

Low residual dissolved phosphate in spent medium bioleaching enables rapid and enhanced solubilization of rare earth elements from end-of-life NiMH batteries

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ABSTRACT

Successful heterotrophic bioleaching with high metal yields requires an efficient leaching agent production and minimization of secondary reactions such as precipitation of leached metals with growth medium components. In this study, the role of the secondary reactions on bioleaching of spent nickel-metal-hydride batteries was investigated. Substitution of K_2HPO_4 by yeast extract (YE) reduced precipitation of both base metals and rare earth elements (REEs). REEs were proportionally more affected by precipitation than base metals. Optimizing the ratio of YE to glucose in the growth medium resulted in glucose to gluconic acid conversion yield of 90% by *Gluconobacter oxydans*. In one-day leaching with YE medium, 28.8% Mn, 52.8% Fe, 22.9% Co, 12.0% Ni, and 19.5% of total REEs were extracted. The leach liquor obtained with the YE medium resulted in leaching of 1.5 and 11.0 times more of total base metals and REEs, respectively, than with phosphate medium. Experimental results were consistent with geochemical modeling results corroborating the benefit of low phosphate concentrations in leaching systems at neutral to moderately acidic pH. In summary, substitution of K_2HPO_4 with YE in gluconic acid production phase with *G. oxydans* reduced subsequent base metal and especially REE precipitation during leaching and, thus, enhanced the overall metal extraction.

1. Introduction

Rare earth elements (REEs) are critical metals of high economic importance that suffer from an imminent risk of supply depletion (Barteková and Kemp, 2016). In order to overcome REE deficiency, low-cost and sustainable recycling of REEs from end-of-life products such as spent nickel-metal-hydride (NiMH) batteries, fluorescent lamps, catalysts and magnets and/or waste materials like coal ash, red mud and scrap alloys is in high demand (Auerbach et al., 2019; Borra et al., 2015; Jyothi et al., 2020; Lu et al., 2020; Rasoulnia et al., 2020). Heterotrophic bioleaching, which uses microorganisms to mediate metal mobilization from solid materials, is increasingly explored as a potential REE extraction method (Dev et al., 2020; Fathollahzadeh et al., 2019; Rasoulnia et al., 2020) due to its potential for lowering costs and reducing the use of environmentally hazardous compounds compared to physico-chemical recovery methods (Brierley and Brierley, 2013; Jin

et al., 2019; Thompson et al., 2018). Metabolites produced by heterotrophic bacteria and fungi, can extract REEs from a range of alkaline waste materials that are not amenable for acidophilic bioleaching (Cockell et al., 2020; Pollmann et al., 2018; Rasoulnia et al., 2021b).

Heterotrophic microorganisms oxidize organic compounds such as glucose and sucrose to organic acids that enable metal extraction via complexation and/or proton promoted leaching (Cockell et al., 2020; Fathollahzadeh et al., 2019; Pollmann et al., 2018). Microbial growth and efficient conversion of the supplied organics into desired leaching agents, require nitrogen and phosphorous supply (Brisson et al., 2016; Rasoulnia et al., 2021b). The ratio of carbon to nitrogen and phosphorous in bioconversion processes needs to be optimized for the target products and the selected microorganisms (Poljungreed and Boonyarattanakalin, 2018).

Yeast extract (YE), phosphate and ammonium salts such as K_2HPO_4 , KH_2PO_4 and $(NH_4)_2SO_4$ are typical nitrogen and phosphorous sources

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used for various microorganisms (Barnett et al., 2020; Dewi et al., 2020; Mouna and Baral, 2019; Poljungreed and Boonyarattanakalin, 2018; Rasoulnia et al., 2021b; Reed et al., 2016). YE is typically more costly than inorganic ammonium and phosphate salts (Poljungreed and Boonyarattanakalin, 2018). However, as YE provides a variety of other essential nutrients (Thompson et al., 2017), small quantities of YE are usually supplemented with nitrogen and phosphorous salts. Phosphate salts act not only as a phosphorous source, but also as a buffering agent (De Wulf et al., 2000; Rasoulnia et al., 2021b) that aids in maintaining the medium pH within the desired level (Poljungreed and Boonyarattanakalin, 2018). Due to their positive impact on leaching agent production and their low costs, phosphate salts such as K_2HPO_4 and KH_2PO_4 have been commonly used in heterotrophic REE bioleaching studies (Amin et al., 2014; Barnett et al., 2020; Dewi et al., 2020; Jin et al., 2019; Mouna and Baral, 2019; Park and Liang, 2019; Reed et al., 2016; Reynier et al., 2021). These studies have focused on maximization of leaching agent production for REE leaching, whilst possible REE precipitation with residual dissolved phosphate has not been considered. Precipitation of REEs with phosphate during the leaching stage can lead to a decrease in REE recycling yield and thus reducing the economic benefits of the whole process. To our knowledge, the impacts of such reactions on the attainable REE and base metal leaching yields have not been previously reported. Therefore, the present study focused on enhancing bioleaching of REEs and base metals from spent NiMH batteries by investigating the following:

1. Quantification of REE and base metal precipitation in the presence of various concentrations of K_2HPO_4 and YE.
2. Developing a medium containing YE as the sole phosphorus source for gluconic acid production by *Gluconobacter oxydans* to minimize metal precipitation and enhance REE and base metal leaching from spent NiMH batteries.
3. Evaluating the impacts of presence and absence of phosphate on REE and base metal bioleaching yields with *G. oxydans* supernatants.
4. Modeling of pH and phosphate effects on REE solubility under typical bioleaching conditions.

2. Materials and methods

2.1. Spent NiMH battery powder composition

The spent NiMH battery powder was obtained from battery recycling company AkkuSer Oy in Finland. The material used was the same as previously reported (Rasoulnia et al., 2021b, 2021a). The total REE content of the spent NiMH battery was 21 wt% mostly comprising of La, Ce and Nd while the most abundant base metals were Ni and Co

Table 1

Composition of the spent NiMH battery powder used in the bioleaching experiments.

Element	Spent NiMH battery content (wt%)
Ni	43.0
Co	9.00
Mn	2.30
Cu	1.20
Zn	1.00
Fe	0.50
La	10.2
Ce	6.00
Nd	3.00
Pr	1.00
Sm	0.30
Y	0.15
Yb	0.05
Gd	0.04
Er	0.03
Tb	0.002
Dy	0.001

(Table 1). Spent NiMH battery powder with a particle size of $\leq 630 \mu m$ was used in the bioleaching experiments.

