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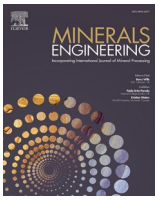

Effects of metal extraction liquors from electric vehicle battery materials production on iron and sulfur oxidation by heap bioleaching microorganisms

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ABSTRACT

This study reports the effects of metal extraction liquors that are used for production of electric vehicle batteries on biological iron and sulfur oxidation. These liquors include ammonium sulfate and organic solvent constituents, and thus are potentially or inhibitory for heap bioleaching microorganisms. The effects of the liquors and their potential constituents were studied in batch bioassays at pH 2 and 27 ± 2 °C. Both metal extraction liquors had a negative effect on biological iron oxidation at >2% (*v/v*), whereas biological sulfur oxidation was enhanced with ≤8% (*v/v*) metal extraction liquor 1. Biological iron oxidation was negatively affected by ammonium sulfate at above 20 g/L. From the studied low-solubility organic solvents (neodecanoic acid, Nessol D100, Cyanex 272, and Baysolvex D2EHPA), neodecanoic acid was the only one negatively affecting biological iron oxidation, and this effect occurred at ≥ 6.3 mg/L (2.5% of its aqueous solubility). Since these extraction liquors and some of their potential constituents inhibited biological iron oxidation, they may also inhibit heap bioleaching and have adverse impacts in recipient waters, if released to the environment. With ammonium limited culture, iron oxidation was stimulated with ≤ 1% (*v/v*) of metal extraction liquor 1 and 2, and therefore, would also likely enhance heap bioleaching.

1. Introduction

Electric vehicles are considered important in combatting global warming, mainly due to their positive influence on controlling greenhouse gas (GHG) emissions and other air pollutants. Their quantity is estimated to considerably increase with the reduction of the production and driving costs and development of battery technology. The increased production of the electric vehicle batteries also increases the demand for the metal-based materials, such as nickel and cobalt sulfate (for reviews, see Marafi and Stanislaus, 2008; Ajanovic, 2015).

Heap bioleaching is used in commercial scale to extract metals particularly from low-grade sulfide ores worldwide (du Plessis et al., 2007). In production of the electric vehicle battery materials from bioleaching liquors, ammonia can be used in metal extraction, resulting in ammonium (NH_4^+) containing side streams (for a review, see Marafi and Stanislaus, 2008). In heap bioleaching, quantity of nitrogen is often growth limiting for the bioleaching microorganisms (du Plessis et al., 2007). Therefore, these NH_4^+ containing side streams are a potential nitrogen source for heap bioleaching. Ores usually consist of sufficient quantities of micronutrients for microbial growth, while the quantities of macronutrients, such as nitrogen, are low (du Plessis et al., 2007; Ahoranta et al., 2017). Nitrogen is the most essential nutrient for the growth and therefore, efficient bioleaching may require nitrogen supplementation (Rawlings, 2007).

Microorganisms in heap bioleaching environments are mainly iron and sulfur oxidizing chemolithoautotrophs, which are responsible for the oxidation of ferrous iron (Fe^{2+}), elemental sulfur (S^0), and reduced sulfurous compounds (Rawlings, 2007). These microorganisms are sensitive to organic compounds (Torma and Itzkovitch, 1976; Tuttle and Dugan, 1976; Alexander et al., 1987; Fang and Zhou, 2006; Rawlings, 2007; Chen et al., 2015). In the electric vehicle battery materials production process, metals are separately removed from the aqueous leach liquor, for example, by using solvent extraction and crystallization (for a review, see Marafi and Stanislaus, 2008). In the solvent extraction, organic solvents are used and therefore, the residual solvents are present in the liquors of the following crystallization processes (Torma and

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Itzkovitch, 1976; Chen et al., 2015). When considering circulation of these liquors to the heap bioleaching for NH_4^+ supplementation, possible inhibitory effect must also be taken into account.

The aim of this study was to evaluate the potential of NH_4^+ containing metal extraction liquors from electric vehicle battery materials production as NH_4^+ source for heap bioleaching microorganisms, and thus for enhancing the bioleaching. Possible inhibitory and stimulatory effects of the liquors and their potential organic solvent constituents on iron and sulfur oxidation were studied in batch bioassays (shake flasks) with iron and sulfur oxidizing microorganisms, enriched from a heap bioleaching irrigation leach liquor.

2. Materials and methods

2.1. Microbial cultures and growth media

Three microbial cultures were enriched from an irrigation leach liquor of a complex sulfide metal heap bioleaching site and used in batch bioassays. The irrigation leach liquor was taken from a flow after a bioheap, with temperatures varying from +10 to +80 °C, depending on the time of a leaching period. The pH of the liquor was 3.0 and it contained all the ions typically leached from the multi-metal sulfide based black shist ore (Halinen et al., 2009a,b) with concentrations varying depending on the leaching period being typically >5.0 g/L for Al, >2 g/L for Ni, >4 g/L for Zn, >23 g/L for Fe, >23 g/L for Mg and >13 g/L for Mn. First, an iron oxidizing culture was enriched from the irrigation leach liquor with Fe^{2+}. Sulfur oxidizing culture was then enriched with elemental sulfur (S^0) from this iron oxidizing enrichment culture. Moreover, one iron oxidizing enrichment culture was also grown under ammonium deficient (AD) conditions for studying metal extraction liquors as potential NH_4^+ supplement. The iron oxidizing AD culture was incubated for 5 weeks with weekly transfers to a fresh medium prior to the experiment. The cultures and media were as listed in Table 1. The medium for iron oxidizing cultures contained mineral salts medium (MSM), trace elements solution (TES), and 22.5 g/L Fe^{2+} stock (Ahoranta et al., 2017). The iron oxidizing AD enrichment culture was similar to the iron enrichment culture, except that the inoculum was reduced to 1% (*v/v*) and the medium contained no ammonium sulfate ($(NH_4)_2SO_4$) to obtain NH_4^+ deficiency. The sulfur oxidizing enrichment culture medium contained MSM, TES, and 10 g/L S^0 (Lee et al., 2000; Ahoranta et al., 2017). Milli-Q water was added to the media to reach 100 mL working volume. The shake flasks containing MSM, TES, and Milli-Q water were autoclaved at 121 °C for 20 min. Fe^{2+} stock was filtered through 0.2 μm sterile polyethersulfone membrane (VWR International, USA). S^0 was sterilised and dehydrated by keeping at 105 °C over-night and stored in a desiccator prior to use.

All bioassays were conducted in duplicates at 150 rpm, initial pH of ~ 2.0 and 27 ± 2 °C. The sub-culturing of all iron oxidizing cultures was weekly, while the sulfur oxidizing enrichment culture was transferred every second week.

