "Analyse der Datenerhebung nach ElektroG über die Berichtsjahre 2009 und 2010 zur Vorbereitung der EU-Berichtspflicht 2012." Fachgebiet III 1.6 (Produktverantwortung), http://www.umweltbundesamt.de/ publikationen/analyse-datenerhebung-nach-elektrog-ueber. (accessed 13 November 2014)

Golev, A., M. Scott, P. D. Erskine, S. H. Ali and G. Ballantyne (2014). "Rare earths supply chains: Current status,constraints and opportunities." Resour Policy 41: 52-59.

Goyne, K. W., S. L. Brantley and J. Chorover (2010). "Rare earth element release from phosphate minerals in the presence of organic acids." Chem Geol 278: 1-14.

Grigoriev, I. V., H. Nordberg, I. Shabalov, A. Aerts, M. Cantor, D. Goodstein, A. Kuo, S. Minovitsky, R. Nikitin, R. A. Ohm, R. Otillar, A. Poliakov, I. Ratnere, R. Riley, T. Smirnova, D. Rokhsar and I. Dubchak (2012). "The genome portal of the department of Energy Joint Genome Institute." Nucleic Acids Res 40: D26-32.

Hassanien, W. A. G., O. A. N. Desouky and S. S. E. Hussien (2013). "Bioleaching of some Rare Earth Elements from Egyptian monazite using *Aspergillus ficuum* and *Pseudomonas aeruginosa*." Walailak J of Sci Technol 11(9): 809-823.

Haucke, E., T. Huckenbeck and R. Otto (2011). Verfahren zur Rückgewinnung seltener Erden aus Leuchtstofflampen. O. AG. Germany. DE102011007669 A1.

Hopfe, S., K. Flemming, F. Lehmann, R. Möckel, S. Kutschke and K. Pollmann (2017). "Leaching of Rare Earth Elements from fluorescent powder using the tea fungus Kombucha." Waste Manage 62: 211-221.

Karasu-Yalcin, S., M. T. Bozdemir and Z. Y. Ozbasc (2010). "Effects of different fermentation conditions on growth and citric acid production kinetics of two *Yarrowia lipolytica* strains." Chem Biochem Eng Q 24(3): 347–360.

Krebs, W., C. Brombacher, P. P. Bosshard, R. Bachofen and H. Brandl (1997). "Microbial recovery of metals from solids." FEMS Microbiol Rev 20(3-4): 605–617.

Lim, S.-R., D. Kang, O. A. Ogunseitan and J. M. Schoenung (2013). "Potential environmental impacts from the metals in incandescent, compact fluorescent lamp (CFL), and light-emitting diode (LED) bulbs." Environ Sci Technol 47: 1040-1047.

Maes, S., W.-Q. Zhuang, K. Rabaey, L. Alvarez-Cohen and T. Hennebel (2017). "Concomitant leaching and electrochemical extraction of Rare Earth Elements from monazite." Environ Sci Technol 51: 1654−1661.

Moeller, T., D. F. Martin, L. C. Thompson, R. Ferros, G. R. Feistel and W. J. Randall (1965). "The coordination chemistry of yttrium and the Rare Earth metal ions." Chem Rev 65(1): 50.

Morss, L. R. (1985). Yttrium, lanthanum, and the lanthanide elements. Standard potentials in aqueous solution. A. J. Bard, R. Parsons and J. Jordan. New York, Basel, Marcel Dekker, Ink. 1: 587-629.

Nakanishi, T. and S. Tanabe (2008). "Preparation of $BaSi_2O_5:Eu^{2+}$ glass ceramic phosphors and luminescent properties." J Light Vis Environ 32(2): 93-96.

Qu, Y. and B. Lian (2013). "Bioleaching of rare earth and radioactive elements from red mud using *Penicillium tricolor* RM-10." Bioresource Technol 136: 16-23.

Rabah, M. A. (2008). "Recyclables recovery of europium and yttrium metals and some salts from spent fluorescent lamps." Waste Manage 28(2): 318-325.

Reck, B. K. and T. E. Graedel (2012). "Challenges in metal recycling." Science 337: 690-695.

Reed, D. W., Y. Fujita, D. L. Daubaras, Y. Jiao and V. S. Thompson (2016). "Bioleaching of rare earth elements from waste phosphors and cracking catalysts." Hydrometallurgy 166: 34-40.

Reichard, P. U., R. Kretzschmar and S. M. Kraemer (2007). "Dissolution mechanisms of goethite in the presence of siderophores and organic acids." Geochim Cosmochim Acta 71: 5635–5650.

