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Bioleaching of trace elements and rare earth elements from coal fly ash

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Abstract Coal fly ash originated from coal combustion has high concentrations of metals. If suitable leaching techniques are identified, then coal fly ash could serve as a useful source of valuable minerals including rare earth elements (REEs). In this study, three microbial strains, *Candida bombicola, Phanerochaete chrysosporium and Cryptococcus curvatus* were tested on their performance of leaching trace elements and REEs from fly ash. Through comparing mineral loss and leaching efficiencies resulting from indirect leaching or use of the culture supernatant, *C. bombicola* was identified to be the best leading to the highest mineral loss and extracting efficiencies of trace elements and REEs among the three strains. The highest mineral loss observed from using the supernatant of this yeast strain was 59.7%. Among all trace elements, As and Mo had the highest leaching efficiency of 80.9% and 79.5%, respectively. The same leaching test led to 67.7% of Yb and 64.6% of Er dissolved from the ash. This study, thus, demonstrated that bioleaching is feasible for leaching metals out of fly ash. The *C. bombicola* strain deserves further investigation due to its robust actions on metal leaching.

Keywords *Cryptococcus curvatus* · *Candida bombicola* · *Phanerochaete chrysosporium* · Coal fly ash · Bioleaching · Rare earth elements · Trace metals

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1 Introduction

To avoid using toxic chemicals that have been traditionally used in leaching of various metals, research and development on bioleaching or biomining have been on the rise. Bioleaching takes advantage of microbial activities to turn valuable but insoluble metal compounds into water soluble forms. Compared to chemical leaching, this technology has the advantages of being: (1) environmentally friendly, (2) low energy input and (3) low chemical cost. Due to these benefits, bioleaching has been used broadly in recovering metals, such as: Cu, Ni, Cr, Zn, Pb, Cd, Au from sulfidic low-grade ores (Watling et al. 2014) (Sutar and Awasare 2015), printed circuit boards (Karwowska et al. 2014), and various industrial solid wastes (Karwowska et al. 2015; Mishra and Rhee 2014). It has been estimated that as much as 15% of copper and 5% of gold production worldwide utilize microbial-assisted extraction technology (Johnson 2014). In terms of coal and coal related products,

bioleaching has been demonstrated to be successful in removing 13%–66% of Mn, Fe, Na, As, Cd, Cu, Ni, Zn, Cr, Co and Pb from Indian coals (Singh et al. 2014, 2015), and leaching at least 70% of Cr, Ni, As, and Pb from coal fly ash generated in China (Jadhav and Hocheng 2015).

Specific to recovery of rare earth elements (REEs), bioleaching has also been shown to be technically feasible. For example, from Egyptian monazite, 75.4% and 63.5% of REEs were leached by *Aspergillus ficuum* at 30 °C and *Pseudomonas aeruginosa* at 35 °C, respectively (Hassanien et al. 2013). In another study, use of the same *A. ficuum* strain dissolved 20% of La, 33% of Ce and 2.51% of Y in thorium–uranium concentrate (Desouky et al. 2011). With regard to Uraniferous Gibbsite ore containing 4,900 ppm of REEs, 55.1% of which was leached by the presence of an *Acidithiobacillus ferrooxidans* strain cultivated at 30 °C (Ibrahim and El-Sheikh 2011). Regarding coal ash studied in Russia, through incubation with an acidophilic chemolithotrophic microbial community, 52%.0, 52.6%, and 59.5% of Se, Y and La were recovered, respectively (Muravyov et al. 2015). Use of other microbial strains, however, resulted in REE leaching efficiencies of $< 0.2\%$ on Monazite-bearing ore (Shin et al. 2015). Thus, the bioleaching performance is highly dependent on selected microorganisms and may also be specific to target ores.

The mechanisms of bioleaching are set upon at least two facts: (1) the majority of leaching microbes grow attached on mineral surfaces (Schippers et al. 2013). This attachment is mediated by the extracellular polymeric substances (EPSs) that surround the cells. The EPSs can be sugars, lipids, proteins, nucleic acids and the combinations of these. Attachment and/or surface contact can stimulate EPS production by up to 100-fold (Noël et al. 2010; Vandevivere and Kirchman 1993); and (2) once cells are attached to minerals, they secrete organic acids, amino acids, peptides, lipids, enzyme complexes or even cyanide ions (Willscher and Bosecker 2003). Organic acids are recognized as the most important leaching agents in bioleaching processes using fungi and bacteria (Yang et al. 2008). The most observed acids are citric, oxalic, tartaric and gluconic. Organic acids promote mineral dissolution by: (1) donating H^+ to proton-promoted dissolution processes; (2) forming inner-sphere surface complexes that dislodge structural metals from the mineral surface; and (3) the formation of aqueous metal–ligand complexes that reduce the relative solution saturation with respect to minerals undergoing dissolution (Goyne et al. 2010).