2.2. Microorganism and growth medium

Gluconobacter oxydans (DSM 3503) was obtained from German Collection of Microorganisms and Cell Cultures (DSMZ). Glucose-yeast extract (GY) medium containing 100 g/l glucose, 10 g/l YE, 20 g/l $CaCO_3$ and 15 g/l agar was utilized to maintain *G. oxydans* stock culture. GY broth excluding $CaCO_3$ and agar was utilized to prepare *G. oxydans* inoculum for the gluconic acid production and bioleaching experiments, in which a modified Pikovskaya medium (from now on referred to as Phosphate medium) was used as the base (Rasoulnia et al., 2021b). However, depending on the aim at different stages of the experiments, Phosphate medium was further modified as shown in Table 2. All the incubations were conducted at $27 \pm 1 \text{ }^\circ C$.

2.3. Precipitation of REEs and base metals

The effects of different concentrations of K_2HPO_4 and YE on the solubility of REEs were studied with a metal containing leachate (MC leachate) prepared by chemical leaching of spent NiMH battery using 12 g/l D-gluconic acid sodium salt (10 g/l gluconate) at an initial pH of 2.6 (adjusted using 65% nitric acid). The leaching was conducted at 150 rpm and $27 \pm 1 \text{ }^\circ C$ in 50 ml metal-free polypropylene tubes containing 20 ml of the leach solution with 1% (wt/v) pulp density of the spent NiMH battery powder. After 24 h of leaching, the MC leachate was harvested by filtration through 0.45 μm disposable syringe filters (CHROMAFIL® Xtra PET-45/25, Germany).

The precipitation experiments were carried out in 50 ml metal-free polypropylene tubes containing 20 ml of the MC leachate with different concentrations of K_2HPO_4 or YE. After 24 h rotating at 150 rpm and $27 \pm 1 \text{ }^\circ C$, the mixtures were filtered through 0.45 μm disposable syringe filters. Metal precipitation was evaluated by analyzing soluble metal content of the samples before and after addition of K_2HPO_4 or YE.

Table 2

Different media compositions used for *Gluconobacter oxydans* in different experiments. All concentrations are given in g/l.

Medium component	Phosphate medium*	Modified media set 1**	Modified media set 2***	Modified media set 3****	YE medium#
Glucose	10	40	40	10	10
K_2HPO_4	0.47	0.1, 0.25 or 0.5	–	–	–
Yeast extract	0.5	0.05	0, 0.1, 0.25, 0.5, 0.75 or 1	0.4, 0.6, 0.8, 1.0	0.8
$CaCl_2 \cdot 2H_2O$	0.044	0.044	0.044	0.044	0.044
$(NH_4)_2SO_4$	0.5	0.5	0.5	0.5	0.5
NaCl	0.2	0.2	0.2	0.2	0.2
KCl	0.2	0.2	0.2	0.2	0.2
$MgSO_4$	0.1	0.1	0.1	0.1	0.1

* Phosphate medium was used as the base medium for the modifications and bioleaching efficiency using this medium was compared with that of optimum modified medium (YE medium).

** Modified media set 1 was used to evaluate gluconic acid production in presence of different concentrations of K_2HPO_4 with a minor amount of YE.

*** Modified media set 2 was used to evaluate gluconic acid production in presence of different YE concentrations without K_2HPO_4 .

**** Modified media set 3 was used to find an optimum YE/G ratio for optimum gluconic acid production.

YE medium (=modified medium from set 3 with 0.8 g/l YE or YE/G ratio of 0.08) was found to be the optimum medium for gluconic acid production and used for bioleaching.

2.4. Substitution of K_2HPO_4 with yeast extract in gluconic acid production

Gluconic acid production by *G. oxydans* was studied in the presence and absence of K_2HPO_4 in two set of experiments using two different modifications of the Phosphate medium with a glucose content of 40 g/l. In the first experiments, the 0.5 g/l YE content of the Phosphate medium was reduced to 0.05 g/l and used in combination with K_2HPO_4 at a concentration of 0.1, 0.25 or 0.5 g/l (Modified media set 1). In the second set of experiments, K_2HPO_4 was omitted and YE was supplied as the sole phosphorous source at a concentration of 0, 0.1, 0.25, 0.5, 0.75 or 1 g/l (Modified media set 2), resulting in yeast extract to glucose (YE/G) mass ratios of 0, 0.0025, 0.0062, 0.0125, 0.0187 and 0.025. The initial pH of all the media was adjusted to 6.5 prior to addition of 1 ml of fresh *G. oxydans* culture (OD_{600} : 1.3, pH: 2.5), which had been grown for seven days in GY broth (100 g/l glucose and 10 g/l YE).

2.5. Optimizing yeast extract to glucose ratio for gluconic acid production

Gluconic acid production by *G. oxydans* was further investigated via applying higher YE/G mass ratios than those used in Modified media set 2 (Modified media set 3). In order to avoid excessive pH reduction, 10 g/l glucose was used. Modified media set 3 was amended with 0.4, 0.6, 0.8, 1.0, 1.5, or 2.0 g/l YE resulting in YE/G mass ratios of 0.04, 0.06, 0.08, 0.1, 0.15, and 0.2. Furthermore, to avoid drastic pH changes, the *G. oxydans* inoculum was grown in a GY broth containing only 10 g/l glucose and 10 g/l YE for four days (OD_{600} : 1.4, pH: 3.0). All the gluconic acid production experiments were carried out in 250 ml shake flasks containing 100 ml of the respective media inoculated with 1 ml of *G. oxydans* and incubated at 27 ± 1 °C and 150 rpm for 6 days.

2.6. Bioleaching of spent NiMH battery material

Bioleaching experiments were conducted in two different media with a glucose content of 10 g/l: 1) Phosphate medium with YE/G ratio of 0.05 containing 0.47 g/l K_2HPO_4 and 2) YE medium with YE/G ratio of 0.08 without K_2HPO_4 (Table 2). The inoculum preparation procedure and the conditions for 4-day gluconic acid production were the same as in section 2.5. Spent medium bioleaching experiments were conducted using cell-free culture supernatants from the gluconic acid production step by separating the cells from the culture media via centrifugation at 10,000 rpm for 10 min (SIGMA, 4-16KS, Germany). Subsequently, 1% (w/v) pulp density of the spent NiMH battery powder was added to the cell-free culture supernatants and was leached for seven days at 27 ± 1 °C and 150 rpm in 50 ml metal-free polypropylene tubes containing 20 ml of the spent medium solution. Control tubes contained 20 ml of the respective sterile uninoculated media with 1% (w/v) pulp density of the spent NiMH battery powder.