2.2. Metal extraction liquors and organic solvents

The studied metal extraction liquors are $(NH_4)_2SO_4$ containing side streams from metal-based materials production, which recovers metals for electric vehicle batteries. Metal extraction liquor 1 and 2 represent mother liquor and feed liquor of metal recovery, respectively. Both metal extraction liquors are concentrated in $(NH_4)_2SO_4$ and contain residual organic compounds, metals, cations and anions from the preceding metal extractions. The metal extraction liquor 1 and 2 contained 428 (117 g/L as NH_4^+) and 288 g/L (79 g/L as NH_4^+) of $(NH_4)_2SO_4$, respectively, and with total organic carbon (TOC) concentrations of 300 and 180 mg/L, respectively. Sodium (Na^+), magnesium (Mg^{2+}) and potassium (Ca^{2+}) concentrations of the metal extraction liquors were above 50 mg/L, while nickel (Ni^{2+}) and chloride (Cl^-) concentrations were below 200 mg/L. Other metal, cation and anion concentrations were below 50 mg/L.

The studied model compounds contained typical organic solvents, used in metal extraction, which may also be present in metal extraction liquors. The studied organic solvents were neodecanoic acid ($C_{10}H_{19}O_2H$), Nessol D100 (mixture of aliphatic hydrocarbons (C_{13}–C_{18})), Cyanex 272 (dialkyl phosphinic acid), and Baysolvex D2EHPA (bis(2-ethylhexyl) hydrogen phosphate) (Fig. S1). Neodecanoic acid, Nessol D100 and Cyanex 272 were 100% concentrated, and Baysolvex D2EHPA was 90–100% (*w/w*) solution. The metal extraction liquors, and the organic solvents were used without sterilisation.

2.3. Shake flask bioassays

Bioassays were carried out in 250 mL duplicate shake flasks (100 mL working volume) at initial pH of 2.0, 150 rpm, and +27 ± 2 °C (Table 2). The pH was adjusted with concentrated H_2SO_4 after autoclaving, and Fe^{2+} stock and studied solution addition, and before inoculation and S^0 addition. In the inhibition experiments with metal extraction liquors and organic solvents, one positive (biotic) control without the studied solution was used. In the ammonium deficiency experiment, controls (positive, 0.11 g NH_4^+/L and 0.0 g NH_4^+/L control) were carried out in duplicates. The positive control of this experiment was similarly prepared as the iron oxidizing enrichment culture, except that the volume of the inoculum was reduced to 1% (*v/v*). The control without NH_4^+ was similarly prepared as the iron oxidizing AD medium. The 0.11 g NH_4^+/L control was similarly prepared as the iron oxidizing AD medium, except that 3.96 mL of 10 g/L $(NH_4)_2SO_4$ stock was added to achieve 0.11 g/L NH_4^+ concentration.

2.3.1. Inhibition experiments with metal extraction liquors

Iron oxidation activity of the iron oxidizing enrichment culture was monitored by measuring Fe^{2+} concentration, redox potential, and pH daily. Sulfur oxidation by the sulfur oxidizing enrichment culture was monitored as pH decrease and sulfate (SO_4^{2-}) production.

2.3.2. Inhibition experiments with organic solvents

In the neodecanoic acid, Nessol D100 and Cyanex 272 experiments, organic solvent stocks, prepared in the laboratory, were used instead of

Table 1
Composition of the growth media for the iron and sulfur oxidizing enrichment cultures.

Culture	Inoculum (%, *v/v*)	MSM[a] (%, *v/v*)	MSM without $(NH_4)_2SO_4$ (%, *v/v*)	TES[b] (%, *v/v*)	Soluble 22.5 g/L Fe^{2+} stock (%, *v/v*)	S^0 (g/L)
Iron oxidizing enrichment culture	10 (or 1)[c]	10		1	25	
Sulfur oxidizing enrichment culture	10	10		1		10
Iron oxidizing AD[d] culture	1		10	1	25	

[a] Mineral salts medium.
[b] Trace elements solution.
[c] 1% (*v/v*) iron oxidizing enrichment culture was used in ammonium deficiency experiments.
[d] Ammonium deficient.

Table 2
Experimental design used in the bioassays.

Experiments	Variables	Inoculum[a]	Control(s)
Inhibition experiments with metal extraction liquors	Metal extraction liquor 1 (0.1, 1, 2, 10, and 20% (*v/v*))	10% (*v/v*) iron oxidizing enrichment culture	Positive control (without metal extraction liquor 1)
	Metal extraction liquor 1 (0.1, 1, 2, 4, and 8% (*v/v*))	10% (*v/v*) sulfur oxidizing enrichment culture	Positive control (without metal extraction liquor 1)
	Metal extraction liquor 2 (0.1, 1, 2, 10, and 50% (*v/v*))	10% (*v/v*) iron oxidizing enrichment culture	Positive control (without metal extraction liquor 2)
Inhibition experiments with organic solvents[b]	Neodecanoic acid (2.5, 6.3, 13, 25, 130, and 250 mg/L)[c]	10% (*v/v*) iron oxidizing enrichment culture	Positive control (without neodecanoic acid)
	Nessol D100 (0.5, 1.0, 3.0, and 10 mg/L)[d]	10% (*v/v*) iron oxidizing enrichment culture	Positive control (without Nessol D100)
	Cyanex 272 (0.8, 1.6, 6.4, and 16 mg/L)[e]	10% (*v/v*) iron oxidizing enrichment culture	Positive control (without Cyanex 272)
	Baysolvex D2EHPA (9.1, 18, 91, and 180 mg/L)[f]	10% (*v/v*) iron oxidizing enrichment culture	Positive control (without Baysolvex D2EHPA)
Ammonium deficiency experiment with metal extraction liquors	Metal extraction liquor 1 (0.09[g], 0.1, and 1% (*v/v*))	1% (*v/v*) iron oxidizing AD[h] culture	Positive control[i] (without metal extraction liquors)
	Metal extraction liquor 2 (0.1, and 1% (*v/v*))		0.0 g NH_4^+/L control (without NH_4^+ and metal extraction liquors)
			0.11 g NH_4^+/L control[j] (without metal extraction liquors)

[a] During the experiments, microbial cultures supplemented as shown in Table 1.
[b] Used neodecanoic acid supplied by ExxonMobil Chemical Company, USA, used Nessol D100 supplied by Neste Corporation, Finland, used Cyanex supplied by Solvay Business Services Latvia SIA, Latvia, and used Baysolvex D2EHPA supplied by LANXESS AG, Germany.
[c] Neodecanoic acid concentrations: 1, 2.5, 5, 10, 50, and 100% of its aqueous solubility. Aqueous solubility of neodecanoic acid: 250 mg/L (at 25 °C) (National Center for Biotechnology Information, 2021).
[d] Nessol D100 concentrations: 5, 10, 30, and 100% of its aqueous solubility. Aqueous solubility of Nessol D100: 10 mg/L (temperature not mentioned) (Neste, 2019).
[e] Cyanex 272 concentrations: 5, 10, 40, and 100% of its aqueous solubility. Aqueous solubility of Cyanex 272: 16 mg/L (at 20 °C) (Solvay Business Services Latvia SIA, 2019).
[f] Baysolvex D2EHPA concentrations: 5, 10, 50, and 100% of its aqueous solubility. Aqueous solubility of Baysolvex D2EHPA: 182 mg/L (temperature not mentioned) (LANXESS AG, 2018).
[g] The 0.09% (*v/v*) metal extraction liquor 1 included same concentration of NH_4^+ than 0.11 g NH_4^+/L control
[h] Ammonium deficient.
[i] 1% (*v/v*) iron oxidizing enrichment culture used with the positive control.
[j] In 0.11 g NH_4^+/L control same NH_4^+ concentration as in the study of Niemelä et al. (1994).

