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Leaching of rare earth elements from fluorescent powder using the tea fungus Kombucha

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ABSTRACT

In most modern technologies such as flat screens, highly effective magnets and lasers, as well as luminescence phosphors, Rare Earth Elements (REE) are used. Unfortunately no environmentally friendly recycling process exists so far. In comparison to other elements the interaction of microorganisms with REE has been studied to a less extent. However, as REE are ubiquitously present in nature it can be assumed that microorganisms play an important role in the biogeochemistry of REE. This study investigates the potential of organic acid-producing microbes for extracting REE from industrial waste.

In Germany, 175 tons of fluorescent phosphor (FP) are collected per year as a distinct fraction from the recycling of compact fluorescent lamps. Because the FP contains about 10% of REE-oxides bound in the so-called triband dyes it is a readily accessible secondary resource of REE. Using the symbiotic mixed culture Kombucha, consisting of yeasts and acetic acid bacteria, REE were leached at a significant rate. The highest leaching-rates were observed in shake cultures using the entire Kombucha-consortium or its supernatant as leaching agent compared to experiments using the isolates *Zygosaccharomyces lentus* and *Komagataeibacter hansenii* as leaching organisms. During the cultivation, the pH decreased as a result of organic acid production (mainly acetic and gluconic acid). Thus, the underlying mechanism of the triband dye solubilisation is probably linked to the carboxyl-functionality or a proton excess. In accordance with the higher solubility of REE-oxides compared to REE-phosphates and –aluminates, the red dye Y_2O_3:Eu^{2+} containing relatively expensive REE was shown to be preferentially solubilized.

These results show that it is possible to dissolve the REE-compounds of FP with the help of microbial processes. Moreover, they provide the basis for the development of an eco-friendly alternative to the currently applied methods that use strong inorganic acids or toxic chemicals.

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1. Introduction

Currently, more and more complex composited and therefore poorly recyclable industrial waste materials, like neodymium-iron-boron magnets or fluorescent phosphor form energy saving bulbs, are accumulated. A promising although to date less studied recycling method is available in the form of bioleaching, which may be defined as microbial metal mobilization. In contrast to conventional leaching, metals in this process are slowly solubilized in succession, and thus may be readily separated from each other (Bosecker, 1997). Besides, it is an energy- and cost-efficient process (Bosecker, 1997; Zhu et al., 2011). There are different types of bioleaching: The "classical" bioleaching with chemolitho-autotrophic microorganisms which is mainly used for the mining of low-grade deposits of sulfidic minerals. In this process, acidophilic chemolitho-autotrophic bacteria transform an inorganic substance via redox-reactions into a soluble form, whilst simultaneously an iron or sulfidic compound is oxidised or reduced for energy production. Another kind is the chemoorgano-heterotrophic bioleaching, where the microorganisms do not interact directly with metal compound. Instead, the metals are mobilized indirectly by metabolites such as organic acids or metal binding molecules, excreted by the microorganisms. In this case, it is possible to incubate the leaching substrate together with the microorganisms in one process, or to produce first the leaching solution, and in a second step, incubate the substrate with the leaching solution. Currently, the latter processes are not applied in industry, even though the process could occur at higher pH,

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and many different substrates could serve as energy and carbon sources (Bosecker, 1997; Krebs et al., 1997).

Earlier studies showed that in principle, bioleaching of waste products is possible: Brandl et al. (2001) described metal mobilization from electronic scrap like printed circuit boards with the aid of *Acidithiobacillus* species as well as chemoorgano-heterotrophic fungi. Also Zhu et al. (2011) and Wang et al. (2016) used acidophilic microorganisms for such materials. In Wang et al. (2016) the leaching rate could be increased by the use of biochar, facilitating the redox action. In a more recent publication the application of cyanogenic bacteria is reported (Brandl et al., 2008). Furthermore, *Aspergillus niger* was used for the bioleaching of fly ash from municipal waste (Bosshard et al., 1996) and a specific thermophilic bacteria was employed in the solubilisation of gallium from ores and semiconductors (Bowers-Irons et al., 1993).

REE are often incorporated in the manufacture of plenty new consumer goods (e. g. computers, LCD screens). Similarly, the manufacture of products of the so called "green technologies" such as wind power plants, electric cars and energy saving fluorescent-bulbs consumes significant amounts of REE (Schüler et al., 2011). Until 2012 the costs of these resources increased due to the monopoly status and also because of Chinese export restrictions. After 2012 the costs declined as a consequence of investor perception to markets forecasts due to the prospect of substitution with other elements, the discovery of new deposits and the opening of new mines (Elsner, 2014; Jung, 2014). Nevertheless, China's market share is still 85% and heavy REE as well as Europium are practically only mined in China (heise online, 2015; USGS Survey, 2016). The Statista GmbH (2013) predicts an increase in REE production in the coming years. Despite these facts, almost no recycling of end-of-life-products is being done (European Commission, 2014; Graedel et al., 2011; Panayotova and Panayotov, 2012; Reck and Graedel, 2012). Considering that mining, especially of REE, consumes high amounts of energy, water and chemicals, often accompanied by the release of radioactive elements and environmental damage (Golev et al., 2014; Schüler et al., 2011), it is obvious that new environmentally friendly strategies to recycle high potential waste materials are needed.

As a case study, we investigated fluorescent phosphor (FP) powder, which is collected as a distinct fraction during the recycling process of fluorescent bulbs and energy saving bulbs (compact fluorescent lamps, CFL), beneath glass and scrap metal. In Germany alone, per year 175 tons of recycling-FP are accumulated (Gallenkemper and Breer, 2012; Riemann, 2014), that needs to be stored as hazardous, because it still contains mercury (Binnemans et al., 2013; Radeke et al., 1998). FPs are synthetically manufactured inorganic compounds, which are used for the conversion of the UV-light from the mercury into visible light and heat. Usually, they consist of two parts: An absorbing one, in which the energy of the light is collected, and an activator-atom, which emits the visible light (Gock et al., 2008; Schimrosczyk, 2004; Srivastava and Sommerer, 1998). A common FP powder from recycling consists of the old halophosphate which emits white light with low efficiency and the newer, so called triband dyes. These dyes are always used in a mixture of a blue, green and a red dye (Gock et al., 2008; Haucke et al., 2011; Radeke et al., 1998). The chemical composition of the dyes is depicted in Table 1.

Table 1
Chemical composition and color of emitted light of typical fluorescent dyes (Gock et al., 2008; Kummer, 1999; Srivastava and Sommerer, 1998).