2.7. Analytical methods

Growth of *G. oxydans* was monitored by optical density at 600 nm (OD_{600}) measured using Ultrospec 500 pro spectrophotometer (Amersham Biosciences, England). Ultrapure water was used as blank for the OD measurements. A pH meter with a Hamilton Slimtrode electrode (pH 3110, WTW 82,362 Wellhelm, Germany) was used to measure pH. Gluconic acid concentration was analyzed by high performance liquid chromatography (HPLC) using a Shimadzu (SIL-40C series, Japan) device equipped with an Aminex HPX-87H column (300 × 7.8 mm) and a diode array detector at 210 nm. The measurement conditions were the following: sample injection volume 2 µl, mobile phase 20 mM sulfuric acid, mobile phase flow rate 0.5 ml/min, column temperature 50 °C, stop time 30 min. A Shimadzu HPLC (SIL-20 series, Japan) equipped with a Shodex column (HILICpak, VG-50 4E) and a refractive index detector was utilized for glucose concentration determination. The mobile phase was a 70–30% (v/v) mixture of acetonitrile and 0.5% NH_3

(aq) with a flow rate of 0.2 ml/min. Prior to injection the samples were mixed with acetonitrile at a 30–70% ratio (v/v) and the sample injection volume was 5 µl. The column temperature during the run was 40 °C and the stop time was 40 min. Inductively coupled plasma mass spectrometry (ICAP RQ ICP-MS, Thermo Scientific, USA) was used to measure the metal concentrations. All measurements were performed in kinetic energy discrimination mode using He as collision gas in the collision/reaction cell and Ar as the carrier gas. On-line addition of an internal standard solution containing 1 µg/l U, 1 µg/l Rh and 5 µg/l Ge in 2% HNO_3 was used for the correction of nonspectral interferences in the analysis. Ionic standard solutions with appropriate concentration range for each analyte were prepared in 2% HNO_3 using ICP grade chemicals (Romil PrimAg®-xtra mono-component reference solutions and 67,349 Rare earth elements mix for ICP, Sigma-Aldrich), and were applied to measure the calibration curves. The samples were diluted 1000 times for the measurement using 2% HNO_3 . Ultrapure H_2O (18.2 MΩ cm, Merck Milli-Q®) and super pure acids (Romil-SpA™) were used for preparation of all the samples. Disposable syringe filters of 0.2 µm (CHROMAFIL® Xtra PET-20/25, Germany) were used to filter the samples for the HPLC and ICP-MS analyses. Phosphate measurement was carried out using ion-chromatography equipped with Dionex IonPac AS22 anion exchange column (Thermo Scientific). A mixture of 117.6 mg/l $NaHCO_3$ and 476.95 mg/l Na_2CO_3 with a flow rate of 1.2 ml/min was used as the eluent. The injection volume of the sample and the column temperature were 10 µl and 30 °C, respectively. To calculate the theoretical gluconic acid production from carbon stemming from YE, a 40% organic carbon content was assumed for YE (Thompson et al., 2017) and considered as glucose equivalents. The glucose to gluconic acid conversion yield was assumed to be 100% based on the following reaction:



2.8. Modeling of REE-phosphate solubility

Modeling was performed with a geochemical speciation program CHESS (Version 2.4) (van der Lee, 1998). Thermodynamic data was from the CHESS formatted version of the EQ3/6 database (van der Lee and Windt, 1999; Wolery, 1992) updated with data for gluconic acid complexation with Nd (Kutus et al., 2017) and La, Pr, Eu, Dy, Er, Lu (Giroux et al., 2002). Complex formation constants of lanthanides with gluconic acid were corrected to ionic strength of 0.0 using the Specific Interaction Theory program (Pettit et al., 2004). All thermodynamic data used for modelling can be found in Table S1 in the supplementary materials. In all calculations, a gluconic acid pK_a value of 3.43 (Giroux et al., 2002) was used. Concentrations of La, Pr, Nd, Eu, Dy, Er, and Lu were set as 138.91 mg/l, 140.91 mg/l, 144.24 mg/l, 151.96 mg/l, 162.5 mg/l, 167.26 mg/l, and 174.97 mg/l respectively. Gluconic acid concentration was set as 9.81 g/l and CO_2 and O_2 fugacity were set at 3.162×10^{-4} and 1.995×10^{-1} and temperature at 25 °C, respectively. Furthermore, the following growth medium components were included: $K^+ = 316$ mg/l, $Mg^{2+} = 100$ mg/l, $Na^+ = 78.62$ mg/l, $NH_4^+ = 68.19$ mg/l, $Cl^- = 251.70$ mg/l, $SO_4^{2-} = 761.76$ mg/l. For calculations of the aqueous fraction of lanthanides, either phosphate concentration or pH was fixed at 94.97 mg/l or pH 3, respectively, while the other parameter was varied in the range of 0–474.85 mg/l PO_4^{3-} or pH 1–7, respectively.

3. Results and discussion

3.1. REE and base metal precipitation in presence of K_2HPO_4 and yeast extract

The effects of different concentrations of K_2HPO_4 and YE on the solubility of REEs were studied using a MC leachate containing 451 mg/l of total REEs including Y, La, Ce, Pr, Nd, Sm, Gd, Er and Yb (Fig. 1a) and the base metals Zn, Mn, Fe, Co, Ni and Cu at a total concentration of 847