the concentrated organic solvents at lower concentrations (below 13 mg/L with neodecanoic acid, below 10 mg/L with Nessol D100, and below 16 mg/L with Cyanex 272). Neodecanoic acid stock of 25 mg/L (10% of its aqueous solubility), Nessol D100 stocks of 5.0 and 6.0 mg/L (50 and 60% of its aqueous solubility, respectively), and Cyanex 272 stock of 13 mg/L (80% of its aqueous solubility) were prepared similarly and on the day of starting the experiment. Room temperature organic solvent was diluted in deionised Milli-Q water (~27 °C) by thoroughly shaking the flask for 2 min. The organic solvent stock was added into the shake flask before pH adjustment and inoculation. With the higher concentrations, concentrated organic solvents were added into the shake flasks after pH adjustment, and Fe^{2+} stock and inoculum addition. Iron oxidation was monitored by measuring Fe^{2+} concentration, redox potential, and pH. Removal of neodecanoic acid was monitored by measuring dissolved organic carbon (DOC) from 2.5 to 13 mg/L (1–5% of its aqueous solubility) and 130 mg/L (50% of its aqueous solubility) neodecanoic acid shake flasks. With 2.5–13 mg/L, DOC was measured from all the replicates, whereas with 130 mg/L, DOC was measured from one replicate, except in three data points.

2.3.3. *Ammonium deficiency experiment with metal extraction liquors*

Iron oxidation and NH_4^+ utilization of the iron oxidizing AD culture was monitored by measuring Fe^{2+} concentration, redox potential, and pH daily, and NH_4^+concentration from the first and the last samples.

2.4. *Analyses*

Redox potential and pH were determined from the non-filtered samples. The pH values were measured with either a pH 3210 m (WTW, Germany), equipped with a pH electrode SenTix 81, or a pH 330i meter (WTW, Germany), equipped with a pH electrode SlimTrode (Hamilton Company, USA). Redox electrode BlueLine 31 Rx (SI Analytics, Germany) with Silamid® reference system (Ag/AgCl) was used to measure the redox potential. The Fe^{2+} concentration was determined with a UV-1900i UV–Vis spectrophotometer (Shimadzu Corporation, Japan) using the modified 3500-Fe *ortho*-phenantroline method (APHA, 1992). NH_4^+ concentrations were determined with a Dionex DX-120 ion chromatography (IC) (Thermo Fischer Scientific, USA), equipped with a Dionex IonPac CG12A (4 × 50 mm) guard column, an IonPac CS12A (4 × 250 mm) analytical cation exchange column, and a Dionex AS40 autosampler. The SO_4^{2-} concentrations were determined with a Dionex IC-1600 IC (Thermo Fischer Scientific, USA), equipped with an IonPac AG42-SC (4 × 50 mm) guard column, an IonPac AS4A-SC (4 × 250 mm) analytical anion exchange column, and a Dionex AS-DV autosampler. The DOC concentrations were determined either with TOC-VCPH/CPN analyzer (Shimadzu, Japan) (2.5–13 mg/L neodecanoic acid) or with high-sensitive TOC-L analyzer (Shimadzu, Japan) (130 mg/L neodecanoic acid) using SFS-EN 1484 standard (Finnish Standards Association, 1997). The methods were non-purgeable organic carbon (NPOC) with the TOC-VCPH/CPN analyzer and TOC (total carbon (TC) – total inorganic carbon (TIC)) with high-sensitive TOC-L analyzer. Prior to the Fe^{2+}, NH_4^+, SO_4^{2-} and DOC analyses, the samples were filtrated through a 0.45 μm polyester filter, Chromafil® Xtra PET-45/25 (Macherey-Nagel, Germany). Prior to the DOC analysis of 130 mg/L neodecanoic acid, the samples were first filtrated with the 0.45 μm filter and then with a 0.2 μm polyethersulfone filter (VWR, USA). The samples were diluted with 0.07 M HNO_3 (Fe^{2+} analysis) or deionised Milli-Q water (NH_4^+, SO_4^{2-} and DOC analyses) when necessary.