In view of these mechanisms, we aimed to investigate three microbial strains on their performance of leaching metals from coal fly ash. Recovering metals from ash takes into consideration of: (1) while coal fly ash is a waste product from coal combustion, this material is enriched with minerals, such as trace elements and REEs; (2) metals, especially REEs including the 15 lanthanides plus yttrium and scandium (Lin et al. 2017) are valuable and extraordinarily useful elements for our society (Hower et al. 2016). REEs have broad uses in clean energy production, health care, automotive, electronics, and defense industries. Therefore, extracting metals from coal ash generates valuables for the society from a waste material. The rationale for studying three microbial strains are: (1) *Phanerochaete chrysosporium* belongs to wood-decay basidiomycetes, collectively referred to as white rot fungi (Fernandez-Fueyo et al. 2012). *Phanerochaete chrysosporium* was the first basidiomycete where lignin peroxidase and manganese peroxidase were discovered from (Fakoussa and Hofrichter 1999). In one study, after 16 days, this fungus was found to convert 85% of low rank coal to a form which was not recoverable by alkali washing and acid precipitation (Ralph and Catcheside 1994); (2) *Candida bombicola* is a non-pathogenic yeast strain and has the ability in producing sophorolipids which are surface active biocompounds (Samad et al. 2015, 2017). The highest yield of 422 g/L was observed when deproteinized whey and rapeseed oil were used as substrates for *C. bombicola* (Daniel et al. 1998); (3) *Cryptococcus curvatus* is an oleaginous yeast whose capability in accumulating intracellular lipids was demonstrated by our and numerous other researchers' studies. In particular, this yeast strain can grow on hydrolysates derived from plant biomass, which may contain a suite of toxic or inhibitory compounds (Liang et al. 2012, 2014a, b); (4) besides being robust, the commonality among the three is that during fermentation, the pH of the culture decreases, which may indicate the release of organic acids. And all three cultures can tolerate low pH between 3 and 4; and (5) none of these strains have been tested for leaching metals from coal fly ash.

2 Materials and methods

2.1 Microorganisms and inoculum preparation

White rot fungus, the basidiomycete, *Phanerochaete chrysosporium* (ATCC 24725) was grown in a liquid medium made from a recipe reported in (Tien and Kirk 1988). Briefly, the medium contained (per liter) of 100 mL of Basal II medium, 100 mL of 10% glucose, 100 mL of 0.1 M 2,2-dimethylsuccinate (pH 4.2), 10 mL of 100 mg/L of thiamin, 25 mL of 8 g/L of ammonium tartrate, 100 mL of veratryl alcohol and 60 mL trace elements. A yeast strain, *Candida* (*Starmerella*) *bombicola* (ATCC 22214) was maintained on agar plates containing (per liter): glucose, 100 g; yeast extract, 10 g; urea, 1 g; and agar, 20 g. Colonies were transferred to fresh plates every 6 weeks and

were used to start inoculum in the same medium but without agar (Samad et al. 2015). Another yeast strain, *Cryptococcus curvatus* (ATCC 20509) was set up by adding frozen stock of this yeast to a medium containing yeast extract (10 g/L), peptone (20 g/L), and glucose (10 g/L). For each of these cultures, the pH of the growth medium was adjusted to 5.5 using HCl or NaOH. All liquid cultures were maintained in a shaking incubator at 120 rpm and 28 °C.

2.2 Coal fly ash

The fly ash was obtained from the combustion of coal at the Southern Illinois University Carbondale power plant. The collected ash was ground in a ball mill for 1 h and sieved through a 200 mesh, where particles less than 74 µm were used in this study. The fly ash at the designated size was characterized in terms of moisture content and mineral content. For the former, the samples were dried in an oven at 103 °C until constant weight. For the latter, the dried samples were placed in a furnace at 550 °C for 6 h. To avoid confusion, the content of those incombustible materials was termed as content of minerals instead of content of ash since fly ash was investigated here.

2.3 Bioleaching experiments

Fly ash leaching was conducted by using supernatant as indicated in Fig. 1. Firstly, the microbial cells were revived from corresponding frozen stocks stored at − 80 °C. Secondly, the revived cells at 10% of the final culture volume were grown on fly ash at 1% (w/v) in the standard medium detailed above for three days. During the fermentation period, the cultures were maintained at 28 °C in darkness with shaking at 150 rpm. The cultures were monitored closely on a daily basis for cell growth by measuring optical density at 600 nm using a spectrophotometer and pH using a pH meter. After three days, the whole culture was centrifuged at 4,500*g* for 15 min to separate solid from liquid. The liquid portion, termed as supernatant was used to leach fresh fly ash. This leaching was conducted at 28 °C for 6 h with shaking at 50 rpm. After 6 h, the solid was separated from the liquid by filtration through a 0.45-µm membrane filter. The solid was then washed with 50 mL of distilled and deionized water (DDW), dried in an oven at 105 °C until constant weight to measure total solid content, and subsequently put in a 550 °C muffle furnace for 6 h to measure inorganic mineral content. The residual minerals were then analyzed by ICP-MS for quantifying individual metal content. It needs to be noted that all of these experiments were performed for each of the three target microorganisms (Fig. 1) and all experiments were conducted with three replicates. Again, since fly ash contained both minerals and volatile materials, the term of mineral loss was used instead of ash loss to count for those losses contributed by minerals or metals only. In addition to the supernatant, DDW and each growth medium were used to leach fly ash, too.