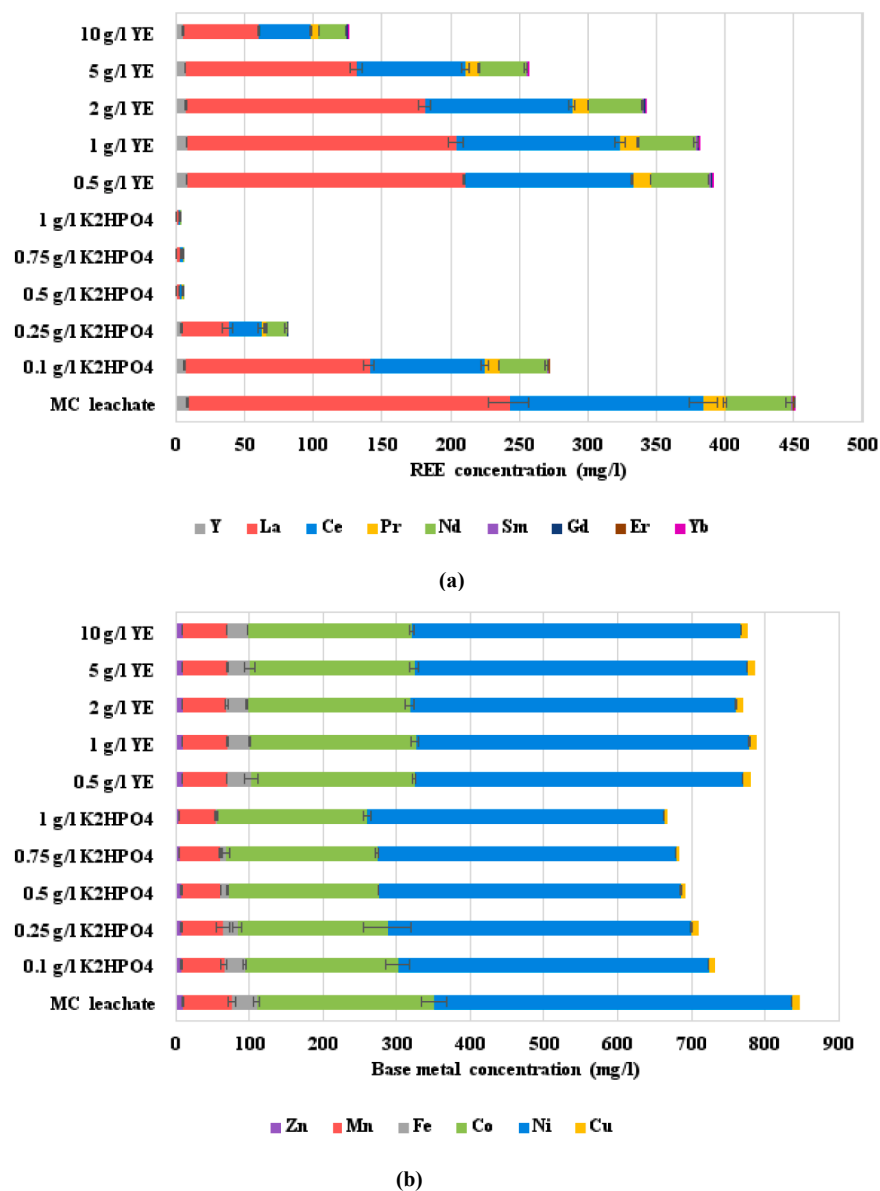


Fig. 1. Residual concentration of soluble (a) REEs and (b) base metals after reacting with different concentrations of yeast extract or K₂HPO₄ for 24 h.

mg/l (Fig. 1b). With addition of different concentrations of K₂HPO₄ and YE to the filtered MC leachate, pH remained reasonably constant (4.8–5.3) within one day reaction time (Table S2). With addition of 0.1 and 0.25 g/l K₂HPO₄, the total REE content of the MC leachate decreased by 39.5% and 81.8%, respectively. Addition of higher K₂HPO₄ concentrations of 0.5, 0.75 and 1 g/l resulted in almost complete precipitation of REEs, with final concentration of 3–6 mg/l in MC leachate. In contrast to REEs, the base metal concentrations were proportionally less impacted by K₂HPO₄. With addition of the highest tested K₂HPO₄ concentration of 1 g/l, the total base metal concentration of the MC leachate decreased by only 21.3%. These results confirmed that even very low concentrations of K₂HPO₄ can lead to precipitation of especially high proportion of REEs. Therefore, eliminating K₂HPO₄ from bioleaching media without compromising high organic acid production is desirable. One option is to supply the required phosphorous as YE.

For both REEs and base metals, YE addition resulted in less precipitation than addition of K₂HPO₄ (Fig. 1). At 10 g/l YE, the total REE content of the MC leachate decreased by 72.1% (Fig. 1a), while the base metal content decreased by only 8.3% (Fig. 1b). Thus, as with K₂HPO₄, the precipitation of base metals after adding YE was proportionally

lower than that of REEs. The precipitation experiment results show that replacing K₂HPO₄ with YE for growth and organic acid production, could decrease metal precipitation in the subsequent spent medium bioleaching as YE in the medium results in lower dissolved phosphate. For example, dissolved phosphate concentration in a medium with 1 g/l YE was 0.02 g/l, while in a medium with 0.5 g/l K₂HPO₄ it was 0.26 g/l. Analysis of residual dissolved phosphate in the media revealed that the dissolved phosphates mostly remain in the spent medium after the growth phase.

Although phosphate is deemed to be the main REE precipitant, at phosphate concentrations below 273 mg/l, REE decrease cannot be fully explained by precipitation as REE phosphates (See Table S3 and Fig. S1). Other anions possibly contribute to REE precipitation. Speciation calculations for La, however, indicate precipitation of La₂(CO₃)₃·8H₂O only at pH > 7 (Fig. S2), while during precipitation experiments pH did not rise above 5.3. Nevertheless, small contributions to REE precipitation at pH < 7 due to presence of other anions cannot be ruled out as previously reported by Hassas et al. (Hassas et al., 2021).

3.2. Medium optimization to minimize residual dissolved phosphate

3.2.1. Replacement of K_2HPO_4 with yeast extract

The effects of K_2HPO_4 and YE on *G. oxydans* growth and gluconic acid production were studied using two different media with 40 g/l glucose: (1) Phosphate medium was supplied with 0.05 g/l YE together with 0.1, 0.25 or 0.5 g/l K_2HPO_4 (Modified media set 1), and (2) Phosphate medium was supplied only with YE at concentrations of 0, 0.1, 0.25, 0.5, 0.75 or 1 g/l (Modified media set 2). With Modified media set 1, gluconic acid production increased from 3.7 g/l to 18.4 g/l with increasing K_2HPO_4 concentration from 0.1 to 0.5 g/l (Fig. 2a). The higher OD_{600} (Fig. S3a) and glucose utilization (Fig. 2c) as well as the faster pH decrease (Fig. S3c) with 0.5 g/l K_2HPO_4 also indicated improved growth and more efficient gluconic acid production with increase of K_2HPO_4 concentration. These results corroborate the importance of K_2HPO_4 as a pH buffer and phosphorous source (Rasoulnia et al., 2021b). Therefore, elimination of K_2HPO_4 from the medium should be done without compromising efficient acid production i.e., due to reduced buffering capacity or lack of sufficient phosphorous. The reduced gluconate production with decrease of K_2HPO_4 was likely linked to limiting phosphate nutrient availability than to reduced buffering capacity as medium initial pH decreased only from 3.8 to 3.3 and final pH was highest with the lowest initial phosphate concentration (Fig. S3c).

With Modified media set 2, the highest gluconic acid production was 27 g/l with 1 g/l YE after 6 days of incubation. With decrease of YE concentration, the gluconic acid production decreased as well, and only 5.2 g/l gluconic acid was produced with 0.1 g/l YE (Fig. 2b). In the absence of YE, no gluconic acid was produced. The highest OD_{600} of 0.35 and the sharpest pH decrease (final pH = 2.3) (Fig. S3b and d) were obtained with 1 g/l YE, confirming the more efficient growth and

gluconic acid production than with lower YE concentrations. With increase of YE concentration, the medium initial pH also increased. However, as with K_2HPO_4 amended cultures, a change in buffering capacity due to lower addition of YE did not seem to considerably influence the gluconic acid production.