Riemann, S. (2014). "Verwertbare Bestandteile von Altlampen.", http://www.lightcycle.de/fileadmin/user_upload/Bilder_neu/Infografiken_Druck_Download/LC_IG_Verwertungsgrafik_Druck.pdf. (accessed 20 June 2013)

Schüler, D. D., D. M. Buchert, D.-I. Ran Liu, D.-G. S. Dittrich and D.-I. Cornelia Merz (2011). Study on Rare Earths and their recycling. Darmstadt, Öko-Institut e.V., Freiburg Head Office: 162.

Selenska-Pobell, S., P. Panak, V. Miteva, I. Boudakov, G. Bernhard and H. Nitsche (1999). "Selective accumulation of heavy metals by three indigenous *Bacillus* strains, *B. cereus*, *B. megaterium* and *B. sphaericus*, from drain waters of a uranium waste pile." FEMS Microbiol Ecol 29: 56-67.

Shin, D., J. Kim, B.-s. Kim, J. Jeong and J.-c. Lee (2015). "Use of phosphate solubilizing bacteria to leach Rare Earth Elements from monazite-bearing ore." Miner 5: 189-202.

Silva, G. P. d., M. Mack and J. Contiero (2009). "Glycerol: A promising and abundant carbon source for industrial microbiology." Biotechnol Adv 27: 30–39.

Talasova, I. I., N. N. Khavski, R. T. Khairullina, G. L. Karavaiko and A. W. L. Dudeney (1995). "Red Mud leaching with fungal metabolites." Biohydromet Proces: 379–384.

Tanaka, M., T. Oki, K. Koyama, H. Narita and T. Oishi (2013). Recycling of Rare Earths from scrap. Handbook on the Physics and Chemistry of Rare Earths. Onogawa, Tsukuba, Ibaraki, Japan, Elsevier B.V. 43: 182-194.

Zhu, N., Y. Xiang, T. Zhang, P. Wu, Z. Dang, P. Li and J. Wu (2011). "Bioleaching of metal concentrates of waste printed circuit boards by mixed culture of acidophilic bacteria." J Hazard Mater 192: 614-619.

Zhuang, W.-Q., J. P. Fitts, C. M. Ajo-Franklin, S. Maes, L. Alvarez-Cohen and T. Hennebel (2015). "Recovery of critical metals using biometallurgy." Curr Opin Biotechnol 33: 327–335.