2.4 ICP-MS

Metals including REEs were analyzed by following procedures outlined in EPA method 200.8, Revision 5.4: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma—Mass Spectrometry (ICP-MS). Briefly, both fly ash and residual ash samples after 550 °C in a furnace were subjected to this analysis. The samples were digested in a diluted mixture of nitric acid and hydrochloric acid for reflux extraction of the analytes at 95 °C for 30 min. After cooling to room temperature, the samples were diluted and analyzed by ICP-MS. Metals were quantified based on calibration curves established for mixtures of trace elements listed in Method 200.8 and those of REEs.

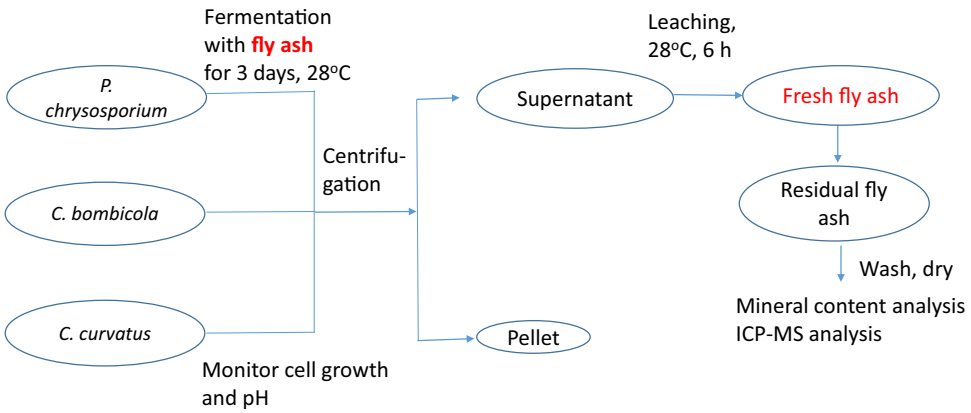

Fig. 1 The overall experimental design

3 Results and discussion

As discussed above, bioleaching has been performed through using different microorganisms. But for the three targeted here, no studies have been conducted yet. Since this is the first bioleaching study on the three strains, our initial questions were: (1) Can these three microorganisms leach metals out of the fly ash? (2) If yes, which one is the best? And (3) What are the efficiencies in leaching different metals? To answer these unknowns, we took a down select approach. At the beginning, all three microbes were cultivated simultaneously in specific standard medium with fly ash as shown in Fig. 1. Based on mineral loss and metals' leaching efficiencies, one microorganism was studied further.

As described above, the mineral loss (%) = [(total minerals in the initial fly ash) − (total minerals in the residual fly ash)]/total minerals in the initial fly ash × 100%. In this case, the mineral is the same as total metals. We used the term minerals instead of ash to avoid confusion since fly ash was the initial substrate. In addition, the fly ash we used contained 85.2% of minerals or metals.

To measure mineral loss, we could directly measure metal concentrations in the leaching liquid. We chose instead to measure metal contents in the initial and final ash. By doing so, we eliminated the need to consider metal recovery efficiency by using EPA method 200.8 since all solid samples would go through the same extraction procedure: solubilization by gentle refluxing with nitric and hydrochloric acids.

In this study, only the performance of the supernatant in metal leaching was investigated. This is also referred to indirect or two-step leaching compared to direct or one-step leaching by using microbial cells (Brandl et al. 2001). This approach considered the difficulty in separating cells from residual fly ash. In particular, if fungi are used, leached metals are often adsorbed to or enclosed by the fungal mycelium (Jain and Sharma 2004) (Hopfe et al. 2017), which makes separating residual solid substrates from fungal biomass nearly impossible. Thus, for the three heterotrophic microorganisms, the strategy we used was to grow the microbes first and then use the metabolites in the supernatant for quantitative leaching.

The fly ash used in this study had a moisture content of 0.419% ± 0.039% (wet weight based). The mineral content was 0.852 ± 0.002 g/g, dry weight (dw) based. Thus, the content of volatile matter was 0.148 g/g (dw based). The major trace elements detected in the fly ash were (Table 1): Fe: 62.0 2 g/kg; Al: 46.72 g/kg; Zn: 240.37 mg/kg; V: 104.30 mg/kg; Ba: 99.31 mg/kg; Mn: 94.09 mg/kg; and Pb: 86.52 mg/kg. The other 14 had content lower than 50 mg/kg. Among REEs, the dominant ones were: Ce, 47.3 mg/kg; Y: 26.0 mg/kg; La, 22.9 mg/kg; Nd, 21.1 mg/kg; and Sc: 16.7 mg/kg. The other 12 had concentrations lower than 10 mg/kg.