Name	Chemical formula	Color
Halophosphate	$(Ca,Sr)_5(PO_4)_3(F,Cl):Sb^{3+},Mn^{3+}$	White
Barium Magnesium Aluminate (BAM)	$BaMgA_{l1}0O_{17}:Eu^{2+}$	Blue
Cerium Magnesium Aluminate (CAT)	$CeMgAl_{11}O_{19}:Tb^{3+}$	Green
Lanthanum Phosphate (LAP)	$LaPO_4: Ce^{3+},Tb^{3+}$	Green
Cer-Gadolinium Magnesium Pentaborate (CBT)	$(Ce,Gd)MgB_5O_{10}:Tb^{3+}$	Green
Yttrium-Europium-Oxid (YOE)	$Y_2O_3:Eu^{3+}$	Red

A direct reuse of the dyes is not reasonable, because the compounds deteriorate when lamps are used. Furthermore, even traces of impurities diminish the luminescence of the FP (Binnemans et al., 2013; Schimrosczyk, 2004). As shown in Table 1, the triband dyes contain REE, mainly Y, Eu, La, Tb, Ce and Gd. A typical recycling FP contains 10% REE-Oxides (Gock et al., 2008; Haucke et al., 2011), which is higher than in natural sources (Binnemans et al., 2013). Hence, there is much potential in the research on recycling of REE from FP. However, all of these technical approaches deal with toxic chemicals or high amounts of inorganic acids (Haucke et al., 2011; Rabah, 2008; Schimrosczyk, 2004; Tanaka et al., 2013; Tunsu et al., 2011). Bioleaching could be an attractive alternative approach to enable an eco-friendly recycling of REE.

To our knowledge, the literature concerning the bioleaching of REE from minerals and waste products, especially FP, is limited. Several studies concerning the biological leaching of REE from minerals such as apatite and monazite (Brisson et al., 2015; Goyne et al., 2010; Hassanien et al., 2013; Mubarok et al., 2015; Shin et al., 2015), hornblende (Brantley et al., 2001), granite (Chen et al., 2001) and zircon (Glombitza et al., 1988) allow the hypothesis that microorganisms could be also used for the recovery of REE from technical products. In Barmettler et al. (2016) an overview about leaching of minerals as well as waste products like ash slag with acidophilic as well as with chemoorgano-heterotrophic microorganisms is given. In the review of Zhuang et al. (2015) citric acid seems to be the most appropriate metabolite for heterotrophic bioleaching. Nancharaiah et al. (2016) describe bioelectrical systems which could be used also for reductive leaching. Thirty-five years ago, Schwartz and Näveke (1980) conducted experiments with REE-oxides and *Aspergillus niger*. Beolchini et al. (2012) described the utilization of acidophilic bacteria in the leaching of cathode ray tubes and other wastes. Talasova et al. (1995) and Qu and Lian (2013) reported the leaching of Y and Sc respectively REEs from red mud by *A. niger* and *Yarrowia lipolytica* or Penicillium tricolor, producing mainly citric and oxalic acid. In the study of Reed et al. (2016) bioleaching of fluorescent phosphor with gluconic acid producing bacteria achieved a maximum leaching rate of 2%.

In this study, chemoorgano-heterotrophs were chosen for leaching of FP, because FP contains no sulphur or iron and buffers pH in a range that inhibits the growth of acidophiles. Furthermore, REE in FPs cannot be solubilized by redox reactions because most of the REE are already at the highest oxidation stage and their redox potential is extremely low (Evans, 1990; Morss, 1985). Another advantage of the use of chemoorgano-heterotrophic microorganisms is the ease with which they are cultivated and the variety of substrates they can use, including waste materials. This is important for a later industrial application. Furthermore, the interaction of the microorganisms with the FP and the production of additional metal binding metabolites apart from organic acids could enhance the leaching effect (Saala and Duckworth, 2010). Therefore, for this study, the inexpensive, organic-acid producing mixed culture Kombucha was chosen for the bioleaching experiments.

This study is one of the first comprehensive investigations of microbial leaching of REE from FP. It forms the basis for the development of an eco-friendly recycling process for an important secondary resource of REE. Moreover, the process may be applicable for REE recovery from other REE-containing waste products, as Reed et al. (2016) showed.

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2. Material and methods

2.1. Fluorescent phosphor

In this study, the used Recycling-FP originates from fluorescent tubes processed with the end cut method of one batch (25/10/2013) supplied by Larec Lampen-Recycling Gesellschaft mbH (Erzstraβe 18, 09618 Brand-Erbisdorf, Germany). In order to obtain identical samples, the FP was portioned by a sample divider (Rotary Micro Riffler, Quatochrome Instruments, United States and DR1000, Retsch, Germany) into fractions of 0.85 g. To reduce mercury content and for sterilization, all FP was autoclaved (1 h, 121 °C) prior to use (Jang et al., 2005). The exhaust air was purified by passing through a water-sulphuric acid solution to precipitate mercury as mercury sulphate (Nethe, 2007). A HELOS (H0735) laser diffractometer (Sympatec, Clausthal-Zellerfeld, Germany) was used for particle size analysis, using the following conditions: wet dispersing system, measurement range R3: 0.9–175 μm.

The elemental composition was measured by X-ray fluorescence analysis (XRF) with a PANalytical Axios mineral spectrometer (Almelo, Netherlands). For data-evaluation, SuperQ-Software (PANalytical) was used. The device was equipped with a threefold collimator changer (150, 300, and 700 μm), a Rh-tube (4 kW), and a crystal changer outfitted with seven crystals. Spectra were obtained in a step scan mode mainly using a 300 μm collimator and a speed of 0.5° 2θ/s. Further optical analysis was done by an Olympus microscope (Shinjuku, Tokyo, Japan, BX61) with the following lamps: visible light: BX-UCB, UV-light: U-RFL-T and fluorescence filters: U-NMU2, U-MSWG2 and GFP.

2.2. Microbial cultures and Characterization

The used Kombucha culture was obtained from a household in Dresden (Germany) in 2013. The microorganisms in the mixed culture were determined by DNA isolation and subsequent Sanger-sequencing. For the DNA-isolation 1 g of a Kombucha pellicle in 15 ml of 0.1% peptone was shredded for 2 min at stage 3 with an Ultra-Turrax® (IKA® T18 basic, Staufen im Breisgau, Germany). After washing with 0.1% peptone, 30% of the obtained solution was used for the DNA isolation with NucleoSpin Tissue DNA-isolation kit (Machery Nagel, Düren, Germany). The ribosomal region of the DNA was amplified by PCR with GoTaq®-kit of Promega (Madison, Wisconsin, USA) and the following primers (Table 2). The sequencing-reactions were performed with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and analyzed using a DNA-Sequencer (ABI PRISM 310 Genetic Analyzer, Applied Biosystems; Waltham, Massachusetts, USA). The obtained sequences were compared using BLASTN 2.2.32+ database for strain identification. Besides, the DNA was digested with MspI (Fermentas, Waltham, Massachusetts, USA) and separated on a 3.5% top vision agarose gel (0.5% TBE-buffer, 4 h, 100 V, DNA-ladder: Gene Ruler™ 1 kb Plus DNA ladder, Fermentas, Waltham, Massachusetts, USA).