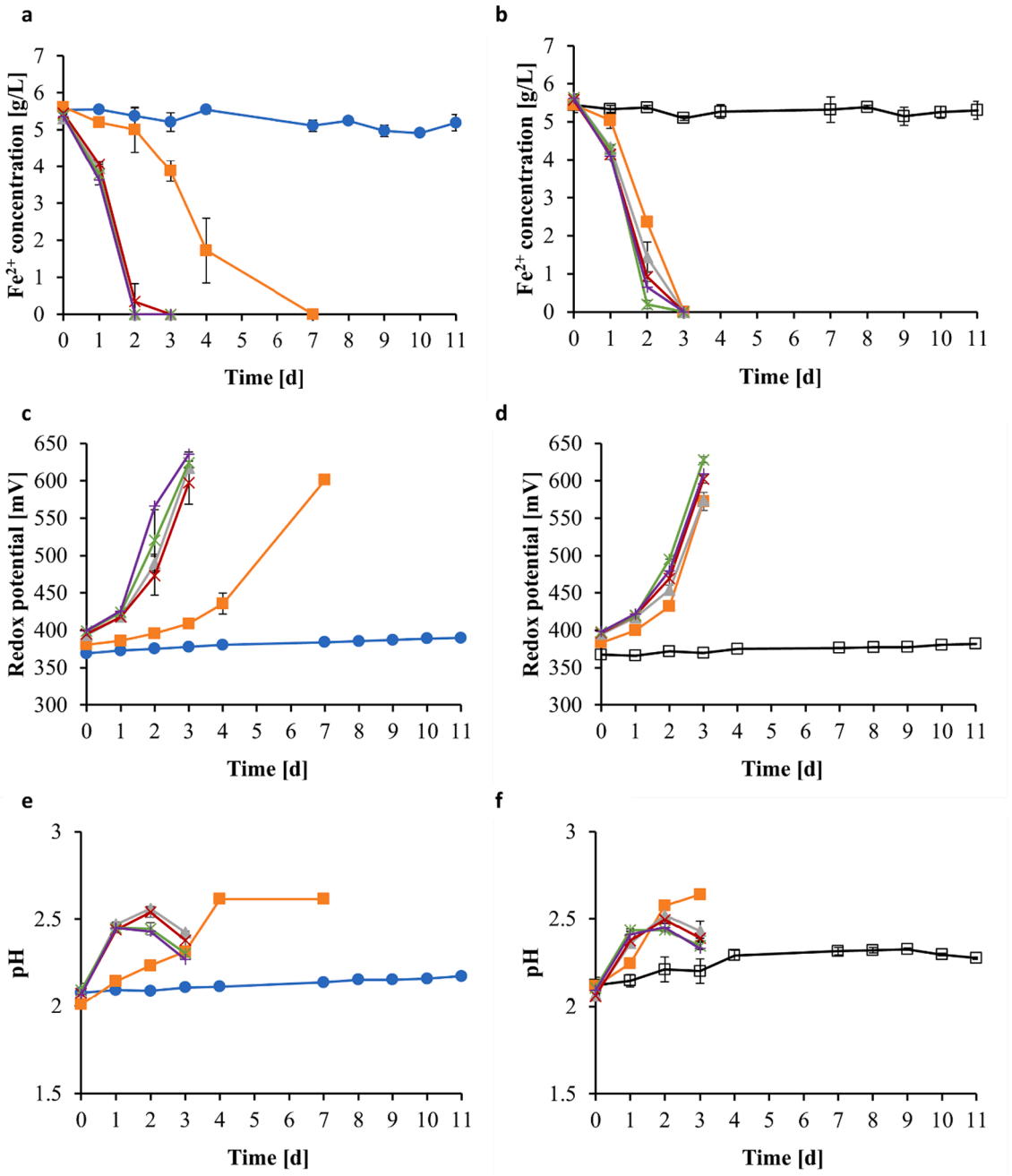

Fig. 1. Effect of metal extraction liquor 1 on the development of (a) Fe^{2+} concentration, (c) redox potential, and (e) pH, and metal extraction liquor 2 on (b) Fe^{2+} concentration, (d) redox potential, and (f) pH, during iron oxidation by the iron oxidizing enrichment culture. (□): 50% (*v/v*); (●): 20% (*v/v*); (■): 10% (*v/v*); (▲): 2% (*v/v*); (×): 1% (*v/v*); (✳): 0.1% (*v/v*); (+): positive control (without metal extraction liquors). Same data marks of the concentrations used with both metal extraction liquors. The 20% (*v/v*) concentration of metal extraction liquor 1 was incubated separately. The error bars present the standard deviations (n = 2).

3. Results and discussion

3.1. Inhibition of iron and sulfur oxidation by metal extraction liquors

The effects of metal extraction liquors on biological iron oxidation by the iron oxidizing enrichment culture were studied, and the results were as shown in Fig. 1. The effects of metal extraction liquor 1 on biological sulfur oxidation by sulfur oxidizing enrichment culture was investigated and the results were as shown in Fig. 2.

With 0.1–2% (*v/v*) metal extraction liquors, iron oxidation associated with redox and pH increases were similar as in the positive control, and therefore did not affect iron oxidation, whereas with concentrations of 10% (*v/v*), iron oxidation rate was decreased, which was as also indicated by redox and pH changes (Fig. 1). Redox potential increase was associated with the increase of ferric iron (Fe^{3+}) to Fe^{2+} ion ratio and the increase in pH was associated with the proton (H^+) consumption. At 10% (*v/v*) liquor 1, 3-day lag phase in iron oxidation occurred, after that iron oxidation rate was slower than in the positive control. At

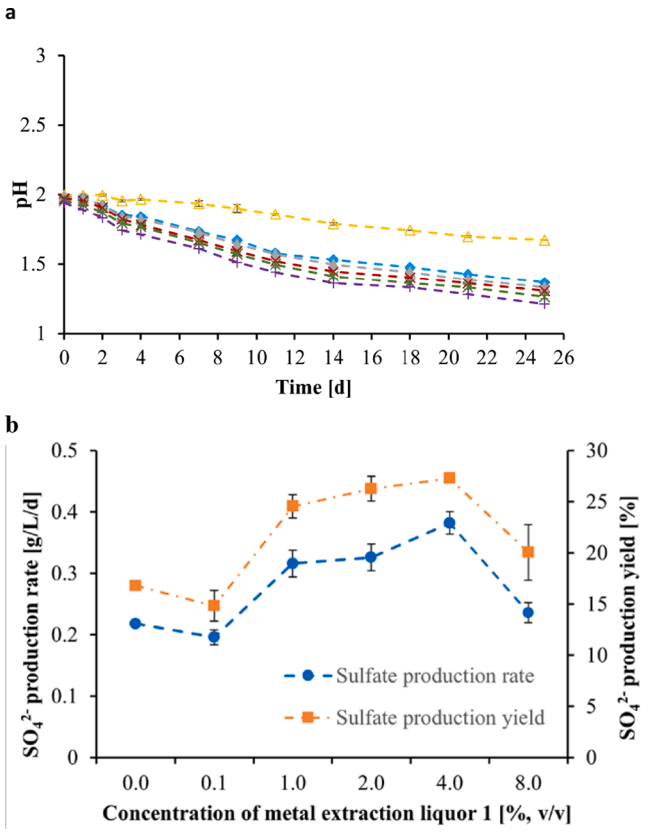

Fig. 2. Effect of metal extraction liquor 1 on (a) pH and (b) sulfate production rate and yield during sulfur oxidation by the sulfur oxidizing enrichment culture. The sulfate production rates were determined from the slope of the linear regression lines of the sulfate production curves ($R^2 > 0.89$). (a): (( ): 8% (*v/v*); ( ): 4% (*v/v*); (( ): 2% (*v/v*); (( ): 1% (*v/v*); ( ): 0.1% (*v/v*); (( ): positive control (without metal extraction liquor 1). The error bars present the standard deviations (n = 2).

10% (*v/v*) liquor 2, after a lag phase of one day, the iron oxidation rate was similar as in the positive control. With 20% (*v/v*) liquor 1 and 50% (*v/v*) liquor 2, iron oxidation was irreversibly inhibited.

In metal extraction liquors 1 and 2, the concentration of $(NH_4)_2SO_4$ were 428 and 288 g/L, respectively, TOC concentrations were 300 and 180 mg/L, respectively with residual metal (Ni^{2+}) and anionic impurity (Cl^-) concentrations below 200 mg/L. As seen in Fig. 1, liquor 1 was more inhibitory for iron oxidation than liquor 2. In 10% (*v/v*) liquor 1 and 2, $(NH_4)_2SO_4$ concentrations of 43 and 29 g/L, respectively, were above toxic level (Fig. S2). The $(NH_4)_2SO_4$ concentration $\leq$ 20 g/L did not cause any inhibition, while concentrations above 20 g/L resulted in reduced iron oxidation rate than in the positive control. Concentration of $\geq$ 42 g/L resulted in lag phase, and 250 g/L inhibited iron oxidation irreversibly. Organic compounds inhibit iron and sulfur oxidizing chemolithoautotrophic bacteria (Torma and Itzkovitch, 1976; Tuttle and Dugan, 1976; Fang and Zhou, 2006), and thus, also metal extraction liquors consisting of various organic solvents, probably inhibited biological iron oxidation. These results show that the inhibition of iron oxidation was due to the high concentrations of $(NH_4)_2SO_4$ and/or the organic solvents content in metal extraction liquor 1 and 2.