Table 1 Elemental composition of fly ash

Metal	Content (dw) [mg/kg]
Be	9.17
Al	46721.54
V	104.30
Cr	53.77
Mn	94.09
Fe	62021.68
Co	14.18
Ni	43.43
Cu	48.60
Zn	240.37
As	4.62
Se	17.77
Mo	8.18
Ag	0.23
Cd	3.82
Sb	0.27
Ba	99.31
Tl	0.14
Pb	86.52
Th	8.43
U	7.05
Sc	16.7
Y	26
La	22.9
Ce	47.3
Pr	5.5
Nd	21.1
Sm	4.8
Eu	1.1
Gd	4.6
Tb	< 1
Dy	4.2
Ho	< 1
Er	2.2
Yb	1.9
Th	6.7
Lu	< 1
Tm	< 1

After mixing fresh fly ash with supernatant at 28 °C, 50 rpm for 6 h, significant loss of minerals was observed. Regarding *C. bombicola*, the mineral loss was 59.74% ± 1.07% of those initially in the fly ash. For *P. chrysosporium* supernatant, it was 51.28% ± 3.63%. In

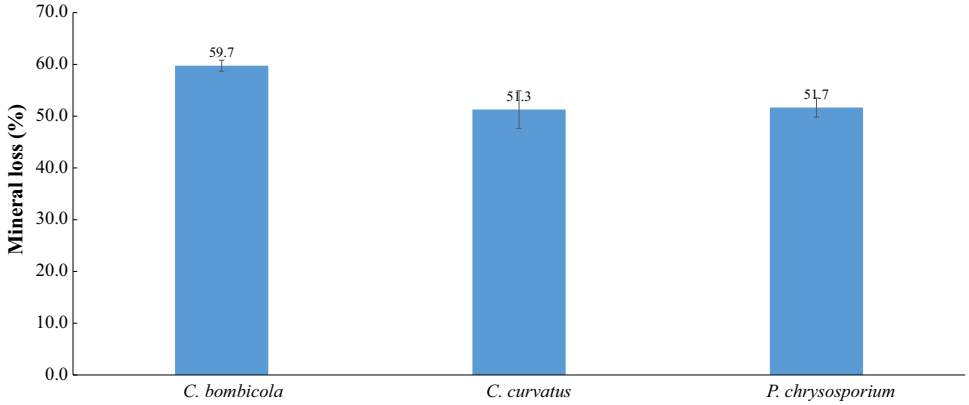

Fig. 2 Mineral loss resulting from using supernatant for leaching fresh fly ash

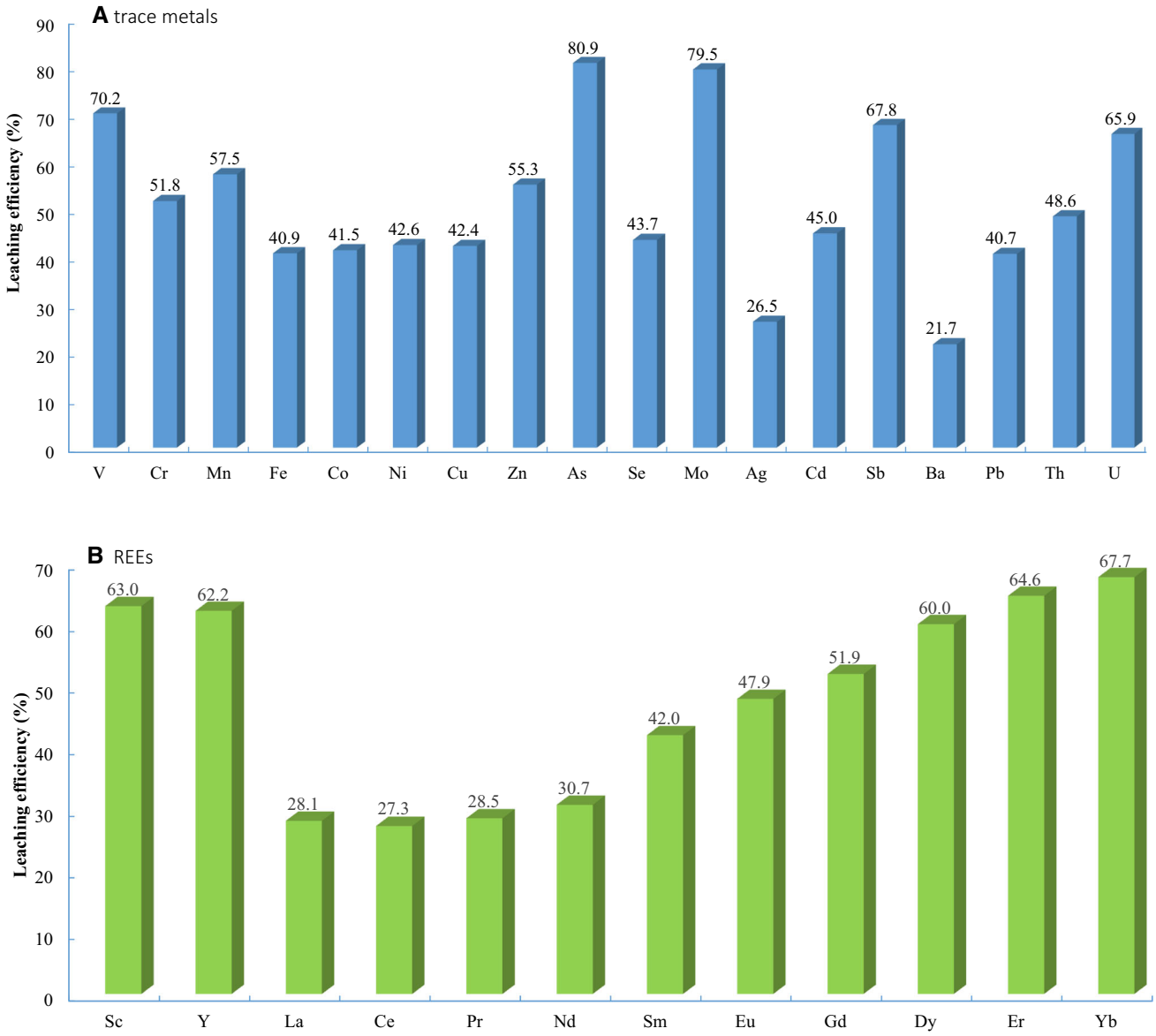

Fig. 3 Leaching efficiency of *C. bombicola*. **a** Trace metals; **b** REEs

terms of *C. curvatus*, it was 51.66% ± 1.84% (Fig. 2). After processing the remaining ash in a muffle furnace at 550 °C, the left material referred to as residual mineral was analyzed by ICP-MS. Based on calibration curves built for trace elements and REEs, all metals were quantified. As indicated in Fig. 3, the