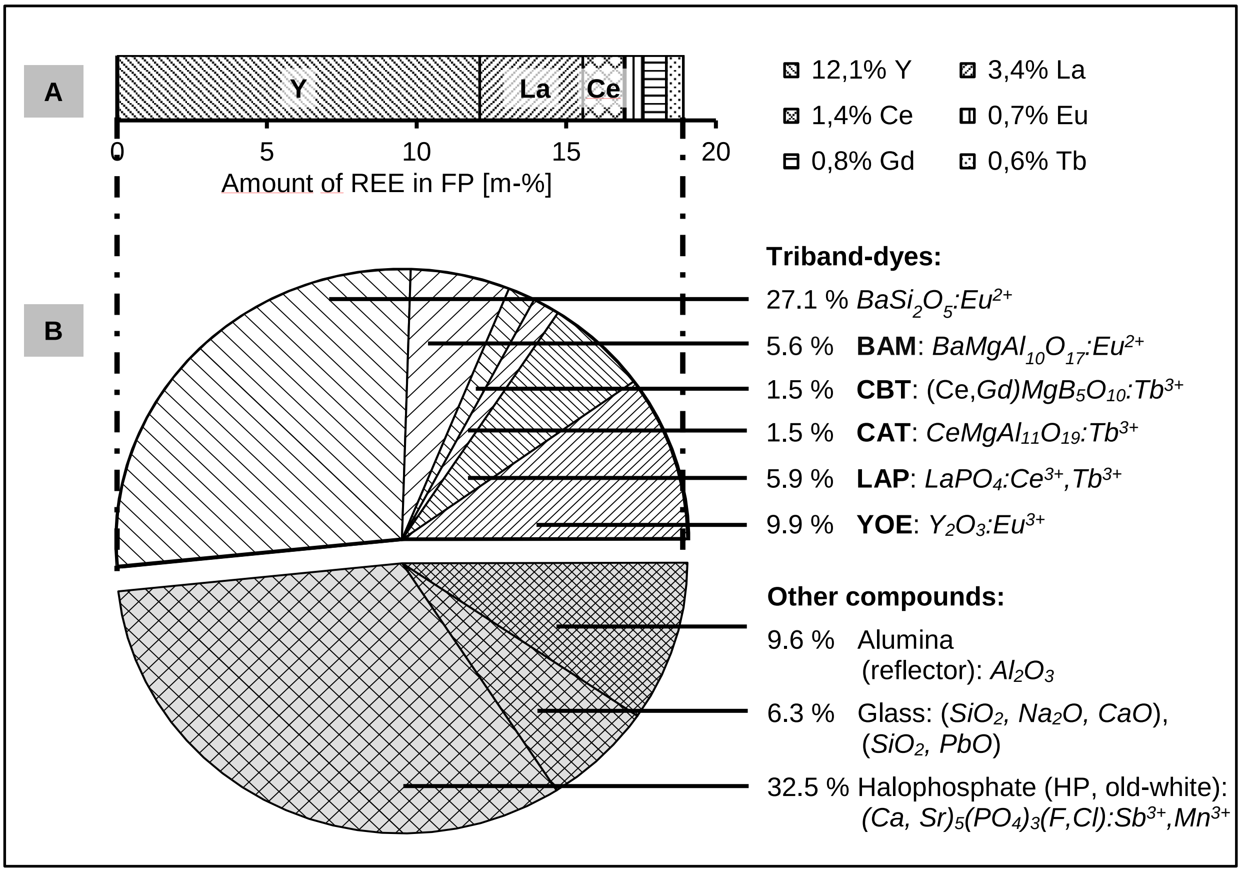

Figure 1: Composition of the used FP. Legend: A: Relative mass concentration of REE based on XRF-measurements, B: amount of the different compounds estimated by mathematical calculations.

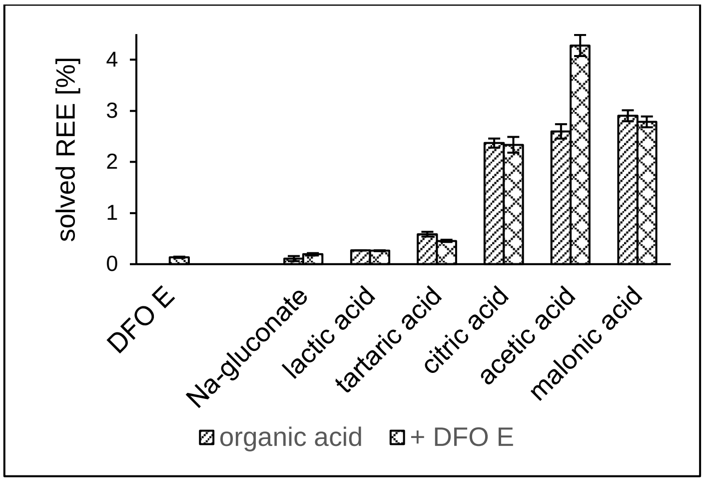

Figure 2: Chemical leaching of FP. 0.1 g FP were incubated with 0.83M COOH in case of organic acids or 0,83M deferoxamine E (DFO E) and combinations of this after one week.

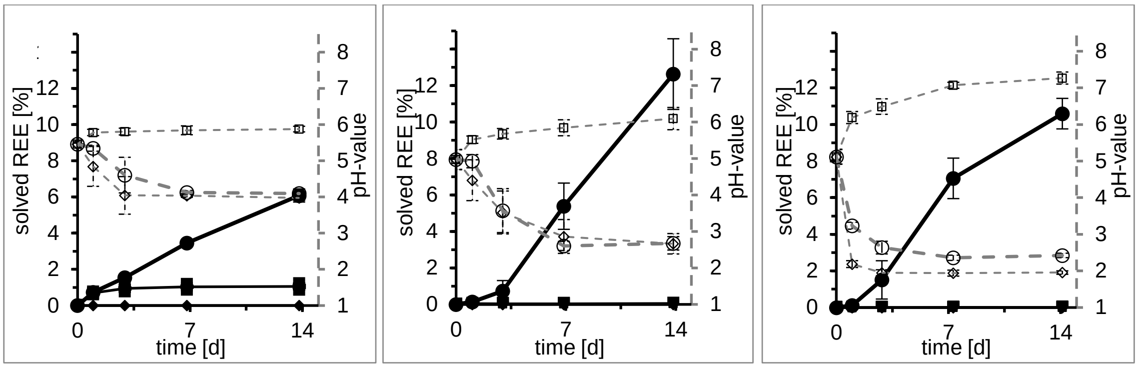

Figure 3a: Relative mass-concentrations of REE and pH-values in supernatants during direct leaching of FP with *K. xylinus* (left), *L. casei* (middle), and *Y. lipolytica* (right). Legend: ———, filled symbols: relative mass-concentration of REE; - - - -, open symbols: pH-value.◇…Control with media and microorganism, ☐…control with media and FP, ○…sample with media, microorganism and FP. Graphs are averages of 6 to 17 measurements each.