Single microbial strains from Kombucha were isolated, using 0.3 g Kombucha in 0.5 ml 0.1% peptone and some quartz sand (d ≈ 0.4 mm) or glass beads (d ≈ 1 mm) in an Omni Bead Rupter24 (biolab products, Bebensee, Germany) at 2.1 m/s for 15 × 1 min with a 5 min break in-between. The obtained suspension was plated at different dilutions on bacterial- (100 mg/l oxytetracyclin) or yeast selective (50 mg/l cycloheximide) nutrient agar-plates (Sievers et al., 1995; Jayabalan et al., 2010). Colonies were counted and picked after three days incubation. The DNA was isolated analogous to the Kombucha samples, but without any pre-treatment. DNA-amplification, -sequencing and digestion were also carried out as on the Kombucha samples.

Table 2
Applied primers for PCR and sequencing.

	Forward	Backward
Bacteria	5′-A AGA GTT TGA TCN TGG CTC AG-3′	5′-TAC GGY TAC CTT GTT ACG ACT T-3′
Yeasts	5′-GTA GTC ATA TGC TTG TCT C-3′	5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′

2.3. Cultivation conditions and leaching experiments

Kombucha was cultivated on a medium having the following composition: 10 g/l Green Tea (Grüntee-Mischung, Wilkon Tee GmbH, Aurich, Germany) and 140 g/l sucrose were added to boiled water. After cooling to room temperature, both solutions were mixed in a ratio of 1:1. The obtained medium was used without sterilization, because autoclaving led to flocculation of the green tea and, according to Marsh et al. (2014) formation of toxic compounds. The isolated strains of the Kombucha were cultivated on autoclaved DMSZ-medium 105 without $CaCO_3$ for the bacteria (100 g/l glucose, 10 g/l yeast extract) and DMSZ-medium 393 for yeasts (20 g/l glucose, 10 g/l yeast extract, 10 g/l peptone). The leaching-experiments were performed in batch mode: 0.85 g REE containing FP were mixed with 27 ml medium plus 3 ml (10%) culture supernatant of the starting culture and a 1 × 1 cm piece of the Kombucha-pellicle taken from a one week old culture in a 100 ml Erlenmeyer flask. Alternatively, in case of the isolates, the leaching substrate was mixed with 30 ml medium and 1 ml three-day old culture broth. The cultures were grown either by shaking at 300 rpm (type: 3015, Gesellschaft für Labortechnik mbH, Burgwedel, Germany) or stationary at room temperature under a fume cupboard. As a control, approaches either without FP or without microorganisms were used. For the experiments using culture supernatant, the cells were cultivated stationary or by shaking at 300 rpm in a 500 ml wide neck Erlenmeyer flask with 200 ml medium for two weeks analogous to the experiments in the 100 ml flasks. Upon completion of the cultivation, the culture was centrifuged. Subsequent, the supernatant was separated and sterilized by filtration (0.22 μm Durapore membrane, Merck KGaA, Darmstadt, Germany). In the leaching experiments, 30 ml supernatant were mixed with the substrate to be leached. In case of control, sterile medium was used instead of supernatant. Apart from FP, the model substances Y_2O_3 (Aldrich, St. Louis, Missouri, USA) for the red dye YOE and $LaPO_4$ (Alfa Aesar, Ward Hill, Massachusetts, USA) for the green dye LAP were used as substrates to be leached. Furthermore, as a control, FP was incubated with solutions of commercial organic acids, which were identified by HPLC. For these experiments the average concentrations of organic acids that were detected after two weeks of bioleaching in an exemplary approach were applied, as the measured acid concentrations varied between the experiments. (see Fig. 6; 0.13 M gluconate, 0.16 M acetic acid, mixture of both).

2.4. Analytical methods

After 1, 3, 7 and 14 days leaching substrate was separated from supernatant by descending and samples of 1 ml supernatant were taken from each leaching-approach. After centrifugation for 10 min at a speed of 15,000 rpm (Mirko 12–24, Hettich-Zentrifugen, Tuttlingen, Germany) the pH-value (WTW inoLab with a SenTix Micsond, Weilheim, Germany) was determined and the concentration of Mg, Al, Si, P, Ca, Y, Ba, La, Ce, Eu, Gd and Tb was measured by ICP-MS in 3 replicates at normal resolution, using an internal standard of 5 μg/l Rh at rf-power of 1100 W quadrupole mass filter (Elan 9000, Perkin Elmer, Waltham, Massachusetts, USA). An analysis of the solid leaching residues was not possible, because the parti-

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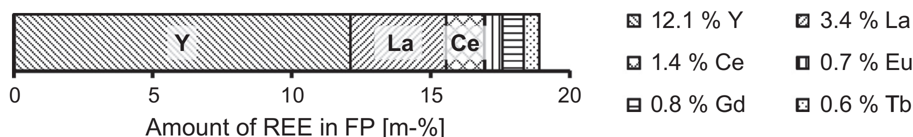

Fig. 1. Elemental composition of the used FP measured by XRF in weight-%.

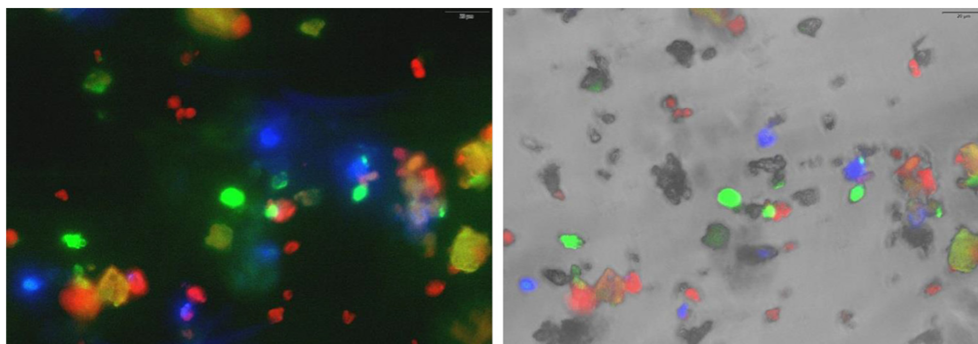

Fig. 2. Fluorescence microscopic analysis of the used FP. Left: Overlay of different florescent microscopic pictures, right: additional overlay of light microscopic picture. Visible are blue, green and red glowing particles, belonging to the group of triband dyes and containing REE. Beneath, there are non-glowing particles, most probable white halophosphates and supporting substances.