leaching efficiency of trace elements by *C. bombicola* supernatant varied between 21.7% and 80.9%. Metals, such as Ba and Ag had the lowest extraction efficiency while As and Mo had the highest. With regard to REEs, the loss of La, Ce, Pr and Nd was the lowest, ranging between 28.1% and 30.7%. The highest loss was for Yb (67.7%), Er (64.6%), Sc (63.0%) and Y (62.2%). In terms of *P. chrysosporium* (Fig. 4), among trace elements, As and V had the largest extraction efficiency of 76.8% and 65.2%, respectively while Ag and Co had the lowest of around 20%. For REEs, Yb, Sc, Er and Dy had the highest loss in the range of 47%–50.6%. Similar to *C. bombicola* supernatant, La, Ce, Pr and Nd were leached the least with losses between 22.3% and 26.0%. Regarding *C. curvatus*, the highest leaching efficiency was observed for As (75.2%) and Mo (72.6%) and the lowest was for Ba (8.3%) among trace elements. Among REEs, the highest loss was detected for Yb (56.1%), Er (53.2%), Sc (52.1) and Dy (52.1%). The lowest loss was again for Ce, La, Pr, and Nd in the range of 19.5% and 24.4% (Fig. 5).

Comparing leaching results from using the supernatant derived from the three microbial strains, the exaction