Nurmi et al. (2009) reported that Ni^{2+} did not inhibit iron oxidation even at 60 g/L, which exceeded the Ni^{2+} concentration in the metal extraction liquors. Cl^- ions have been reported to inhibit iron oxidation at > 7 g/L concentrations (Harahuc et al., 2000; Gahan et al., 2010; Huynh et al., 2020), while the Cl^- in the metal extraction liquors remained below the inhibitory concentration. These results show that Ni^{2+} and Cl^- were not inhibitory to iron oxidation in this study. The high salinity of the leaching solution may affect the oxygen transfer rate, and therefore, control the iron oxidation rate. Bioassays of this study were aerated by constant shaking. Further the salinity of $\geq$ 10% (*v/v*) liquors (<10 g/L) was below salinity that would have negative effect on iron oxidation (Sadeghieh et al., 2020). For these reasons, the inhibitory effect of metal extraction liquor concentrations above 10% (*v/v*) on iron oxidation was not due to oxygen transfer limitation or the salinity.

In sulfur oxidation experiment, pH decreased similarly as in the positive control with 0.1–4% (*v/v*) metal extraction liquor 1, during 25-days of incubation (Fig. 2a). With 8% (*v/v*) liquor 1, pH reduction was slower than in the positive control. The pH decrease was associated with H^+ production during sulfur oxidation. Concentrations higher than 0.1% (*v/v*) increased the SO_4^{2-} production rate and yield (Fig. 2b). At concentration of $\leq$ 4% (*v/v*), the SO_4^{2-} production rate and yield increased with increasing liquor 1 concentration, whereas with concentrations above 4% (*v/v*), they decreased. However, below 8% (*v/v*) liquor 1, SO_4^{2-} production rate and yield remained higher than in the positive control. The decrease in the SO_4^{2-} production rate and yield at concentration above 8% (*v/v*) shows that metal extraction liquor 1 had a negative effect on sulfur oxidation.

As the negative impact of metal extraction liquor 1 on biological iron oxidation was evident already with concentrations above 2% (*v/v*), it was shown that the sulfur oxidizing enrichment culture was more resistant to liquor 1. Halinen et al. (2012) demonstrated that the microbial community of demonstration-scale bioheap of the same heap bioleaching site as of this study's contained a diverse community including facultative chemolithotrophs belonging to genus *Sulfobacillus* (Robbins, 2000), heterotrophs belonging to genus *Alicyclobacillus* (Wisotzkey et al., 1992) and *Ferrimicrobium acidiphilum* (Johnson et al., 2009). The demonstration-scale bioheap also harboured sulfur oxidizer *Acidithiobacillus* (*A.*) *thiooxidans*, which have been demonstrated to be more resistant to inhibition by certain organic compounds than *A. ferrooxidans* by Fang and Zhou (2006). Therefore, a more diverse microbial community may be more resistant to organic compounds of metal extraction liquor 1 in the sulfur oxidizing enrichment culture than in the iron oxidizing enrichment culture, even though sulfur oxidizing microorganisms were enriched from the iron oxidizing enrichment culture.

3.2. Inhibition of iron oxidation by organic solvents

The effects of neodecanoic acid on biological iron oxidation by the iron oxidizing enrichment culture were studied. The results were as shown in Fig. 3. Neodecanoic acid concentrations in iron oxidizing culture medium was monitored as DOC (Fig. S3).

With 2.5 mg/L neodecanoic acid, iron oxidation, pH and redox increased similarly as in the positive control, and therefore did not affect iron oxidation (Fig. 3a). With 6.3 mg/L neodecanoic acid and above, iron oxidation rate was decreased. At concentration of 6.3 mg/L, iron oxidation rate, redox potential and pH increases were slightly slower than in the positive control, whereas at concentration of 13 mg/L, 1-day lag phase occurred, and after that iron oxidation rate was slightly slower than in the positive control. With 25 and 250 mg/L neodecanoic acid, the lag phases were different between the parallel flasks, therefore the results of the flasks are separately presented in Fig. 3b. With concentration of 25 mg/L in flask A and B, after the l0- and 14-day lag phase, respectively, iron oxidation rate, redox potential and pH increases were slightly slower compared to the positive control. With concentration of 130 mg/L, after 18-day lag phase, iron oxidation rate, redox potential and pH increases remained slightly slower than in the positive control. With 250 mg/L neodecanoic acid in flask A and B, iron oxidation lag phase took 23 and 21 days, respectively, and after that iron was oxidized, and redox potential and pH increased, yet the change was slower than in the positive control.

With 2.5–13 mg/L and 130 mg/L neodecanoic acid, subtle fluctuation of DOC occurred during the incubation (Fig. S3). These results show

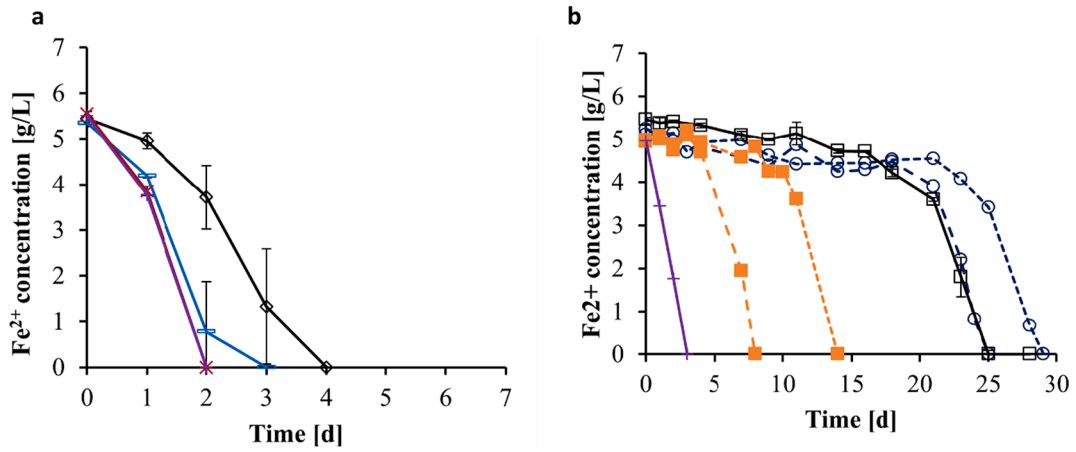

Fig. 3. Effect of neodecanoic acid concentrations of (a) 2.5–13 mg/L (1–5% of its aqueous solubility) and (b) 25–250 mg/L (10–100% of its aqueous solubility) on Fe^{2+} concentration during iron oxidation by the iron oxidizing enrichment culture. (◯): 250 mg/L; (□): 130 mg/L; (▬): 25 mg/L; (◇): 13 mg/L; (——): 6.3 mg/L; (✕): 2.5 mg/L; (+): positive control (without neodecanoic acid). (- - -): shake flask A; (– – –): shake flask B; and (——): mean of shake flasks A and B. Neodecanoic acid concentration of 130 mg/L was incubated separately. The error bars present the standard deviations (n = 2).

that DOC was not removed from the neodecanoic acid culture media during the incubation, rather stayed quite stable. At different concentrations of neodecanoic acid, the DOC fluctuated to some extent (Fig. S3) but without any indication of biodegradation.