In Modified medium from set 2 with 1 g/l YE, approximately 14 g/l glucose out of the 40 g/l initially supplied glucose remained unused (Fig. 2d) and the glucose to gluconic acid conversion yield was 62% as compared to the 42% conversion yield of Modified medium from set 1 with 0.05 g/l YE and 0.5 g/l K_2HPO_4 . Theoretical gluconic acid production from 1 g/l YE would equal to about 0.4 g/l (see section 2.7, reaction (1)). This is insignificant compared to, for example, 27 g/l gluconic acid produced in Modified medium from set 2 with 1 g/l YE. More efficient gluconic acid production by *G. oxydans* in complex medium containing YE compared to chemically defined medium containing phosphate salts of K_2HPO_4 and KH_2PO_4 has been also reported by Olijve and Kok (Olijve and Kok, 1979).

3.2.2. Optimizing yeast extract to glucose ratio

The results of the previous experiments indicated that increasing YE/G mass ratio, enhances gluconic acid production. Therefore, to optimize gluconic acid production by *G. oxydans* in medium with YE as the sole phosphorous source, different YE/G mass ratios ranging from 0.04 to 0.20 were studied in Modified media set 3. The media with higher YE/G mass ratio, had a higher initial pH. However, the pH of the media with different YE/G ratios decreased from 4.9 to 5.7 to approximately 2.7 during the incubation (Fig. 3a). Despite the similar pH reductions, OD_{600} of the different media increased differently with increase of YE/G ratio. Cultures with higher YE/G ratio had a higher OD_{600} during and at the end of incubation (Fig. 3b). This indicates that *G. oxydans* growth was affected by different YE/G ratios. With increase of YE/G mass ratio from

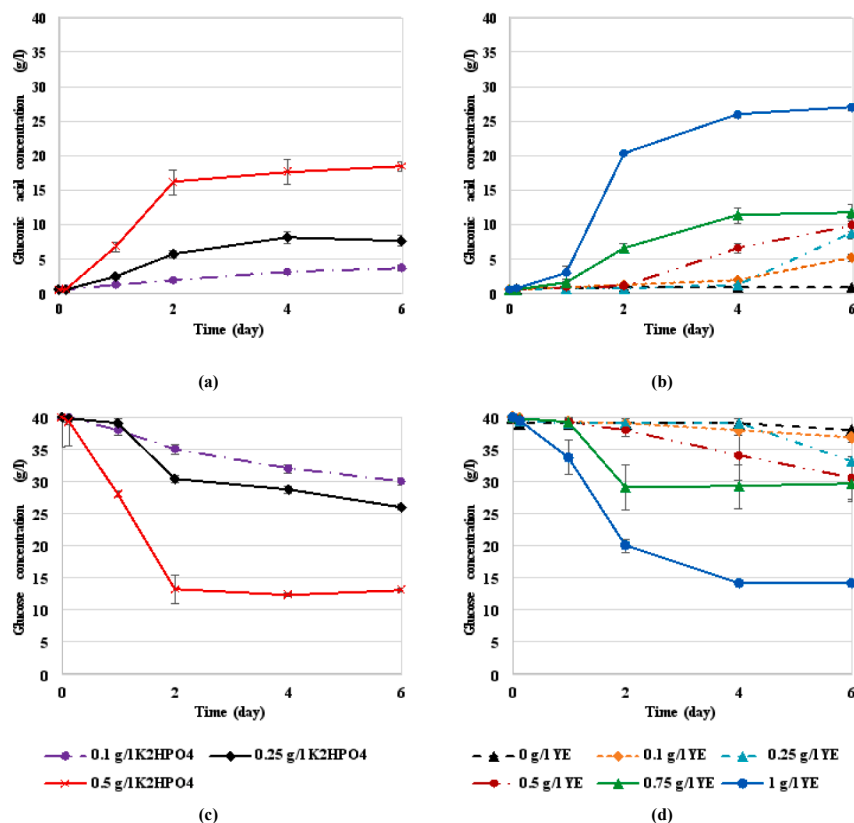


Fig. 2. Gluconic acid production and glucose consumption by *Gluconobacter oxydans* (a, c) in Modified media set 1 and (b, d) in Modified media set 2 (Error bars not visible fall within the symbols).

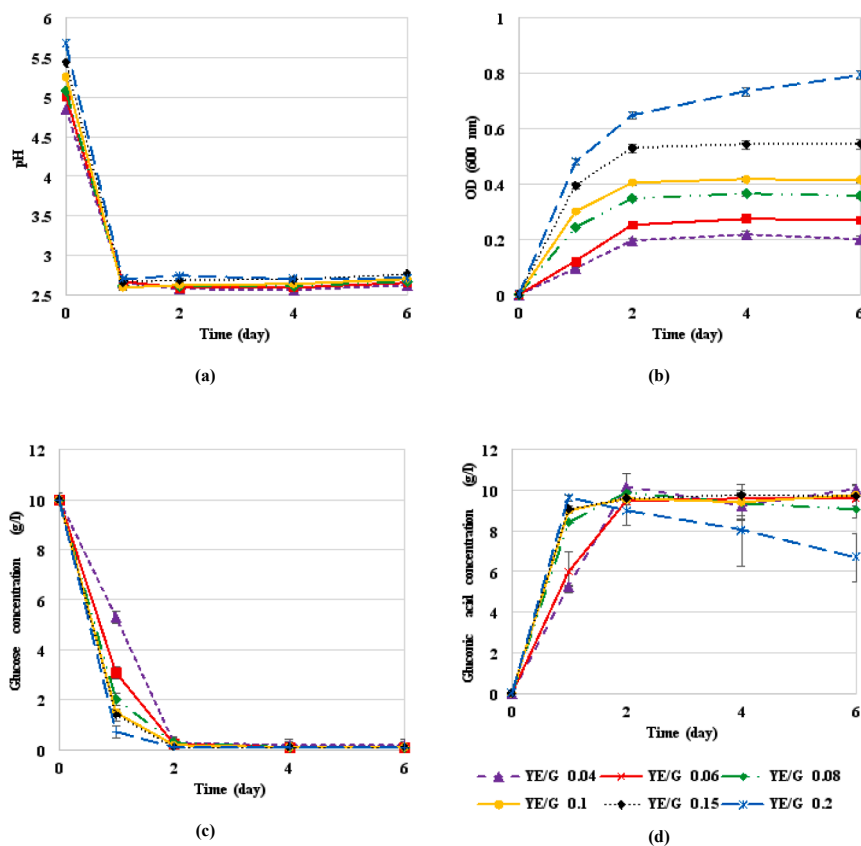


Fig. 3. (a) pH, (b) OD_{600} , (c) glucose concentration and (d) gluconic acid concentration during incubation of *Gluconobacter oxydans* in Modified media set 3 at different yeast extract to glucose (YE/G) mass ratios.

