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Utilisation of acid-tolerant bacteria for base metal recovery under strongly acidic conditions

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Abbreviations

AAS, atomic absorbance spectrophotometry; AP, agar plate; DDBJ, DNA Data Bank of

Japan; e-waste, electronic device waste; R2A, Reasoner's 2A; TSB, trypticase soy broth

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Declarations

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Conflict of interest

No conflict of interest declared.

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51 Draft, Visualisation. **Kazunori Nakashima:** Resources, Writing - Review & Editing,
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54

Abstract

Hydrometallurgical bioprocesses for base metal recovery in environmentally friendly electronic device waste (e-waste) recycling are typically studied under neutral pH conditions to avoid competition between metals and hydrogen ions. However, metal leachate is generally strongly acidic, thus necessitating a neutralisation process in the application of these bioprocesses to e-waste recycling. To solve this pH disparity, we focused on acid-tolerant bacteria for metal recovery under strongly acidic conditions. Four acid-tolerant bacterial strains were isolated from neutral pH environments to recover base metals from simulated waste metal leachate (pH 1.5, containing 100 or 1000 mg L⁻¹ of Co, Cu, Li, Mn, and Ni) without neutralisation. The laboratory setting for sequential metal recovery was established using these strains and a reported metal-adsorbing bacterium, *Micrococcus luteus* JCM1464. The metal species were successfully recovered from 100 mg L⁻¹ metal mixtures at the following rates: Co (8.95%), Cu (21.23%), Li (5.49%), Mn (13.18%), and Ni (9.91%). From 1000 mg L⁻¹ metal mixtures, Co (7.23%), Cu (6.82%), Li (5.85%), Mn (7.64%), and Ni (7.52%) were recovered. These results indicated the amenability of acid-tolerant bacteria to environmentally friendly base metal recycling, contributing to the development of novel industrial application of the beneficial but unutilised bioresource comprising acid-tolerant bacteria.

Keywords: acid-tolerant bacteria, bioaccumulation, biosorption, e-waste, metal recycling

1. Introduction

The consumption of metals in electrical and electronic devices is increasing with the widespread use of the Internet of Things and electric vehicles (Xu *et al.* 2020). Appropriate management and treatment of electronic device waste (e-waste) containing large amounts of base metals, such as lithium-ion batteries (LiBs) and printed circuit boards (PCBs), are required to prevent environmental pollution, ensure a stable metal supply to satisfy increasing demands, and meet the United Nations' Sustainable Development Goals (Zhang *et al.* 2017). E-waste contains valuable base metals such as Co, Cu, Li, Mn, and Ni in high concentrations (Chen *et al.* 2021, Roy *et al.* 2021).

Conventionally, acid leaching and chemical recovery processes (such as solvent extraction and chemical precipitation) are widely applied to base metal recycling (Krol *et al.* 2020, Han *et al.* 2023). Considering the high environmental load of these processes using large amounts of neutraliser and organic solvent, biosorption and bioaccumulation are considered environmentally friendly alternatives (Tian *et al.* 2013, Isildar *et al.* 2019). In most cases, these bioprocesses are studied under neutral pH conditions, under which biomass construction is stable and competitive adsorption does not occur between metal ions and H^+ to obtain high metal recovery efficiency (Markou *et al.* 2015, Rizvi *et al.* 2020). However, metals are leached from e-waste by strong inorganic acids in the upstream process (Krol *et al.* 2020). The leachate is strongly acidic pH (1–2), with high base metal concentrations (100–10000 mg L⁻¹) in most cases (Xin *et al.* 2016, Naseri *et al.* 2019). Although the recovery of metals under acidic conditions has been attempted, most of such studies are focused on rare metal elements, which occur in e-waste in trace amounts (Liang and Shen 2022). There have been few attempts to recover the highly concentrated base metals, which are major components of e-waste leachate under strongly

acidic conditions. Therefore, a neutralisation process is necessary to recover base metals from acidic leachate. Such processes consume large amounts of neutraliser and generate alkaline wastewater. Novel base metal recovery technology is expected to solve the pH disparity between acid leaching and base metal recovery processes.

To establish such a process, we focused on acid-tolerant bacteria, which can grow under neutral pH conditions and survive under acidic conditions to serve as metal recovery biomaterial under strongly acidic conditions. Biomass production of acid-tolerant bacteria is considerably easier than that of acidophiles because it can be conducted under neutral pH conditions. Despite these beneficial characteristics, acid-tolerant bacteria are an insufficiently utilised bioresource (Takano and Aoyagi 2022). Here, we propose the concept of a metal recycling process without neutralisation (**Fig. 1**) as a novel application of acid-tolerant bacteria. In this study, acid-tolerant bacteria recovering base metals under acidic conditions were isolated and applied to metal recovery from simulated waste metal leachate (pH 1.5) containing 100 or 1000 mg L⁻¹ each of Co, Cu, Li, Mn, and Ni, imitating the leachate of LiBs and PCBs (Chen *et al.* 2015, Demarco *et al.* 2019).