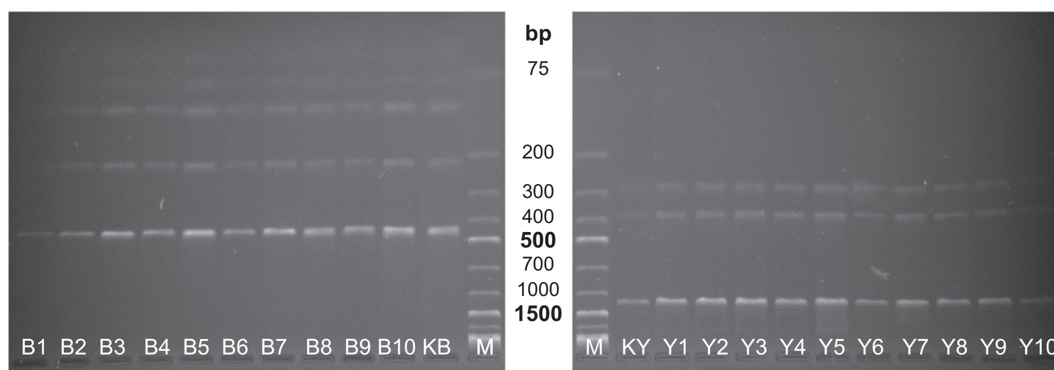

Fig. 3. Agarose gel-electrophoresis of restriction enzyme digest with MspI of amplified ribosomal DNA of the bacterial (left) and yeast (right) isolates of Kombucha, K: PCR-product of Kombucha-ribosomal DNA (mixed culture) amplified with bacterial (B) or yeast (Y) primer.

cles were incorporated into the Kombucha-pellicle thus preventing a quantitative separation. The microbial produced organic acids were identified and measured by high pressure liquid chromatography (HPLC) using an Agilent 2000 (Santa Clara, California, USA) device equipped with a DAD detector at 210 nm (column: Nucleogel® ION 300 OA (Machery Nagel, Düren, Germany), conditions: 70 °C, 90 min, 5 mM H_2SO_4 isocratic, 0.4 ml/min). The growth of the microorganisms in the control without FP was monitored by OD600 measurement. After termination of the leaching experiments the microbial composition of the culture and the distribution of the leaching substrate were determined microscopically (Olympus, Shinjuku, Tokyo, Japan, BX61, lamps: visible light: BX-UCB, UV-light: U-RFL-T, fluorescence filters: U-NMU2, U-MSWG2 and GFP).

3. Results

3.1. Fluorescent phosphor

The present study investigates, if REE from FP could be leached microbially using the symbiotic mixed culture Kombucha as test case. Detailed knowledge of the composition of FP is essential for interpretation of results. In addition, a large accessible surface of the FP is essential to obtain a high leaching rate, thus requiring a high concentration of suspended particles. For these analyses the particle size was measured by laser diffraction. The particles are roughly equally spread in a fraction of about 0.7 μm and of 4–5 μm (data not shown). In shake cultures of FP, particles are optically fully suspended at a speed of 300 rpm.

The elemental composition of the leaching material was analyzed by XRF (Fig. 1). According to this, the FP used in this study contains 19.05 wt-% REE, which are only part of the so called triband dyes. The main part of the REE content is made up of yttrium with 64%, which is completely bound in the solely red dye YOE. But also lanthanum (18%) and cerium (7%) are present in larger quantities and are probably distributed across the green dyes LAP, CAT and CBT. Additionally, BAM could for example occur as blue dye and contains europium as activator-atom. Further fluorescent microscopic analysis revealed, that besides the blue, green and red glowing particles, non-glowing particles also exist (Fig. 2). These compounds could belong to the halophosphate dyes, as indicated by the high amount of calcium (16.3%) and strontium (3.1%) (XRF analysis, data not shown). But also glass residues and additives could contribute to this fraction.

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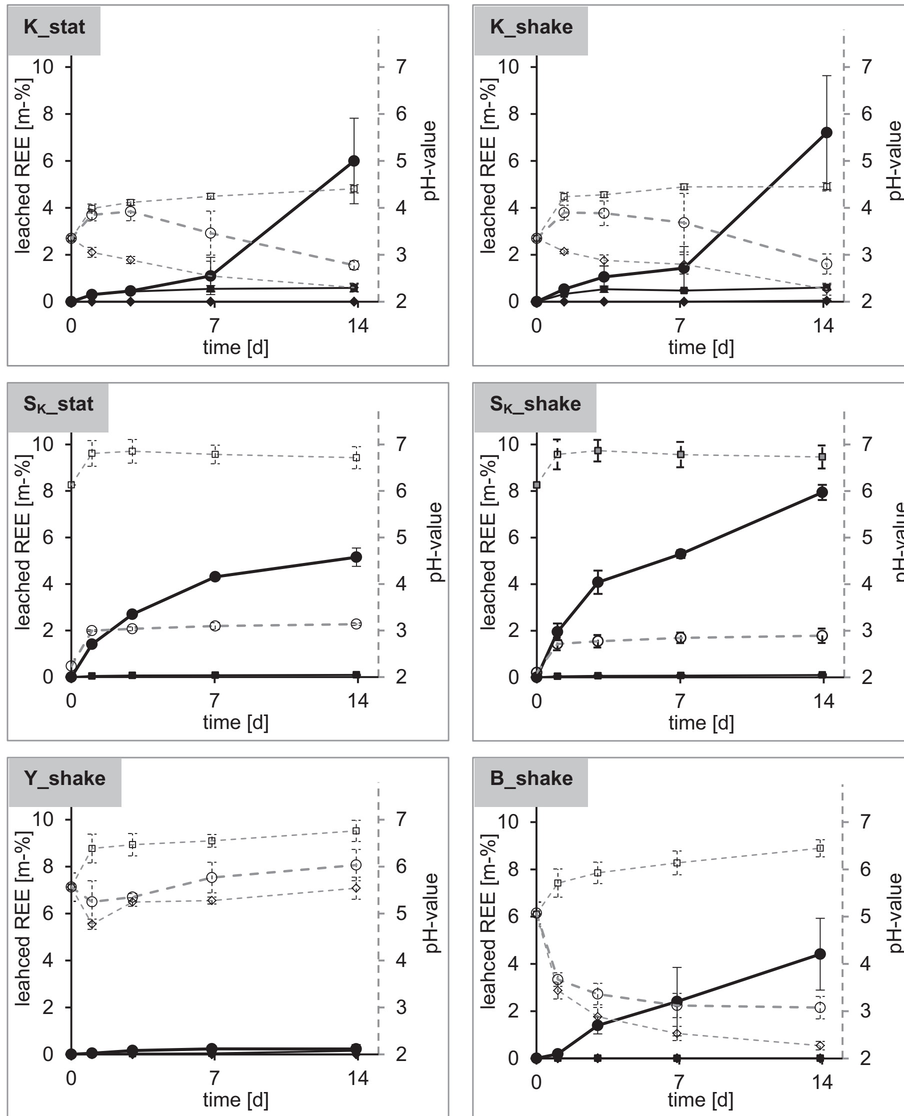
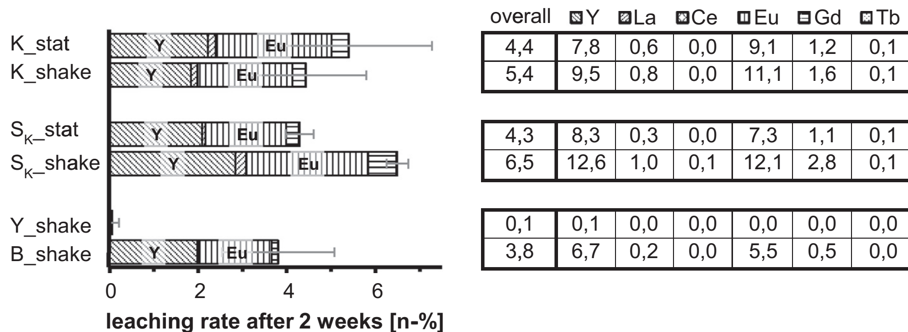