The effects of other organic solvents (Nessol D100, Cyanex 272, and Baysolvex D2EHPA) on iron oxidation by the iron oxidizing enrichment culture were also investigated, and the results were as shown in Fig. 4.

With all the studied Nessol D100, Cyanex 272 and Baysolvex D2EHPA concentrations, iron was oxidized, and redox potential and pH increased similarly as in the positive control, and therefore did not affect iron oxidation (Fig. 4).

The iron oxidation results of the organic solvent experiments show that neodecanoic acid was the only solvent negatively affecting biological iron oxidation. This inhibitory effect was, however, reversible. The DOC results also showed that, neodecanoic acid was not removed. Although no biodegradation of neodecanoic acid occurred with the iron oxidizing culture, it is possible that the heterotrophic microorganisms in bioleaching communities could degrade organic solvents (Gu and Wong, 2007; Wang et al., 2010; Li et al., 2011). Demonstrating this would require enrichment of heap bioleaching microorganisms using the organic solvents.

With Nessol D100, Cyanex 272, and Baysolvex D2EHPA, iron oxidation was not affected, indicating that these organic solvents remained non-bioavailable due to their very low aqueous solubilities. Non-bioavailability of these organic solvents, in these experimental conditions, was also confirmed by the visual perceptions. With Nessol D100, a very slight liquid phase was observed on top of the culture medium, during the incubation, while with Cyanex 272 and Baysolvex D2EHPA, white precipitate was formed on top of the aqueous phase. Cyanex 272 reacted with come constituent of TES, whereas Baysolvex D2EHPA reacted with the inoculum. Since the inoculum was the only medium constituent that contained ferric iron (Fe^{3+}), Baysolvex D2EHPA likely reacted with the Fe^{3+}.

Nessol D100 is a hydrocarbon solvent, whereas other studied organic solvents are surfactants with functional groups resulting in hydrophilic and hydrophobic ends. These differences in the chemical structures could result in different function and effects on microorganisms. Effects of carboxylic acids, Cyanex 272, and Baysolvex D2EHPA on bioleaching microorganisms have been previously reported by Tuttle and Dugan (1976), Torma and Itzkovitch (1976) and Chen et al. (2015). Tuttle and Dugan (1976) showed inhibition by various monocarboxylic acids towards iron and sulfur oxidation by *A. ferrooxidans* (formerly *Thiobacillus ferrooxidans*), which was also demonstrated in our study with neodecanoic acid. The negative effects of the organic compounds can, however, be reduced by using diverse microbial cultures containing, in addition to chemolithoautotrophs, heterotrophs and/or mixotrophs (Gu and Wong, 2007; Wang et al., 2010; Li et al., 2011). Tuttle and Dugan (1976) suggested that the major factor resulting in the inhibition of iron and sulfur oxidation was electronegativity of the compound, and possible inhibitory mechanisms would include, for example, abiological reaction with Fe^{2+} of the environment and direct influence on iron oxidizing enzyme system. The inhibitory mechanisms of organic acids to acidophilic bioleaching microorganisms have been summarized in Table 3.

Chen et al. (2015) reported that Cyanex 272 was not inhibitory to biological iron oxidation by acidophilic microorganisms at its saturation concentration (in 9 K medium), and in our study this was also demonstrated. Torma and Itzkovitch (1976) showed that Baysolvex D2EHPA was inhibitory to chalcopyrite oxidation at its saturation concentration (0.264 g TOC/L). The results of our study, however, differed from the results of their study, as no inhibition of iron oxidation at this concentration was seen. The experimental conditions of our study and the study of Torma and Itzkovitch (1976) were different (in our study no sulfide ore was used and Fe^{3+} was formed during iron oxidation), likely serving as explanation to the observed differences.

3.3. Enhancement of iron oxidation by metal extraction liquors

The possible stimulation of metal extraction liquors on biological iron oxidation and NH_4^+ utilization by the iron oxidizing AD culture was studied. The results were as shown in Fig. 5 and in supplementary material Fig. S4.

With all the studied concentrations of the metal extraction liquors, iron was oxidized, and redox potential and pH increased in similar way as in the positive and 0.11 g NH_4^+/L controls, and therefore stimulating biological iron oxidation (Fig. 5a and b). On day 1, in control without NH_4^+, iron was oxidized, and redox potential and pH increased similarly to the positive and 0.11 g NH_4^+/L controls. However, after day 1, iron oxidation rate, redox potential and pH increases declined in the control without NH_4^+ and was slower than in the positive and 0.11 g NH_4^+/L