0.04 to 0.20, higher glucose utilization (Fig. 3c) and gluconic acid production (Fig. 3d) rates were observed within 1 day of growth. After 2 days, glucose was almost completely consumed in all the media and the gluconic acid production for all the media except Modified medium from set 3 with 0.20 YE/G mass ratio, increased to above 9.4 g/l by day 2 and remained almost constant for the next 4 days. In Modified medium from set 3 with 0.20 YE/G, OD_{600} continued to increase by day 6, while gluconic acid concentration decreased from 9.6 g/l on day 1 to 6.7 g/l on day 6 likely due to conversion of gluconic acid to its keto-derivatives (Fig. S4a, b) (Elfari et al., 2005; Weenk et al., 1984). This indicates that, similar to previously reported residual glucose concentration and medium pH effect (Elfari et al., 2005; Weenk et al., 1984), the YE/G ratio also affects the conversion rate of gluconic acid to keto-gluconic acids by *G. oxydans*. Conversion of gluconic acid to its keto-derivatives should be avoided as gluconic acid is more efficient in REE leaching from spent NiMH batteries than its keto-derivatives (Rasoulnia et al., 2021a).

In this study Modified medium from set 3 with YE/G mass ratio of 0.08 (YE medium) was selected for bioleaching experiments as 0.08 was the lowest YE/G ratio that resulted in fast and high gluconic acid production without further oxidation of gluconic acid to keto-gluconic acids. In heterotrophic bioleaching, the major cost driver is the organic carbon source glucose, which makes up approximately 98% of the total medium component costs (Thompson et al., 2018). Therefore, high glucose to gluconic acid conversion yield highly impacts the process profitability via reduced glucose requirement, while ensuring sufficient gluconic acid production for efficient metal solubilization. With YE medium, a 90% glucose to gluconic acid conversion yield was achieved within 2 days. This conversion yield is similar or higher compared to previous studies investigating gluconic acid production from glucose using *G. oxydans* (Olijve and Kok, 1979; Reed et al., 2016; Seiskari et al., 1985; Thompson et al., 2018; Velizarov and Beschkov, 1994). Those

studies used either a combination of different phosphate salts such as K_2HPO_4 and/or KH_2PO_4 with/without YE (Reed et al., 2016; Thompson et al., 2018) or a GY broth with varying YE/G mass ratios (0.05–1) (Olijve and Kok, 1979; Seiskari et al., 1985; Velizarov and Beschkov, 1994) and were mostly conducted in bioreactors, compromising a direct kinetic comparison with our batch bioassays.

3.3. Spent medium bioleaching of the NiMH battery material

Leaching of spent NiMH battery was studied using cell-free supernatants of *G. oxydans* produced in Phosphate and YE media containing 10 g/l glucose (Table 2). The aim was to determine the effect of residual dissolved phosphate on REE and base metal leaching yields from spent NiMH battery powder. Prior to bioleaching experiments, *G. oxydans* growth was monitored via measuring pH, OD_{600} , glucose and gluconic acid concentration (Fig. S5a–d). The final achieved glucose to gluconic acid conversion yields in the Phosphate and YE media were 90% and 83%, respectively (Fig. S5c). Acid production decreased the pH of both media to 2.8 in 1 day and thereafter the pH remained constant for the next 3 days (Fig. S5d). With addition of the spent NiMH battery, pH of both media increased to 3.2 (Fig. S5e). During leaching, the pH increase due to the alkalinity of the spent NiMH battery powder was higher for the YE medium than for the Phosphate medium. In 7 days, pH of YE and Phosphate media increased from 3.2 to 6.1 and to 5.8, respectively.

One day spent medium bioleaching of the spent NiMH battery with the YE medium solubilized 28.8% Mn, 52.8% Fe, 22.9% Co, 12.0 % Ni, 0.9 % Cu, 7.8% Zn and 19.5% of total REEs, while with the Phosphate medium 17.3% Mn, 28.8% Fe, 19.5% Co, 7.7 % Ni, 0.6 % Cu, 8.5% Zn and only 3.3% of total REEs were leached (Fig. 4a and b). With prolonging leaching period to 7 days, the concentrations of most of the elements only slightly increased or remained constant in both media

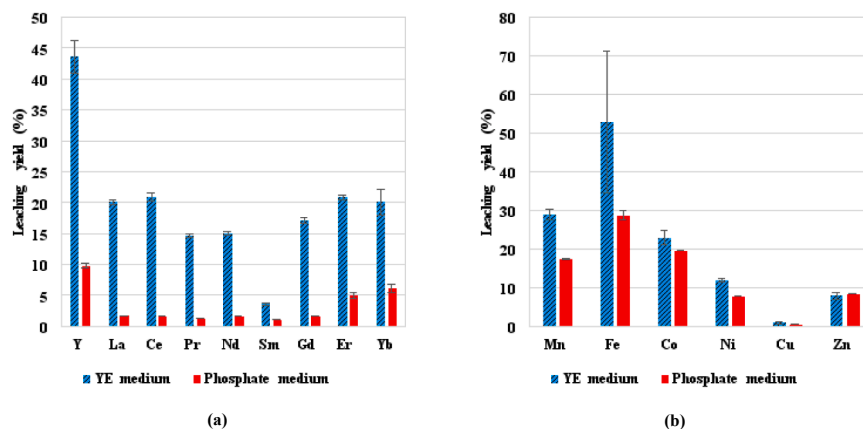


Fig. 4. (a) REE and (b) base metal leaching yields obtained with *Gluconobacter oxydans* culture supernatants of YE and Phosphate media after 1 day of spent medium leaching.