Fig. 4. Relative mass-concentration of REE and pH-value in supernatant during leaching of FP with entire Kombucha, Kombucha supernatant and single isolates from Kombucha. Legend: Black, solid line, filled symbols: relative mass-concentration of REE; grey, dotted line, open symbols: pH-value. K: cultivation of whole Kombucha on Kombucha-media, S_K: cultivation supernatant of a two weeks lasting cultivation, Y: yeast isolate of Kombucha *Z. lentus* on YPD media, B: bacteria isolate *K. hansenii* on DMSZ media 105, stat: stationary, shake: shaked inkubation. ◇ Control with media and microorganism, □ control with media and FP, ○ sample with media, microorgnanism and FP. Graphs are averages of 4–10 measurements each.



	overall	Y	La	Ce	Eu	Gd	Tb
K_stat	4,4	7,8	0,6	0,0	9,1	1,2	0,1
K_shake	5,4	9,5	0,8	0,0	11,1	1,6	0,1
S_K_stat	4,3	8,3	0,3	0,0	7,3	1,1	0,1
S_K_shake	6,5	12,6	1,0	0,1	12,1	2,8	0,1
Y_shake	0,1	0,1	0,0	0,0	0,0	0,0	0,0
B_shake	3,8	6,7	0,2	0,0	5,5	0,5	0,0

Fig. 5. Comparison of different leaching regimes. Relative molar leaching rate of REE minus the leaching effect of the medium after 2 weeks in mole-%. Legend: K: cultivation of whole Kombucha on Kombucha-media, S_K: cultivation supernatant of a two weeks lasting cultivation, Y: yeast isolate of Kombucha on YPD media, B: bacteria isolate on DMSZ media 105, stat: stationary, shake: shaked inkubation.

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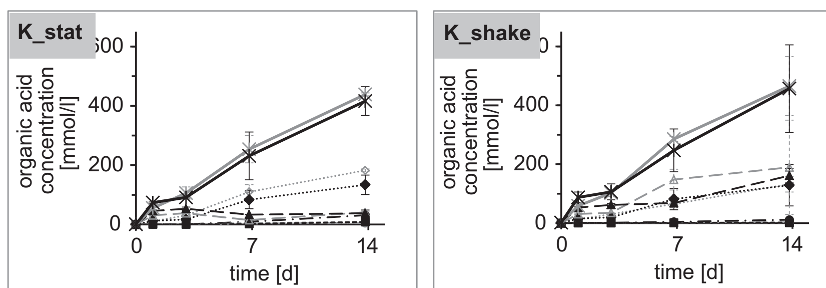

Fig. 6. Exemplary depiction of organic acid production in Kombucha-cultures during leaching of FP. Left: stationary, right: shaked cultivation. × Overall COOH production, △ acetic acid, ◇ gluconic acid, ○ citric acid, □ tartaric acid; black, filled symbols: sample with microorganisms and FP, grey, open symbols: control with microorganisms.

3.2. Analyses of Kombucha consortium and isolation of microorganisms

The chemoorgano-heterotrophic, symbiotic mixed culture Kombucha was used for the leaching process. As the composition of this "tea fungus" varies according to the source and cultivation conditions, prior to leaching, the microbial community was characterized by DNA-analysis. The microorganisms of the Kombucha were separated by mechanical treatment and enriched on bacterial- or yeast-selective agar plates. DNA was extracted from the obtained isolates, amplified with PCR using yeast and bacteria-specific primers and digested with the enzyme Msp I. The results are depicted in Fig. 3. All yeast isolates as well as all bacterial isolates show the same restriction pattern, indicating the presence of only one yeast and bacterial species. These results were confirmed by subsequent DNA sequencing (accession numbers at European Nucleotide Archive: LT546163 and LT546164). According to these findings, the Kombucha used in this study consisted of the bacterium *Komagataeibacter hansenii* (DSM-103118), which is closely related to *K. kombuchae*, and the yeast *Zygosaccharomyces lentus* (DSM-103078).

3.3. Leaching experiments

Leaching experiments were performed with entire Kombucha as well as with single microbial strains isolated from Kombucha. Furthermore, supernatant from Kombucha culture was used for leaching experiments. Literature only describes stationary cultivation of Kombucha (Blanc, 1996; Marsh et al., 2014; Sievers et al., 1995). On the other hand, leaching usually can be enhanced by a thorough mixing of medium, substrate (FP) and microorganisms (Mular et al., 2002; Xue et al., 2014). Therefore, experiments were carried out with shaking as well as in a stationary mode. The amounts of dissolved REE in the aqueous phase as well as the associated pH-values are depicted in Fig. 4.