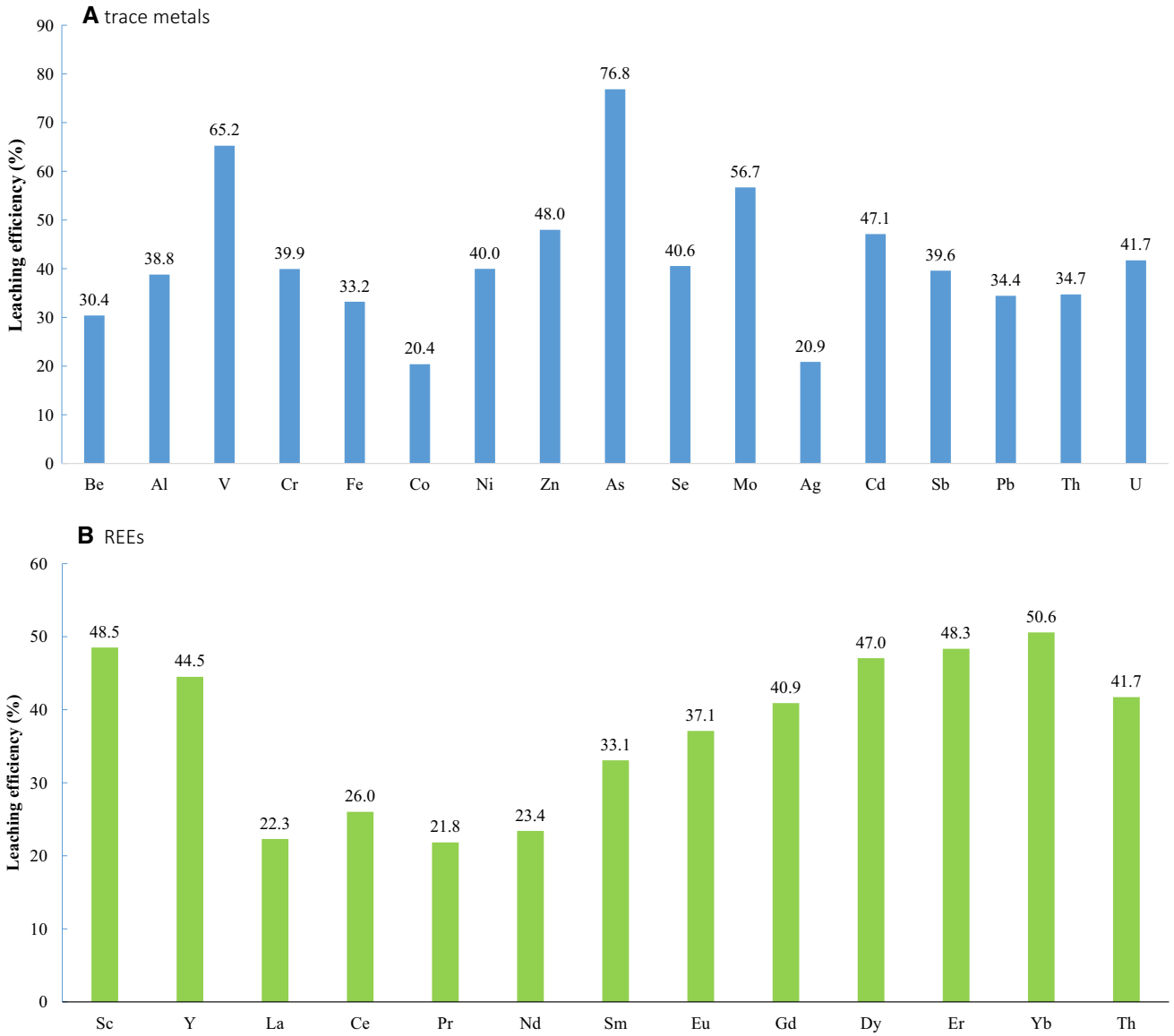

Fig. 4 Leaching efficiency of *P. chrysosporium*. **a** Trace metals; **b** REEs

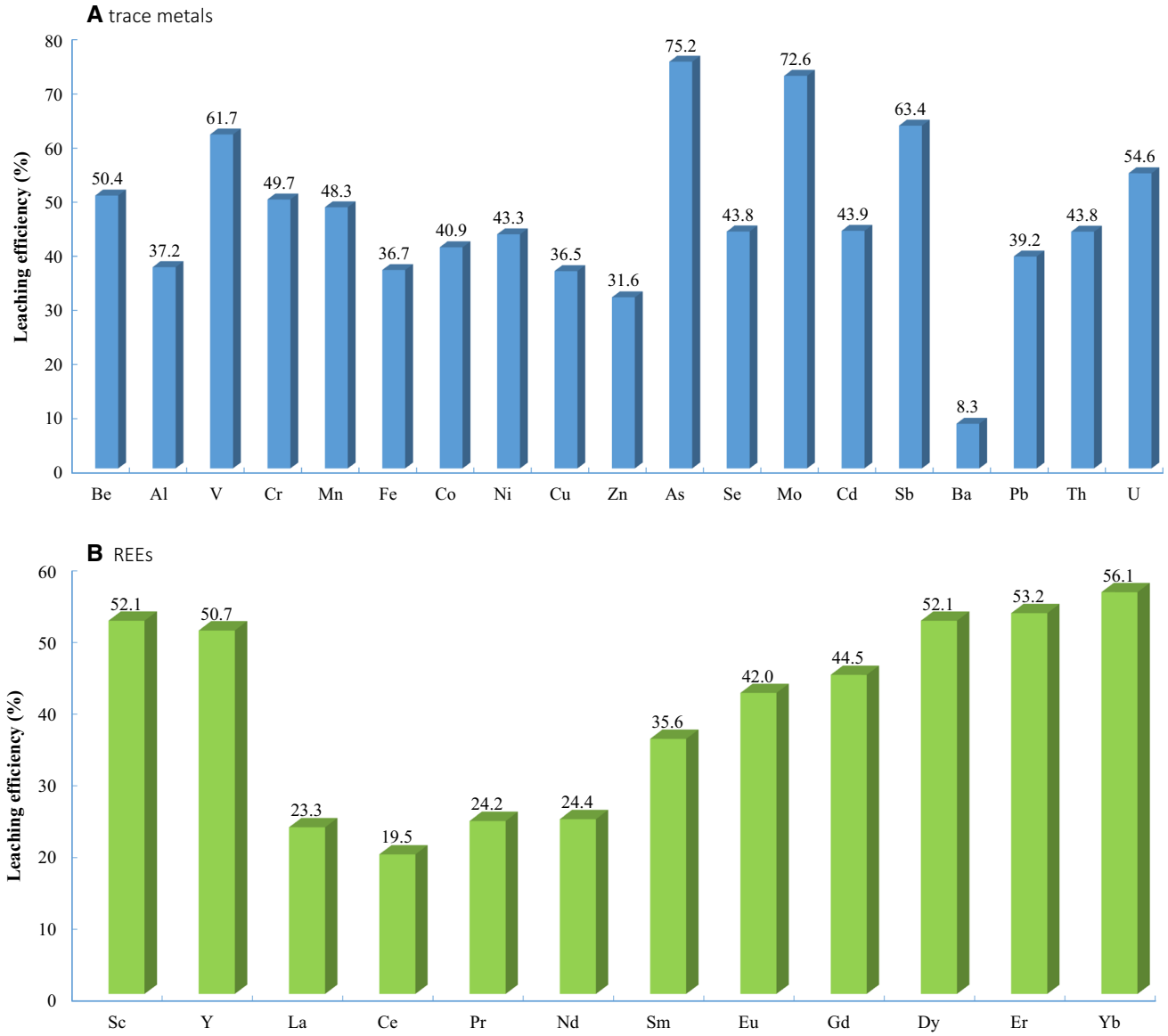

Fig. 5 Leaching efficiency of *C. curvatus*. **a** Trace metals; **b** REEs

profile of trace elements was different (Figs. 3a, 4a and 5a), but As and Mo had the highest loss and Ag and Ba had the lowest for all of the three strains. The leaching profile of REEs (Figs. 3b, 4b and 5b) was very similar although the exact extraction efficiency was different.

Based upon all of these results, we concluded that *C. bombicola* was the best microorganism for leaching metals out of fly ash. To understand whether fermentation time had an effect on leaching, *C. bombicola* was cultivated in the standard medium with fly ash for 3, 6, or 9 days. During fermentation, compared with controls without fly ash, the presence of fly ash did lead to decreased cell growth as reflected from lower optical density (OD). But, the extent of pH decrease was similar between those two sets (Fig. 6). Supernatant from those with fly ash was also used to leach fresh fly ash. As demonstrated by Fig. 7, fermentation broth from longer cultivation time did not lead to statistically higher leaching efficiencies. Therefore, for achieving the highest leaching efficiencies, a fermentation period of 3 days is enough.

Candida bombicola is known to tolerate low pH. For the purpose of producing sophorolipids-compounds comprising a sophorose head whose reducing end is connected to a terminally or subterminally hydroxylated fatty acid through a beta-glycosidic bond (Ciesielska et al. 2014), the culture pH is normally maintained at 3.3–3.5 (Davila et al. 1997; Pekin et al. 2005; Daniel et al. 1998; Solaiman et al. 2004). The pH decrease could be due to the addition of a fatty acid which is a co-substrate for sophorolipid synthesis and/or autoacidification (Rau et al. 2001). For the latter when a