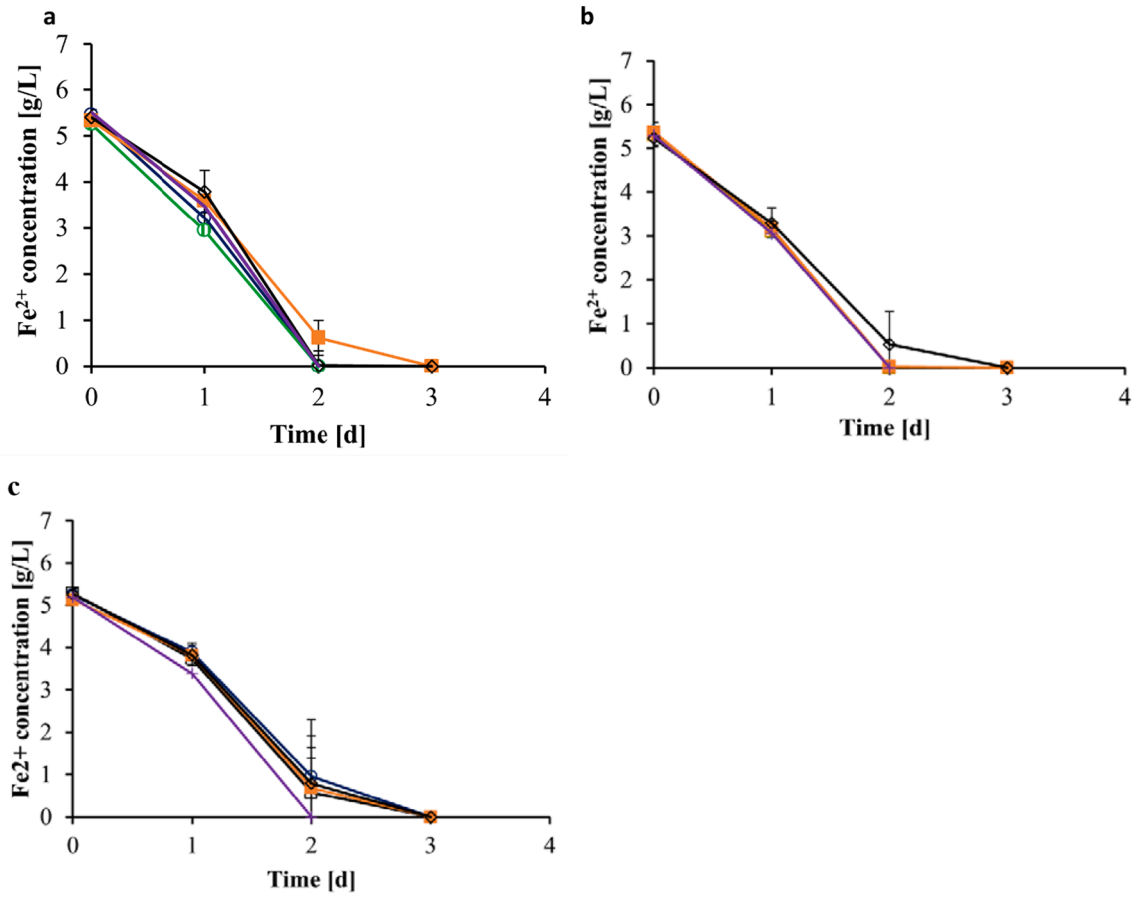

Fig. 4. Effect of (a) Nessol D100, (b) Cyanex 272, and (c) Baysolvex D2EHPA on Fe^{2+} concentration during iron oxidation by the iron oxidizing enrichment culture. (a): (◯): 10 mg/L (100% of its aqueous solubility); (▯): 3.0 mg/L (30% of its aqueous solubility); (■): 1.0 mg/L (10% of its aqueous solubility); (◇):0.5 mg/L (5% of its aqueous solubility); (+): positive control (without Nessol D100). (b): (◯): 16 mg/L (100% of its aqueous solubility); (▯): 6.4 mg/L (40% of its aqueous solubility); (■): 1.6 mg/L (10% of its aqueous solubility); (◇): 0.8 mg/L (5% of its aqueous solubility); (+): positive control (without Cyanex 272). (c): (◯): 180 mg/L (100% of its aqueous solubility); (□): 91 mg/L (50% of its aqueous solubility); (■): 18 mg/L (10% of its aqueous solubility); (◇): 9.1 mg/L (5% of its aqueous solubility); (+): positive control (without Baysolvex D2EHPA). The error bars present the standard deviations (n = 2).

controls.

With all the metal extraction liquor concentrations, moderate NH_4^+ utilization by the iron oxidizing culture was seen (Fig. S4). All the NH_4^+ concentrations slightly decreased during the incubation with the excess NH_4^+ remaining in the media, while the initial NH_4^+ concentrations varied between 0.11 and 1.3 g/L with metal extraction liquor 1 (Fig. S4a) and 0.09–0.84 g/L with liquor 2 supplementations (Fig. S4b).

Previous studies have shown that NH_4^+ supplementation stimulates biological iron oxidation and bioleaching (Niemelä et al., 1994; D'Hugues et al., 1997; Ahoranta et al., 2017). Niemelä et al. (1994) reported that 0.11 g/L NH_4^+ concentration enhanced biological iron oxidation by a mixed microbial culture. In our study this enhancement of iron oxidation with 0.11 g/L NH_4^+ concentration, added as metal extraction liquor 1, was also demonstrated. D'Hugues et al. (1997) and Ahoranta et al. (2017) showed that 1.03 g/L and 0.41 g/L of NH_4^+ concentrations, respectively, enhanced bioleaching and biological iron oxidation. Ahoranta et al. (2017) also reported that increasing the NH_4^+ concentration above 0.41 g/L did not significantly further enhance iron oxidation. Our study demonstrated that NH_4^+ concentration already above 0.079 g/L (with liquor 2) and 0.11 g/L (with liquor 1) did not significantly further enhance the biological iron oxidation.

Previous nitrogen supplementation studies have shown that nitrogen compound used greatly impacts on the growth and bioleaching by iron and sulfur oxidizing microorganisms. NH_4^+ enhanced biological iron and sulfur oxidation and bioleaching, while nitrate (NO_3^-) had a negative effect (Niemelä et al., 1994; D'Hugues et al., 1997; Harahuc et al., 2000; Ahoranta et al., 2017). Niemelä et al. (1994) showed that NO_3^- concentration of 0.38 g/L inhibited biological iron oxidation, while Harahuc et al. (2000) reported that 0.62 and 12 g/L NO_3^- inhibited biological iron and sulfur oxidation, respectively. In addition to inorganic nitrogenous compounds, the influence of organic nitrogenous compounds on bioleaching and biological iron oxidation have been reported by Puhakka & Tuovinen (1987) and D'Hugues et al. (1997). Puhakka & Tuovinen (1987) demonstrated that 0.22 g/L yeast extract drastically increased nickel, zinc, copper, and cobalt recovery from a multi-metal sulfide ore material, originating from the same site as the liquors of the study. D'Hugues et al. (1997) showed that organic nitrogen supplementation with urea and diammonium phosphate (DAP), did not enhance bioleaching as effectively as NH_4^+. Hence, NH_4^+ and organic nitrogenous compounds are the most suitable forms for the bioleaching microorganisms, and that NH_4^+ containing metal extraction liquors are potential NH_4^+ sources at low concentrations (≤1% (*v/v*)).

3.4. *Use of metal extraction liquors in heap bioleaching*

The iron oxidation rate results with metal extraction liquors (Fig. 6)

Table 3
Inhibitory mechanisms of organic compounds towards acidophilic bioleaching microorganisms.

Inhibitory mechanisms	Studied organic compounds	Reference
1. Direct influence on iron oxidizing enzyme system 2. Abiological reaction with Fe^{2+} of the environment 3. Interference with the function of SO_4^{2-} in iron oxidation 4. Disruption of structure of cell membrane	Low molecular weight organic compounds (monocarboxylic acids, α-keto acids, dicarboxylic acids, and urea)	Tuttle and Dugan (1976)
1. Dissociation of the compound: in the microbial cell, organic acids become dissociated, when they provoke acidification of the cytosol and disruption of the transmembrane pH gradient 2. Osmotic damages of the cell caused by elevated concentration of anions in cytosol	Organic acids (proprionate, acetate, lactate, chloroacetate and pyruvate)	Alexander et al. (1987)

showed different effects on the biological iron oxidation. The highest iron oxidation rate decreased by increasing the metal extraction liquor concentrations. These results showed that liquor 1 more negatively affected biological iron oxidation than liquor 2, and therefore maybe also be harmful to the heap bioleaching. Due to the results of the experiments with the metal extraction liquors and the organic solvents, the negative effects and possible accumulation of organic solvents to the bioleaching system must be considered prior to using the metal extraction liquors as NH_4^+ source at low concentrations (≤1% (*v/v*)) in the full-scale heap bioleaching process.