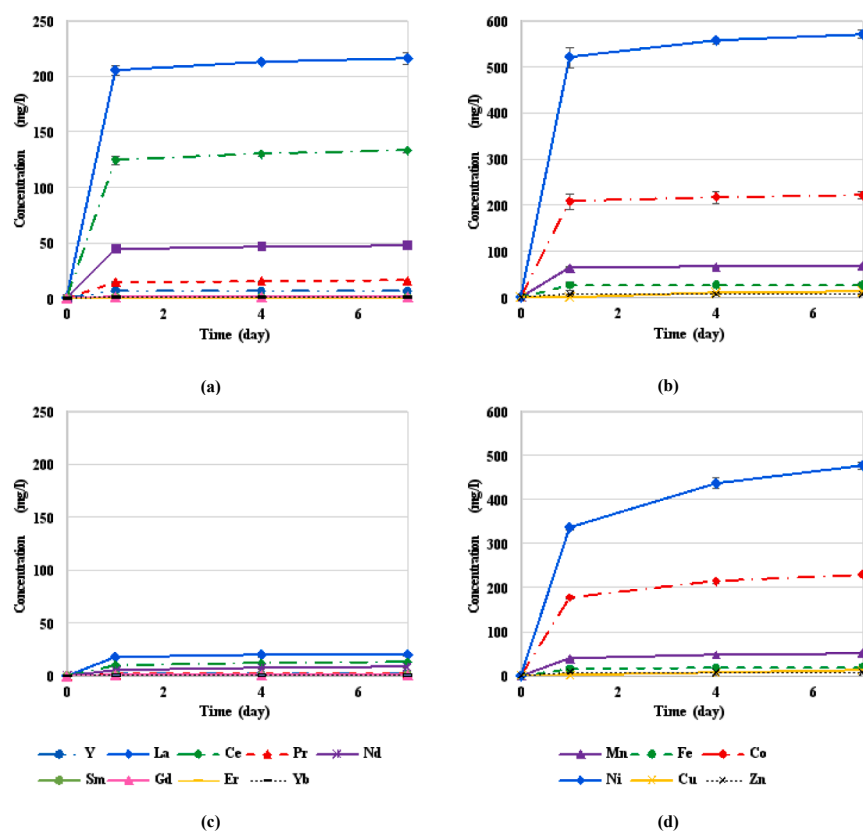


Fig. 5. REE and base metal leaching from spent NiMH battery material in (a, b) YE medium and (c, d) Phosphate medium (Error bars not visible fall within the symbols).

(Fig. 5a-d). The results show that despite presence of similar concentrations of gluconic acid in both culture supernatants (10 g/l in YE medium versus 9 g/l in Phosphate medium) minimizing residual dissolved phosphate improved the solubilization yields. After one day, the YE medium leaching liquor contained 1.5 and 11.0 fold higher concentrations of total base metals (825 mg/l vs. 574 mg/l) and REEs (398 mg/l vs. 36 mg/l), respectively, than the Phosphate medium.

A variety of factors such as the type of the microorganism(s), growth and leaching conditions, type and amount of organic carbon source and produced metabolites as well as REE bonding within solid material can have an impact on the efficiency of a bioleaching process (Rasoulnia et al., 2020). This study showed that the concentration of residual

dissolved phosphate in the medium also crucially affects the REE bioleaching yields. The achieved total REE leaching yield of 19.5% was higher than the yields obtained from fluorescent phosphors (FP) with various microorganisms such as *G. oxydans*, the symbiotic mixed culture *Kombucha* and *Komagataeibacter xylinus* (Hopfe et al., 2018, 2017; Reed et al., 2016), but lower than the yields obtained from spent fluid catalytic cracking (FCC) catalysts using *G. oxydans* or *Aspergillus niger* (Mouna and Baral, 2019; Reed et al., 2016) (Table 3). The differences in REE leaching efficiency are influenced by differences in REE bonding within the waste materials (Table S4), released by-products such as phosphorus from FP, as well as the concentration and type of the produced organic acids, compromising direct comparison between different

Table 3
Comparison of REE bioleaching studies with different waste materials and microorganisms.

Waste material (Total REE content as mg/g)	Microorganism	Pulp density (% wt/v)	Leaching period (day)	Sugar used (conc. as g/l)	Phosphate salt (conc. as g/l)	Total REE bioleaching yield (%)	%REE leached/g sugar used day	mg/l REE leached/g sugar used day	Reference
FCC catalyst (14.4)	<i>Gluconobacter oxydans</i>	1.5	1	Glucose (10)	KH ₂ PO ₄ (0.37)	49	4.9	10.5	(Reed et al., 2016)
FCC catalyst (La 18.8)	<i>Aspergillus niger</i>	5	3	Glucose (60)	KH ₂ PO ₄ (1) K ₂ HPO ₄ (1)	74	0.4	3.8	(Dewi et al., 2020)
FCC catalyst (La -)	<i>Aspergillus niger</i>	1	21	Sucrose (100)	KH ₂ PO ₄ (0.5)	63	0.03	-	(Mouna and Baral, 2019)
Fluorescent Phosphors (71.4)	<i>Gluconobacter oxydans</i>	1.5	1	Glucose (10)	KH ₂ PO ₄ (0.37)	2	0.2	0.2	(Reed et al., 2016)
Fluorescent Phosphors (-)	Symbiotic mixed culture of Kombucha	2.85	14	Glucose (100)	-	6.5	0.005	-	(Hopfe et al., 2017)
Fluorescent Phosphors (-)	<i>Komagataeibacter xylinus</i>	2.85	14	Glucose (100)	-	12.6	0.009	-	(Hopfe et al., 2018)
Spent NiMH battery (206.8)	<i>Gluconobacter oxydans</i>	1	14	Glucose (10)	K ₂ HPO ₄ (0.47)	9	0.07	18.6	(Rasoulnia et al., 2021b)
Spent NiMH battery (206.8)	<i>Gluconobacter oxydans</i>	1	1	Glucose (10)	-	19.5	1.95	40.3	This study

studies. The lower REE leaching yield obtained with spent NiMH battery material compared to FCC catalysts could be due to the higher metal grade of spent NiMH battery potentially causing saturation effects in solution (Table 3). In addition to the high REE content, spent NiMH

batteries contain considerable amounts of base metals and acid consuming materials competing with REEs to react with the leaching agents.