Fig. 4_K_stat and K_shake indicate that during the first week of the leaching with the entire Kombucha only minor amounts of REE were liberated (stationary: 1.1%, shaking: 1.4%). The accompanying pH-measurements correspond to these observations. Shortly after starting the experiment, the pH-value increased slightly, probably due to the buffering effect of the FP, like the controls without microorganisms showed. After approximately three days of leaching, the pH-value dropped significantly from 3.9 to pH 2.8 after 14 days (both modes). It can be assumed, that this decrease was caused by the production of organic acids. Simultaneously, the leaching rate increased: After two weeks, the leaching rate in the shaken mode was slightly higher, about 7.2% compared to 6.0% in the stationary mode. In both cases saturation was not detectable. The growth of the Kombucha was monitored optically. Notably, during the first week, the microorganisms in the control without FP usually grew better than those of the samples with FP. However, at the end of the second week, the pellicle of the sample with FP was larger than that of the control.

Bacterial leaching processes can be divided into direct leaching (direct interaction of microorganisms with the substrate) and indirect leaching, where only metabolic products in the medium are responsible for element mobilization. In order to distinguish between these processes, leaching experiments were carried out with supernatant from a two weeks old culture. Fig. 4_S_K shows that the pH-value increased in both batches within the first day from 2.2 to 3.0 resp. from 2.1 to 2.7 (stationary/shaken cultured supernatant), after which the pH remained roughly constant. Nevertheless, the REE-concentration increased during the whole investigation period: After two weeks the leaching rate reached 5.2% in case of stationary and 7.9% in case of shaken mode. Thus, the leaching in the shaken mode was significantly higher than that in the stationary one, which is comparable to the results obtained with the entire Kombucha. Especially with the supernatant form the stationary culture, the leaching levelled off after two weeks, as saturation occurred.

Similar leaching experiments were performed with isolated yeast and bacteria strains from Kombucha. The results showed, that the yeasts cased almost no leaching of REE (0.23% after two weeks). In contrast, with 4.4% a significant amount of REE were released from FP while two-week cultivation of bacteria. The pH-measurements showed a similar behavior to that of the entire Kombucha. However, the pH-value of the attempts using the bacterial isolate dropped significantly from 5.1 to 3.7 during the first day and the REE-amount in the supernatant started to increase after one day. Remarkably, after two weeks the overall REE release in pure bacterial cultures was with only 4.4% significantly lower than those approaches using the entire Kombucha or its supernatant.

Usually, detailed analyses of the residues allow a reliable information and evaluation of the success of bioleaching. In the case of the leaching experiments with Kombucha, the residual FP particles were incorporated into the cellulosic pellicle that is formed during cultivation, thus hindering such analyses. Consequently, to validate the leaching-selectivity, the data of the supernatant measurements were used once again. Fig. 5 shows the distribution of leaching rates of the single elements in mole-percent in addition to the overall. Compared to the composition of the entire FP, the elements yttrium and europium were dissolved primarily. The leaching rates ranged between 6.7 and 12.5 mole-% for Y and 5.5 and 12.1 mole-% for Eu. All other elements were released in only very small amounts by the microorganisms or its supernatant. This indicates that the red dye YOE (Y_2O_3:Eu^{3+}) was leached selectively. This result was confirmed by experiments using entire Kombucha on pure Y_2O_3 and on pure $LaPO_4$ (data not shown). After two weeks, 60.1% (stationary mode) respectively 67.9% (shaken mode) of Y from Y_2O_3 were leached. Throughout the same time period, only 0.01% of La was released (both modes).

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During all experiments in which significant leaching took place, a decrease in pH was noted. It can be assumed that this decrease is caused by the production of organic acids. In order to prove this, the nature and amount of organic acids was measured using HPLC. In Fig. 6, duplicate plots of the production of organic acids during an exemplary leaching experiment with entire Kombucha are shown. Mainly acetic and gluconic acid, but also in smaller amounts citric and tartaric acid, were formed. In contrast to pH- and leaching-results, the measurements of organic acids were less reproducible. Neither the proportions of single acids nor the amounts of total acid (number of COOH groups) remained constant. Only the type of acid formed was constant, with a tendency to larger amounts of acetic and gluconic acids to be generated. In the supernatants similar results were obtained, nevertheless the amount of acetic acid in comparison to gluconic acid was lower (data not shown). Based on these results FP was incubated with commercially available organic acids in order to investigate the biological influence of REE extraction. In these experiments FP was incubated with 0.13 M gluconic acid and 0.16 M aetic acid, respectively. After two weeks, in average 0.13% and 1.30% of REE were dissolved in case of gluconic acid and acetic acid, respectively. In approaches containing both gluconic and acetic acid, about 1.44% of REE were solubilized. In contrast no leaching was detected when using destilled water or water adjusted to pH 2

4. Discussion

4.1. Characterization of the material

The aim of the study was to investigate the potential of the chemoorgano-heterotrophic "tea fungus" Kombucha to leach REE from spent FP. The target material was analyzed in detail before starting the experiments. In a first step, the particle size distribution of the FP was determined: Two main fractions were observed, one with particles of approximately 0.7 μm and a second with roughly 4–5 μm. These results conflict with literature in which two fractions with larger particle sizes are described. According to Schimrosczyk (2004) and Wojtalewicz-Kasprzak (2007), the triband-dyes have a size of 4–8 μm and the halophosphates have a size of 10–15 μm. The difference in particle size could be explained by different measuring procedures being used. In this work laser diffraction was used. Particle size is usually calculated by assuming the particles are spheres, but the FP particles have irregular shapes. Therefore the real particle size was perhaps underestimated by assuming the particles were spherical. In the other works mechanical classifying were used. In this case particles with a wide size-range may have influenced the classification results. Because of the small particle size, it was relatively easy to fully suspend the FP in Erlenmeyer flasks on a rotary shaker, thus maximizing the surface accessible to the leaching.

The elemental composition of the FP was determined using XRF. Compared to other waste FPs studied, the REE-content of 19.05% in our FP was practically twice as high (Gock et al., 2008; Otto and Wojtalewicz-Kasprzak, 2007). This difference is likely a consequence of the end-cut method employed in which fewer impurities were introduced. According to the elemental analysis using XRF and the optical investigation using the fluorescence microscope, the FP contains blue, green and red fluorescent dyes, and most probably also halophosphates, which is typical for recycling FPs (Binnemans et al., 2013; Gock et al., 2008; Radeke et al., 1998; Tanaka et al., 2013). Therefore, although the compositions of recycling FPs always depend on the source of the lamps, recycling companies and their recycling strategy (Binnemans et al., 2013; Gock et al., 2008; Haucke et al., 2011), the chosen FP appears to be appropriate for the leaching experiments and the results should be also transferable to other recycling FPs.

4.2. Characterization of microorganisms

For the leaching experiments, the "tea fungus" Kombucha was used. Kombucha is normally cultured in households for the production of a supposedly wholesome beverage. As the mixed culture produces high amounts of organic acids and is relatively stable against contaminants (Jayabalan et al., 2010; Marsh et al., 2014), it could be suitable for chemoorgano-heterotrophic leaching processes.