When considering supplementing a full-scale heap bioleaching process with organic solvents and NH_4^+ containing metal extraction liquors, possible environmental effects of residual nitrogen and organic solvents should be also considered. NH_4^+ in the wastewater would result in increase of oxygen consumption and eutrophication in the recipient waters (for reviews, see Vitousek et al., 1997; Schindler, 2006), whereas organic compounds would result in oxygen consumption and floating organic phases, and thus, adversely affect the aquatic ecosystem (Zhang and Li, 2010; Schawarzenbach et al., 2016).

4. Conclusions

In this work, the potential use of metal extraction liquors from electric vehicle battery materials production as NH_4^+ source for heap bioleaching microorganisms was demonstrated. The following conclusions of the studied liquors and the potential constituents on biological iron and sulfur oxidation can be drawn:

- Both metal extraction liquors negatively affect biological iron oxidation at concentration above 2% (*v/v*), and with metal extraction liquor 1 and 2 concentrations of 20% (*v/v*) and 50% (*v/v*), respectively, the inhibition is irreversible.
- Biological sulfur oxidation is enhanced with 8% (*v/v*) concentration of metal extraction liquor 1 and below.
- Neodecanoic acid negatively affects biological iron oxidation at concentration of 6.3 mg/L (2.5% of its aqueous solubility) and above, whereas Nessol D100, Cyanex 272 and Baysolvex D2EHPA do not affect biological iron oxidation.
- $(NH_4)_2SO_4$ has a negative effect on biological iron oxidation at concentration above 20 g/L, and with concentration of 250 g/L, the inhibition is irreversible.
- Under NH_4^+ limited growth conditions, 0.09% (*v/v*) metal extraction liquor 1 and 0.1% (*v/v*) metal extraction liquor 2 concentrations stimulate biological iron oxidation, and therefore may also enhance the leaching efficiencies of heap bioleaching.

CRediT authorship contribution statement

Linda Määttä: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Réka Hajdu-Rahkama:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Carita Oinonen:** Conceptualization, Resources, Writing – review & editing. **Jaakko A. Puhakka:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration, Funding acquisition.

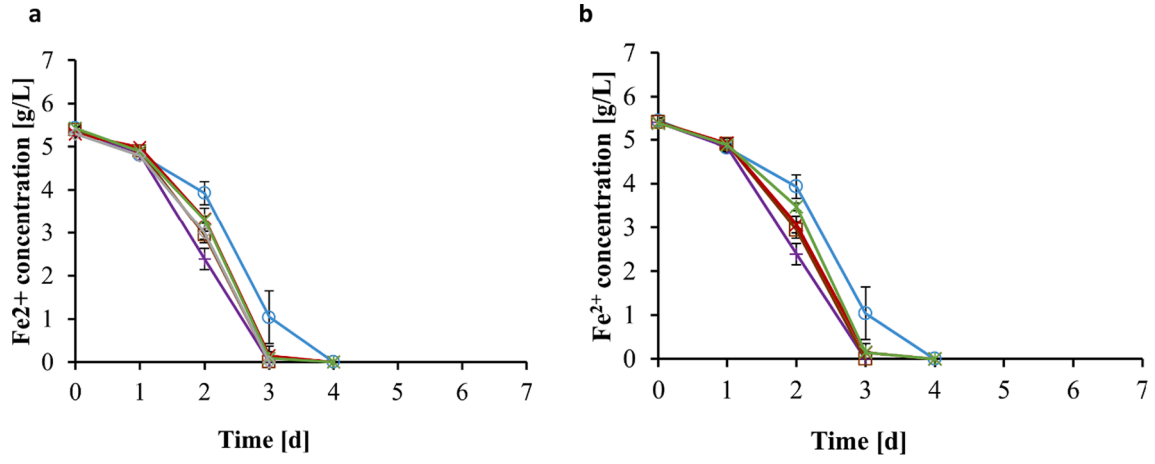

Fig. 5. Effect of (a) metal extraction liquor 1 and (b) 2 on Fe^{2+} concentration during iron oxidation by the 1% (*v/v*) iron oxidizing ammonium deficient (AD) culture. The iron oxidizing AD culture was incubated without NH_4^+ prior to the experiment. The 1% (*v/v*) iron oxidizing enrichment culture was used within the positive control. (◯): 0.0 g NH_4^+/L control; (+): positive control (1.0 g/L of NH_4^+); (□): 0.11 g NH_4^+/L control (0.11 g/L of NH_4^+); (×): 1% (*v/v*) metal extraction liquor 1 or 2; (✳): 0.1% (*v/v*) metal extraction liquor 1 or 2; (◇): 0.09% (*v/v*) metal extraction liquor 1. The error bars present the standard deviations (n = 2).

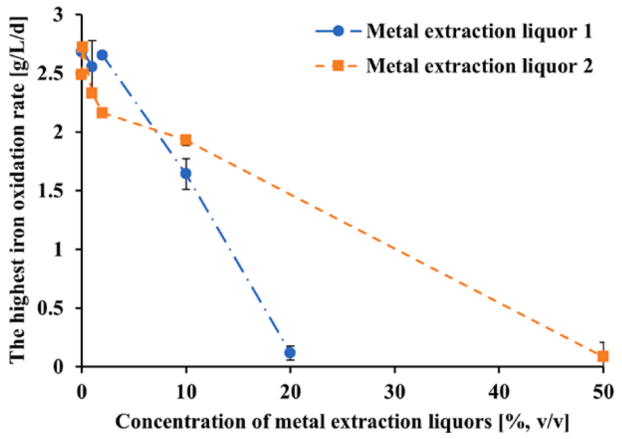

Fig. 6. Effect of concentration of metal extraction liquor 1 and 2 on the highest biological iron oxidation rate by the iron oxidizing enrichment culture. The iron oxidation rates were calculated from the slope of the linear regression lines determined from the exponential part of the Fe^{2+} oxidation curves ($R^2 > 0.90$, except 20% (*v/v*) metal extraction liquor 1: $R^2 = 0.51$, and 50% (*v/v*) metal extraction liquor 2: $R^2 = 0.48$). The error bars present the standard deviations (n = 2).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Carita Oinonen reports a relationship with Terrafame Oy that includes: employment.

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Appendix A. Supplementary material

E-supplementary material (Chemical structures of the studied organic solvents, influence of $(NH_4)_2SO_4$ on biological iron oxidation, neodecanoic acid concentrations measured as DOC, and influence of metal extraction liquor 1 and 2 on NH_4^+ concentration) of this work is available in the online version of this paper.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mineng.2022.107409.

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