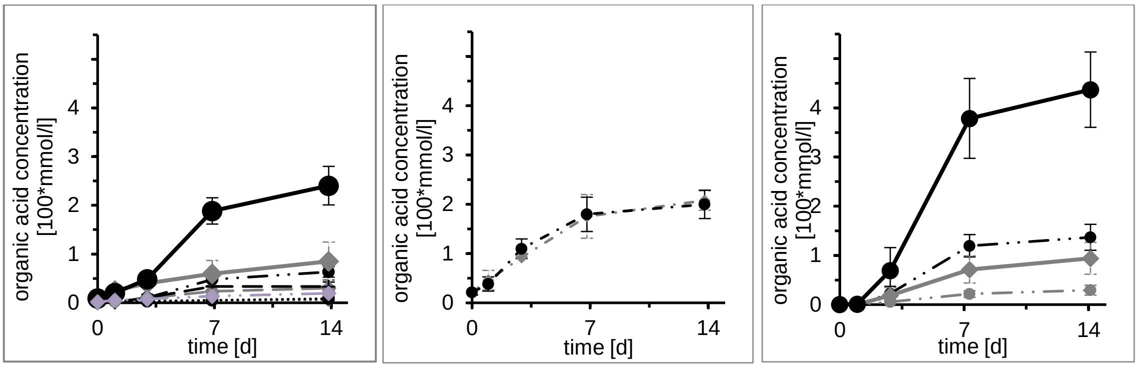

Figure 3b: Produced organic acids during direct leaching of FP with *K. xylinus* (left), *L. casei* (middle), and *Y. lipolytica* (right) (measured in supernatant). Legend: ——— … overall COOH (*K. x.* and *Y. l.*, in case of *L.c.* identical with lactic acid measurements), — · · – … (iso)citric acid (*K. x.*, *Y. l.*), — — … gluconic acid (*K. x.*), ··········· … tataric acid (*K. x.*), – · – · … lactic acid (*L. c.*). ◇…Control with media and microorganism, ○ …sample with media, microorganism and FP. Graphs are averages of 6 to 17 measurements each.

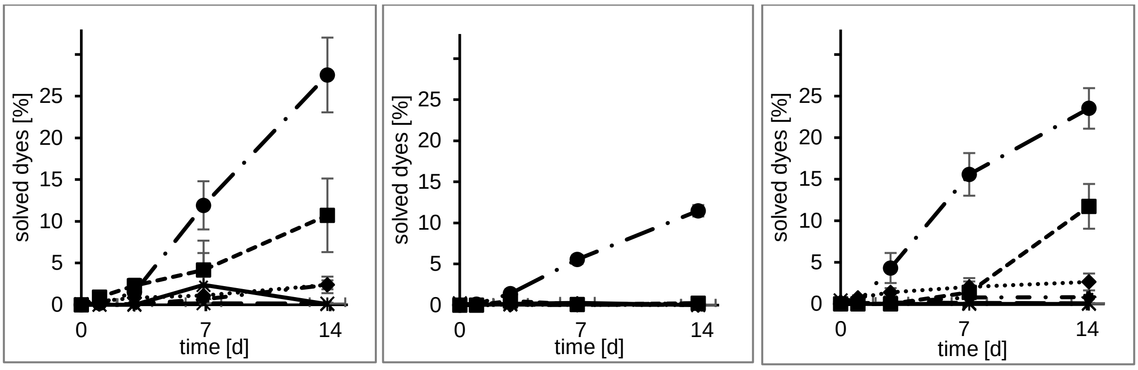

Figure 3c: Calculated relative mass concentration of bioleached REE-containing triband dyes in supernatant during direct leaching of FP with *K. xylinus* (left), *L. casei* (middle), and *Y. lipolytica* (right). Legend: ○ …YOE, □ :…$BaSi_2O_5:Eu^{2+}$, ◇..BAM, △… LAP, × … CBT, ✳…CAT.