In the literature, Kombucha is characterized as a symbiotic culture of acetic acid bacteria and yeasts: The bacteria consist of different *Komagataeibacter* species, mainly *K. xylinus* and *K. kombuchae*. By comparison, the variety of yeasts is significantly greater: They include different *Zygosaccharomyces* species, *Candida, Saccharomyces* and *Shizosaccharomyces* forms are found. However, the exact microbiological composition depends on the source of the tea fungus culture. In households, Kombucha is cultured in green or black tea with added sucrose. The yeasts cleave the sucrose into glucose and fructose, and further oxidise it to ethanol. The acetic acid bacteria use the glucose for the production of bacterial cellulose in which all the other microorganisms are embedded. The Kombucha thus forms a pellicle at the surface of the culture broth. The bacteria also use the glucose and the ethanol of the yeasts for the production of organic acids such as acetic, gluconic and glucuronic acid (Blanc, 1996; Chen and Liu, 2000; Jayabalan et al., 2010; Malbaša et al., 2011; Marsh et al., 2014; Sievers et al., 1995).

The analysis of the Kombucha used in this study revealed the presence of only two microorganisms: the bacterium *Komagataeibacter hansenii*, which is phylogenetically strongly related to *K. kombuchae,* and the yeast *Zygosaccharomyces lentus*. This was confirmed by the isolation of single cells and their analysis. During the cultivation mainly acetic and gluconic, but no glucuronic acid were produced, which is likely related to the low microbial diversity. Similar to Malbaša et al. (2011) small amounts of tartaric acid were identified too. Besides these peculiarities, the culture grows in a stable manner, stays free of contaminants and produces typical metabolites. It can thus be assumed, that similar would be obtained with other types of Kombucha cultures.

4.3. Leaching experiments

In this study, an indirect leaching approach using the chemoorgano-heterotrophic, organic acids producing mixed culture Kombucha was selected, due to the buffering and electrochemical properties of the leaching substrate FP (see also introduction). As an initial step, the mixed culture Kombucha was grown in liquid medium (green tea with added sucrose) with FP. After 1, 3, 7, and 14 days the culture liquid was sampled and characterized regarding the REE-concentration and pH-value. It was noteworthy that the observed increase in REE concentration was always accompanied by a lowering of pH due to production of organic acids by the microorganisms. Similar results were obtained for the experiments with the bacterial isolate *K. hansenii* of the studied Kombucha.

The pH-value in the controls with microorganisms initially decreased rapidly, but after 3–7 days more slowly. According to Malbaša et al. (2011), the reason for this behavior is due to the buffering impact of the weak organic acids which interact with the mineral components of the tea. On the other hand, the pH-value of the uninoculated control with FP initially increased rapidly but then more slowly. Thus, the measured pH change in the inoculated medium with FP fell between that of inoculated controls

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without FP and uninoculated controls with FP. Accordingly, for the experiments with supernatant, the pH-value in the sample increased due to the buffering action of the FP. Brandl et al. (2001) and Qu and Lian (2013) made similar observations concerning pH trend with regard to organic acid production in bioleaching of electronic scrap material or red mud. In Reed et al. (2016), investigating the leaching of FP and cracked catalysts, about 60% of the organic acids, mainly gluconic acid, were produced within the first 24 h. Also Hassanien et al. (2013) used organic acid producing microorganisms (*Aspergillus ficuum* and *Pseudomonas aeruginosa* producing primarily citric and oxalic acid respective ketogluconic acid) for the bioleaching of Egyptian monazite and thorium-uranium concentrate. Further publications dealing with bioleaching of ores such as monazite (Brisson et al., 2015; Mubarok et al., 2015; Shin et al., 2015) and granitite (Chen et al., 2001) as well as waste products like red mud (Talasova et al., 1995) are in agreement with these findings. Goyne et al. (2010) reported on the direct leaching of monazite and apatite ores by organic acids and offered a mechanism for the dissolution of REE-compounds by proton-donation and complex formation. Nevertheless, a direct quantitative correlation between the organic acid formation and the leaching rate was not observed because unlike the leaching rate, the rate of organic acid production was not reproducible. Brandl et al. (2001) reported that leaching of electronic scrap was greater for *P. simplicissimum* than for *Aspergillus niger*, although *P. simplicissimum* produced smaller amounts of organic acids. In Reed et al. (2016) higher leaching rates could be observed for direct leaching with microorganisms and spent culture broth than for commercial organic acids. Thus, it could be assumed that other metabolites also support the leaching process.

In addition to stationary cultivation of Kombucha, which is described in the literature as standard growth condition (Blanc, 1996; Marsh et al., 2014; Sievers et al., 1995), the mixed culture was also grown on a shaker in absence of FP, for the production of culture-supernatant or together with FP in case of direct leaching experiments. In these shaken approaches, after two weeks, the leaching rate was both with entire Kombucha, as well as with supernatant significantly higher than that for the stationary alternative. The explanation of these findings are certainly the improvement in oxygen supply and thus the enhanced production of metabolites. In the approaches with entire Kombucha the enhanced mixing of the substrate FP and leaching solution was also able to promote leaching (The leaching of FP with supernatant was always done with shaking). Mubarok et al. (2015) also observed that shaking enhanced leaching.

From closer examination of the growth of the microorganisms, it appears that Kombucha grew well in stationary culture in the presence as well as absence of FP. However in shake culture, growth of Kombucha was occasionally disturbed in the presence of FP, suggesting a slight toxicity of FP. On the other hand, the Kombucha-pellicles in undisturbed approaches were larger in presence of FP than in its absence, both for the shake flask experiments as well as for the parallel submerse cultures. Similar results were obtained by Brantley et al. (2001) for the leaching of hornblende-minerals. After 18 days, the concentration of microorganisms in presence and absence (control) of the substrate hornblende were similar, but after 28 days, more growth had occurred in the presence of the leaching substrate. Furthermore, the solid content of the medium was rather high (28.3 g/l) in the present study compared to other studies described in the literature (Brandl et al., 2001; Hassanien et al., 2013; Ishigaki et al., 2005; Zhu et al., 2011). Therefore, toxic effects of FP could have been enhanced in the present study.