In this study leaching time of only one day was employed while other

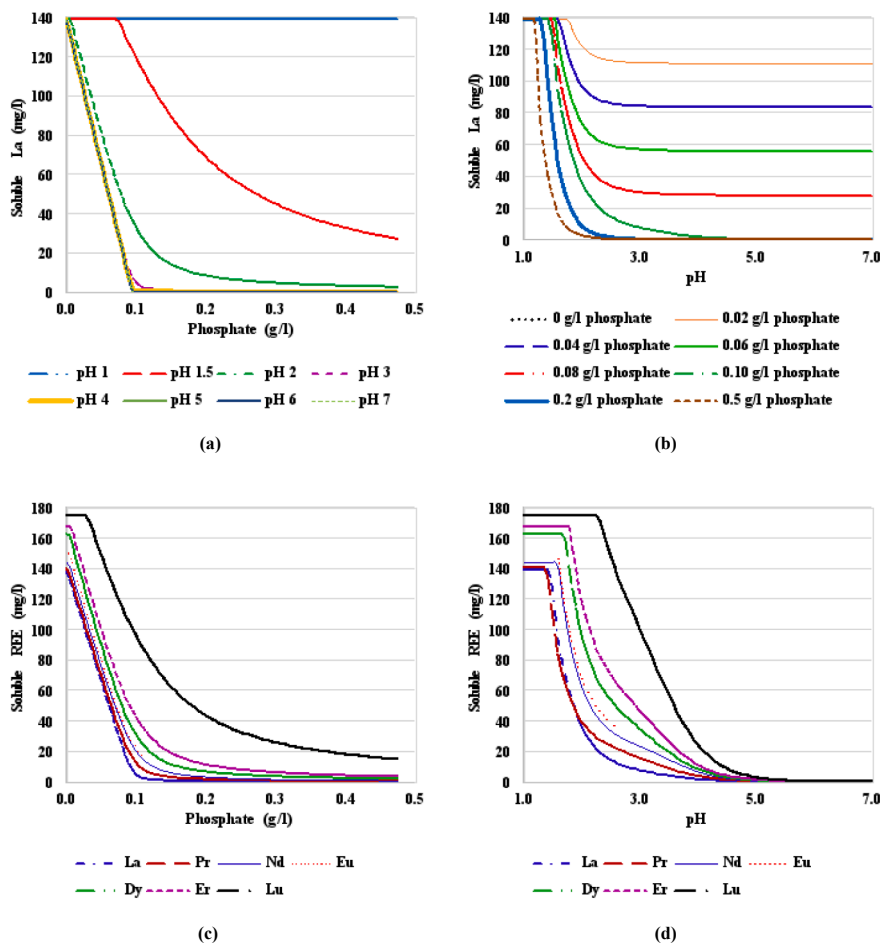


Fig. 6. La solubility based on the applied speciation modeling (a) as a function of phosphate concentration at different solution pH, (b) as a function of pH at different phosphate concentrations; and solubility of various REEs (c) as a function of phosphate concentration at pH 3, (d) as a function of pH at phosphate concentration of 95 mg/l.

studies reported leaching times of up to three weeks (Table 3). The obtained high glucose to gluconic acid conversion yield allows the use of lower glucose concentrations. The used 10 g/l of glucose was considerably lower than in other studies reporting the use of up to 100 g/l of sugar (Table 3). In this study 40.3 mg REE/g sugar used was leached in one day, while in previous studies the value ranged between 0.2 and 18.6 (Table 3). However, it should be also noted that in the previous bioleaching studies with various REE sources such as FCC catalysts, FP, NiMH batteries, carbonaceous shales and bauxite (Amin et al., 2014; Barnett et al., 2020; Reed et al., 2016) different concentrations of K_2HPO_4 or KH_2PO_4 (0.3–1 g/l) were used, which might result in underestimation of the attainable REE bioleaching yields.

In summary, the bioleaching experiments of this study revealed that use of the YE medium results in higher leaching rates and yields of both REEs and base metals from the spent NiMH battery powder than the Phosphate medium. In a similar manner as in the precipitation experiments, the bioleaching results showed that REEs were more susceptible to precipitation with surplus of phosphate than the base metals.

3.4. REE-Phosphate solubility modeling

Depending on the applied microorganism, the substrate and the medium composition, different phosphate concentrations and pH levels may be relevant in bioleaching. In this study, during leaching pH of both media increased gradually from 2.8 to around 6.0 due to the alkalinity of the spent NiMH battery powder. REEs can potentially precipitate with phosphate ions at such pH ranges (Han, 2020). Therefore, to predict precipitation of REEs within a broader pH and phosphate concentration range than those of the precipitation experiments, REE-phosphate modeling was conducted by simulating the bioleaching conditions. La as the major element present in the spent NiMH battery was selected for the pH-phosphate concentration dependency modeling graphs (Fig. 6a and b) but modeling results for other lanthanides are similar (data not shown). The results indicate that La solubility correlated with both pH and phosphate concentration and that for an efficient La solubilization only one parameter (pH or phosphate concentration) can be freely chosen (Fig. 6a and b). In general, a low phosphate concentration enabled higher metal leaching yields at neutral to moderately low pH values. In contrast, at very low pH values below 1.0, also high phosphate concentrations are tolerated and precipitation does not occur. The obtained modeling results are in accordance with results of Lin et al. (2021) and Hassas et al. (2021) also reporting formation of insoluble phosphate compounds in a wide pH range. Interestingly, Kim and Osseo-Asare (2012) reported a decreased REE phosphate precipitation due to formation of amorphous instead of crystalline precipitates. This would allow for higher REE concentrations in solutions and thus, less stringent control of pH and/or phosphate concentration in bioleaching processes. Comparison of REE bioleaching results (Fig. 5a and b) with modeling (Fig. 6) shows that La concentrations in the leaching liquor were higher than the predicted maximum La solubility whereas the concentration of other REEs were within the predicted concentration range. This indicates that the model may overestimate REE phosphate precipitation. Nevertheless, the modeling results support the experimental results and emphasize that at moderately acidic conditions relevant for heterotrophic bioleaching systems already small amounts of dissolved phosphate can hamper REE solubilization. This is especially relevant for light REEs as they are more prone to precipitate as phosphates than heavy REEs (Fig. 6c and d). This difference in precipitation tendency between light and heavy REEs also has been reported with other precipitants such as oxalate, carbonate and sulfate (Han, 2020).

4. Conclusions

Bioleaching of REEs and base metals from end-of-life NiMH battery material using culture supernatants of *Gluconobacter oxydans* allowed the following conclusions to be drawn:

- Base metals and especially REEs precipitate with surpluses of dissolved phosphate in microbial culture supernatants, considerably decreasing the subsequent leaching yields. REEs are proportionally more affected by precipitation with phosphate than base metals.
- With optimal YE to glucose ratio, high glucose to gluconic acid conversion yield of 90% is achieved with *G. oxydans*. Use of YE medium minimizes surplus of dissolved phosphate and enhances total REEs leaching yield within one day from 3.3% to 19.5% compared to the phosphate-containing medium.
- Geochemical modeling illustrates the combined influence of pH and phosphate concentrations on attainable REE leaching yields. At neutral to moderately acidic pH values, already small amounts of phosphate impede REE mobilization.

Thus, this study demonstrated the need of phosphate usage optimization in bioleaching systems. The outcomes including efficient glucose usage, high gluconic acid production yield, short leaching period, lowered REE precipitation during leaching and enhanced metal leaching efficiency have the potential to enable development of more economically feasible bioleaching processes in the future.

CRediT authorship contribution statement

Payam Rasoulnia: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Robert Barthen:** Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing. **Aino-Maija Lakaniemi:** Conceptualization, Project administration, Supervision, Validation, Writing – review & editing. **Harri Ali-Löyty:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Jaakko A. Puhakka:** Conceptualization, Project administration, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mineng.2021.107361>.

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