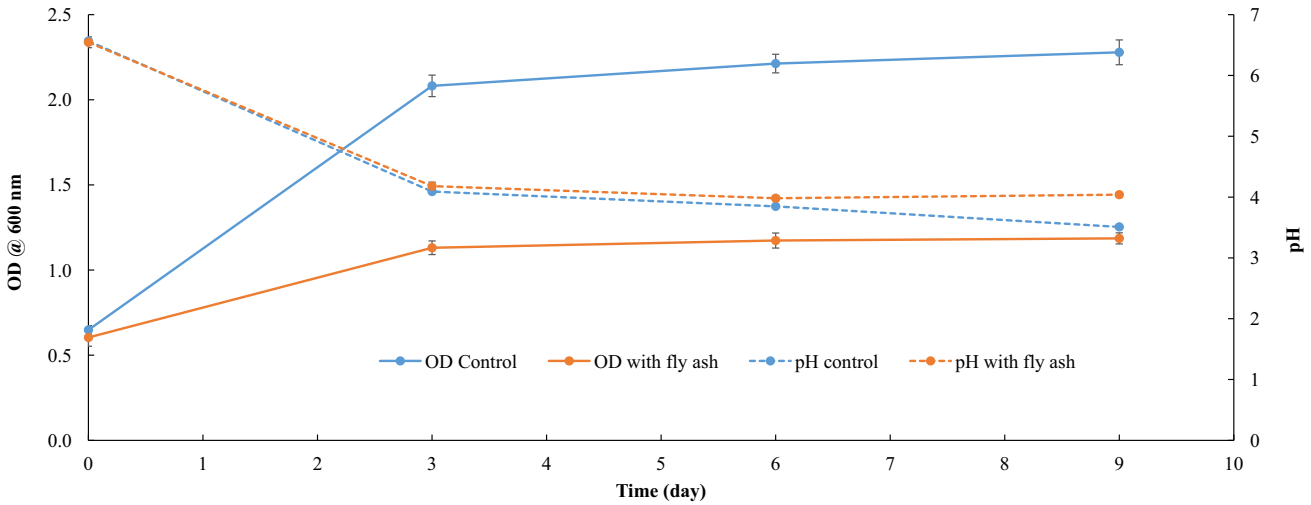

Fig. 6 *Candida bombicola* growth and pH change with time

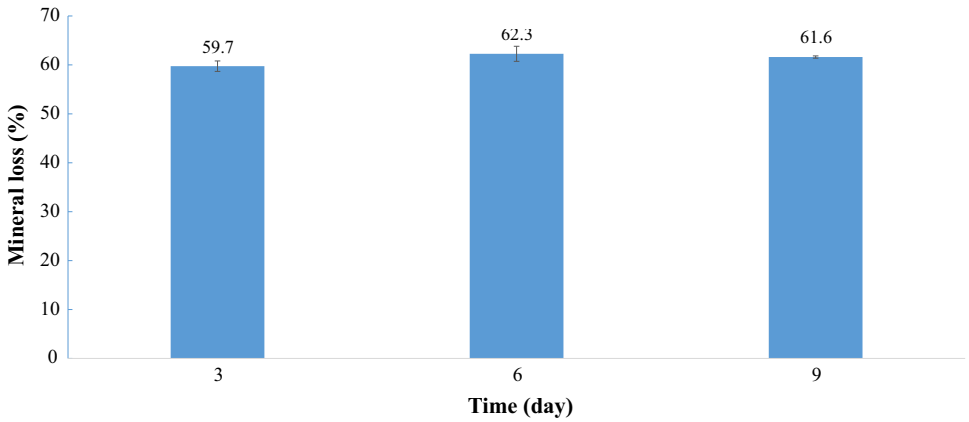

Fig. 7 Leaching efficiency of *C. bombicola* broth on fresh fly ash-effect of fermentation time

fatty acid, such as oleic acid is not used, the pH decrease could be explained by the formation of acidic sophorolipids versus lactonic forms or the release of other organic acids. However, although production of acidic sophorolipids has been reported by different studies, whose concentration depends on the specific fermentation condition, formation of other organic acids by *C. bombicola* has not been disclosed yet. The similar leaching profile of REEs by the three tested microorganisms suggest that the leaching agents released by the three may be similar in properties. However, further research is warranted to isolate and characterize the chemicals that are responsible for metal-leaching.

The leaching efficiencies obtained for the three microorganisms were similar to the highest ones reported and much higher than the others (Table 2). The highest REE extraction efficiency of 75.4% was attained from Monazite by use of *Aspergillus ficuum* (Hassanien et al. 2013). In the same study, by using *Pseudomonas aeruginosa*, the REE leaching efficiency was 63.5%. The only study that focused on coal ash was reported by Muravyov et al. (2015) where leaching efficiency of three REEs were listed. All other studies have target different raw materials (Desouky et al. 2011; Ibrahim and El-Sheikh 2011; Shin et al. 2015) and the results are difficult to be compared.

Although significant amounts of trace elements and REEs were leached by the studied microbial strains in this study, several questions still remain. For example, what are the optimal condition for leaching metals from fly ash? What are the mechanisms involved in such leaching? Can the leaching process be controlled to dissolve specific metals of interest? And can this bioleaching process through using heterotrophs be scaled up at commercial levels? To answer these questions, in-depth studies must be performed.

Table 2 Comparison of leaching efficiency among different studies

Microorganism	Raw material	Temperature (°C)	Duration (d)	Pulp density (%)	Shaking speed (rpm)	Leaching efficiency (%)	References
Acidophilic chemolithotrophic community	Ash-slug waste	45	10	10	NA	Sc: 52.0%, Y: 52.6%, La: 59.5%	Muravyov et al. (2015)
Aspergillus ficuum	Monazite	30	9	0.6	NA	75.4% of REEs	Hassanien et al. (2013)
Pseudomonas aeruginosa		35	8	0.6	175	63.5 REEs	
Aspergillus ficuum	Thorium–uranium concentrate	28	1	0.75	175	La 20%; Ce 33.0%; Y: 2.51%	Desouky et al. (2011)
Acidithiobacillus ferrooxidans	Uraniferous Gibbsite ore	NA	30 Cycles	NA	NA	67.58% of REEs	Ibrahim and El-Sheikh (2011)
Acetobacter aceti	Monazite bearing ore	30	9	16.7	180	Ce: 0.13%; La: 0.11%	Shin et al. (2015)
Candida bombicola	Coal fly ash	28	6 h[a]	1	50	Yb: 67.7%; Er: 64.6%; Sc: 63.0%	This study

[a]Supernatant from the culture was used for leaching

4 Conclusion

This study demonstrated that recovering trace elements and REEs from coal fly ash through bioleaching is possible through using different microorganisms. Among the three tested, the fermentation broth of *C. bombicola* gave the highest leaching efficiency of 59.7%. But comparing the results from the three microorganisms, it is interesting that the leaching profiles of REEs were similar. Certain REEs, such Yb, Er, Sc and Y had the highest loss independent of the culture supernatant used. The mechanisms of bioleaching warrant further investigation. Given the promising results obtained from this study and the low cost feature of bioleaching, this approach deserves to be explored further for extracting metals from solid waste materials beyond coal fly ash. By doing so, we can achieve the dual purposes of waste minimization and resource recovery.

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