A comparison of the results with entire Kombucha and supernatant of spent medium leads to ambiguous conclusions. In the case of experiments run in stationary mode, leaching with microbes mobilized more REE than leaching with spent medium. In contrast, with shaking, more REE were mobilized by spent medium than by microbes. A possible explanation could be that in the approaches mainly with the stationary cultured supernatant, leaching of REE levelled off after two weeks owing to saturation of the solution. However, leaching with entire Kombucha continued beyond two weeks. Therefore, leaching for prolonged periods with intact Kombucha yields more mobilized REE than leaching with spent medium. Experiments with solutions of commercial organic acids lead to relatively small amounts of REE in supernatants, indicating that the organic acids contribute to the leaching effect, but only to a minor amount. Consequently a biogenic influence, possibly of other not yet identified other metabolic or cellular compounds, can be assumed. Pure distilled water and an inorganic solution of pH 2 could not extract REE, indicating that an increase of proton-concentration does not solely contribute to leaching.

These findings correspond to other studies. In most cases, it is described, that, direct leaching leads to more extensive mobilization than leaching with spent medium (Baglin et al., 1992; Beolchini et al., 2012; Brisson et al., 2015; Groudev, 1987; Qu and Lian, 2013) or commercial organic acids (Brisson et al., 2015; Glombitza et al., 1988; Hassanien et al., 2013). Glombitza et al. (1988), for example, investigated the mobilization of REE from zircon by a gluconic acid-producing bacterium *Acetobacter* species. In their experiments, REE release was significantly higher in the presence of biomass than in presence of commercial gluconic acid. In Reed et al. (2016) roughly similar leaching rates with microorganisms and spent supernatant were observed. In contradiction, Bosshard et al. (1996) and Brandl et al. (2001) reported that leaching using commercial organic acids increased when compared to bioleaching. Emmanuel et al. (2011), Selvam et al. (2012) and Qu and Lian (2013)reported, that REE enhanced the production of organic acids, thus enhancing the mobilization of REE. Other metabolites that could enhance leaching could be for example metal binding molecules like siderophors, as shown in the studies of Bau et al. (2013), Desouky et al. (2011) and Hassanien et al. (2013).

Furthermore, leaching experiments with single strains, isolated from Kombucha, were performed. With the yeast *Z. lentus* almost no leaching could be detected, likely due to the fact that no organic acids were produced. In contrast, with single cells of the bacterial isolate, a significant dissolution of REE compounds was achieved. However, the leaching rate after two weeks was considerably lower than the leaching rate using entire Kombucha or its supernatant. As such, it seems that the interaction of the different microorganisms benefit the bioleaching activities. It can be assumed that the yeast contributes to the bioleaching primarily through its metabolic activities and thus providing substrates for the bacterium *K. hansenii* (e. g. splitting glucose from sucrose) as well as supporting the production of organic acids as well as other, yet unidentified bioleaching enhancing metabolites.

When comparing the leaching rate of the single elements, a clear tendency is noticeable for preferential leaching of yttrium and europium. Therefore, the red dye Y_2O_3:Eu^{3+} was primarily leached. The experiments with pure model substances also confirmed these results: more than half of the applied pure Y_2O_3 was solubilized by the entire Kombucha within two weeks. In contrast, only minor amounts of $LaPO_4$ were leached during the same time. These results are in agreement with the literature: Also in Reed et al. (2016) mainly yttrium and europium were leached from FP. Furthermore, several papers (Binnemans et al., 2013; Cetinera et al., 2005; Kim and Osseo-Asare, 2012) report higher solubility of REE-oxides in comparison to REE-phosphates and aluminates. Wojtalewicz-Kasprzak (2007) and Haucke et al. (2011) described a sequential leaching process using inorganic acids. During the first steps, halophosphate-dyes and YOE were leached. The phosphate-

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and aluminate-dyes were solubilized afterwards under more aggressive conditions. Furthermore, Rabah (2008) reported a process for the selective recovery of yttrium and europium again by inorganic acids.

Experimentally it could be shown that with a solid content of more than 28 g/l FP, up to 6.5-mol% of REE could be solubilized by the mixed culture Kombucha within two weeks. These results provide a proof of principle demonstrating the possibility to extract REE from secondary resources using microorganisms. It can be assumed that the dissolved REE are partly immobilized via a secondary bio-mediated precipitation by adsorption to the biomass or at the surface of the remaining FP (Brantley et al., 2001; Delvasto et al., 2009; Desouky et al., 2011; Emmanuel et al., 2011; Zhuang et al., 2015). A precipitation as REE-phosphates (Evans, 1990) seems also possible, but not in the form of REE-hydroxides (Moeller and Kremers, 1944). Therefore the actual REE extraction may be even higher. Nevertheless, this part of REE is not solved the supernatant and therefore is not provided for a subsequent extraction step.

In comparison to chemical leaching, the bio-leaching rate is relatively low (Binnemans et al., 2013; Tanaka et al., 2013; Tunsu et al., 2011). However, chemical leaching involves numerous steps using very strong acids or toxic chemicals (Haucke et al., 2011; Rabah, 2008; Schimrosczyk, 2004; Tanaka et al., 2013; Tunsu et al., 2011). Furthermore, the bioleaching method described in this study is far from being optimised. Controlled growth in bioreactors, choice of alternative microbial consortia, sequential bioleaching as well as a combination with chemical methods may facilitate a selective dissolution of certain REE compounds from a complex material. In conclusion, the results contribute to the development of a bio-based environmentally friendly recycling process of REE waste materials.

5. Conclusions

In this study, the "tea fungus" Kombucha was investigated with regard to its ability to leach REE from FP. The advantage of using this mixed culture instead of single strains is that the Kombucha keeps itself free from contaminants through the creation of an acetic environment. Cultivation thus becomes much easier, especially in respect to subsequent industrial applications. For the first time, it could be proven for that microorganisms are able to degrade the tough REE-compounds by the production of organic acids and possibly other metabolites. This is the basis for the development of an environmentally friendly recycling-process using microorganisms. These results could also potentially be applied to other technical waste products.

In general, the cultivation by shaking leads to better leaching results than that for Kombucha common stationary approach. The best leaching results were obtained using an indirect leaching process, where the Kombucha is initially cultivated, and afterwards the supernatant is used as a leaching agent. However, with direct leaching procedures, a significant leaching rate could be obtained, too. The lower REE dissolution obtained with the isolates from Kombucha revealed that the interaction of the different microorganisms contributes to the leaching.

In future experiments, the leaching process should be optimized, for example by adjusting the growth conditions in a bioreactor or an increase of the energy source as described by Hassanien et al. (2013). Adaption of the microorganisms to the substrate (Brandl et al., 2001) or a lowering of the solid content could potentially enhance the bioleaching performance, too (Ishigaki et al., 2005). Furthermore, the leaching efficiency might be increased by addition of metal complexing molecules like siderophores (Desouky et al., 2011; Emmanuel et al., 2012). Additional investigations in membrane reactors could help in the understanding of the role of microorganisms in the leaching process.

Compliance with Ethical Standards

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