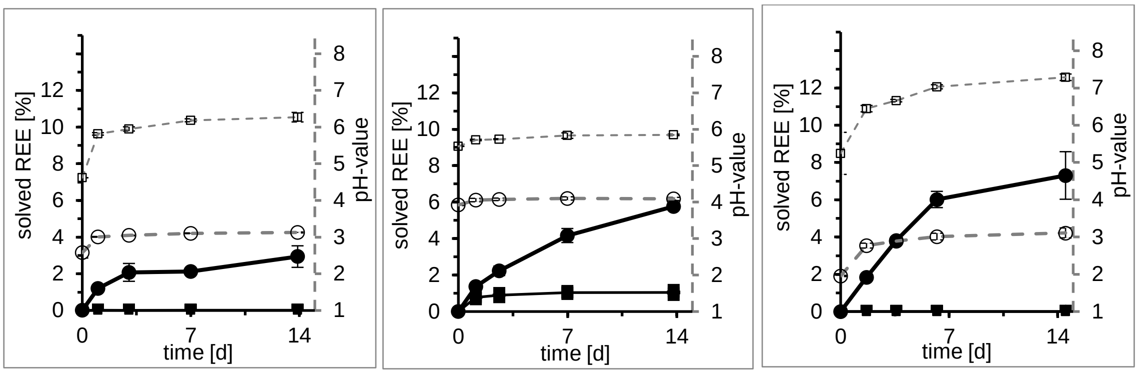

Figure 4a: Relative mass-concentration of REE and pH-value in supernatant during indirect leaching of FP with spent culture broth of a two weeks lasting cultivation with *K. xylinus* (left), *L. casei* (middle), and *Y. lipolytica* (right). Legend: ——, filled symbols: relative mass-concentration of REE; – – – –, open symbols: pH-value. □…control with media and FP, ○…sample with spent culture broth and FP. Graphs are averages of 4 to 8 measurements each.

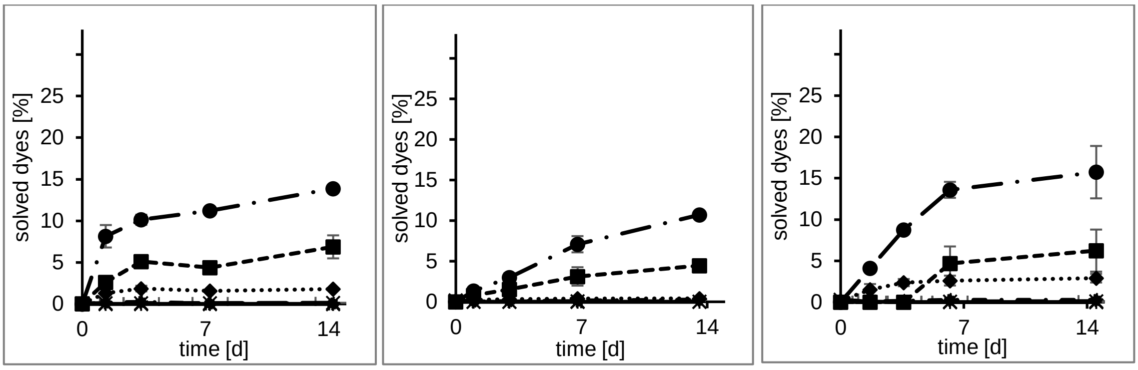

Figure 4b: Calculated relative mass concentration of bioleached REE-containing triband dyes in supernatant during indirect leaching of FP with spent culture broth of a two weeks lasting cultivation with *K. xylinus* (left), *L. casei* (middle), and *Y. lipolytica* (right). Legend: ○ …YOE, □…$BaSi_2O_5:Eu^{2+}$, ◇…BAM, △ … LAP, × … CBT, ✳..CAT.

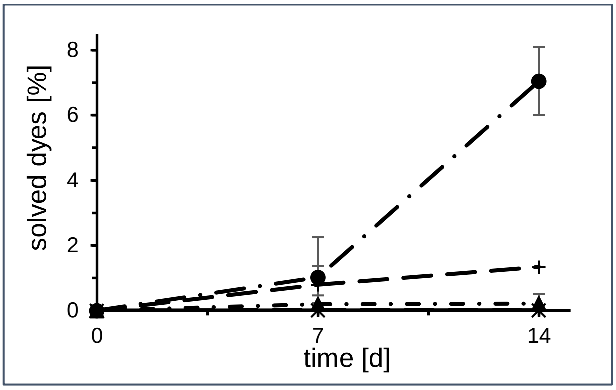

Figure 5: Relative mass concentration of bioleached REE-containing triband dyes in supernatant during direct leaching of FP with *Y. lipolytica*. Legend:○ …YOE, △… LAP, ✕ … CBT,✳ …CAT, and + ….HP