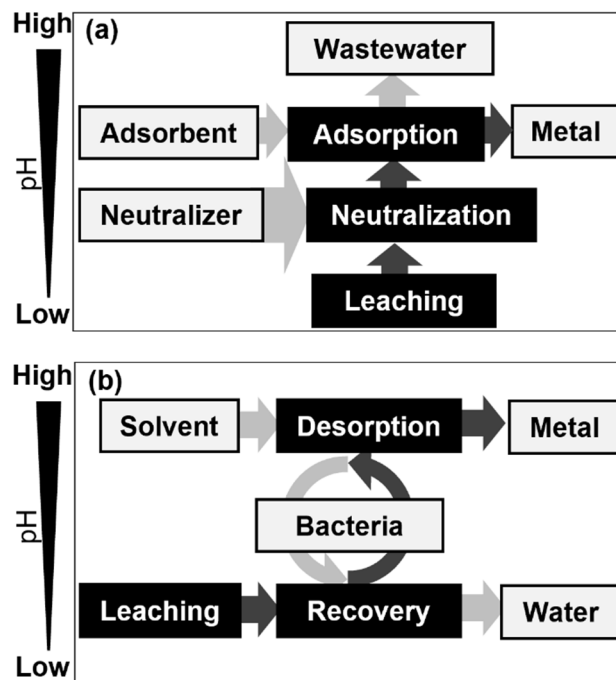


Fig. 1 Conventional and novel concepts of metal recycling

(a) The conventional metal recycling process requires neutralisation because of the optimal pH disparity between leaching and adsorption

(b) Our novel concept resolves this problem by conducting metal recovery under acidic conditions without neutralisation; metal recovery bacteria are transferred to the metal desorption solvent to separate metals

2. Materials and Methods

2.1 Materials

Non-woven fabric and cellulose film-based culturing methods were applied according to our previous studies (Takano and Aoyagi 2022, Aoyagi *et al.* 2022). A metal ion mixture, containing 1000 mg L⁻¹ of Co, Cu, Li, Mn, and Ni each, was prepared as stock solution for the simulated metal leachate and adjusted to pH 1.5 using 10N H₂SO₄. The metal sources were CoCl₂·6H₂O, CuSO₄·5H₂O, LiCl, MnCl₂·4H₂O, and NiSO₄·6H₂O. All chemicals were purchased from FUJIFILM Wako Pure Chemical Co. Ltd. (Osaka, Japan) unless specified otherwise.

The following environmental samples were collected as bacterial isolation sources: activated sludge from the Mikawashima Water Reclamation Center (Tokyo, Japan, 35°44'30.8"N, 139°47'07.8"E), water from a river near the Hitachi abandoned mine site (Ibaraki, Japan, 36°36'41"N, 140°38'45"E and 36°40'29"N, 140°37'20"E), and brackish water and bottom sediment from the Hinuma River (Ibaraki, Japan, 36°17'15"N, 140°30'18"E). *Micrococcus luteus* JCM1464 was purchased from the Riken BioResource Research Center (Ibaraki, Japan) and stored as glycerol stock in the Cell Functions Development Engineering Laboratory at the University of Tsukuba (Ibaraki, Japan).

2.2 Bacterial isolation

The soil samples were suspended in 10 mL of sterilised distilled water, sonicated for 15 min, and filtered using No. 3 filter paper (Advantec Co. Ltd., Tokyo, Japan) as a pre-treatment. Water samples were pre-treated by filtration as described above. Bacterial screening was conducted based on the 'pH shift culture' screening method described in our previous study using Reasoner's 2A (R2A) medium (Takano and Aoyagi 2022).

Briefly, environmental samples were spread on cellulose film placed on non-woven fabric containing R2A medium (pH 7.0) and incubated (30°C, 48 h). Cellulose film was transferred to a fabric containing diluted H₂SO₄ solution (pH 1.5) with ions of Cu²⁺, Co²⁺, Ni²⁺, Mn²⁺, or Li⁺ (final concentration = 0.5–100 mg L⁻¹) and incubated (30°C, 48 h) to exclude non-acidic tolerant bacteria. Then, the film was transferred to fresh R2A medium and incubated (30°C, 48 h) again to promote colony growth. The colonies that formed on the cellulose film were isolated using a disposable plastic loop and streaked on a R2A agar plate (AP) containing 1.5% (w/v) agar. After overnight incubation, the single colony that formed on the AP was streaked on a fresh AP. Streaking and incubation were repeated five times to obtain the purified bacterial strain. PCR amplification of the 16S rDNA gene sequences and phylogenetic analysis of the isolated strains were performed as described in our previous study to identify isolated bacterial strains (Takano and Aoyagi 2022). Sequence data were registered in the DNA Data Bank of Japan (DDBJ; Shizuoka, Japan).

2.3 Evaluation of metal recovery ability of isolated strains under acidic conditions

The simulated metal leachate containing 100 mg L⁻¹ of each of the five metal species was prepared by diluting the metal stock solution using H₂SO₄ solution (pH 1.5), and a 2 mL solution was collected to determine the initial metal concentrations. To prepare the cell biomass, 1 mL of glycerol stock was inoculated into 50 mL of R2A medium and incubated for 12 h (30°C, 200 rpm). One millilitre of the cultured fluid was inoculated into 100 mL of fresh R2A medium and cultured again for 12 h under the same conditions. The cells were harvested by centrifugation (6000 ×g, 10 min) and suspended in 100 mL of simulated metal leachate. The suspension (500 µL) was diluted, and the optical density at 600 nm was measured to calculate cell density based on dry cell weight. Two millilitres

of the suspension was collected, and the cells were removed by centrifugation (14,000 $\times g$, 5 min) every 0.5 h for 3 h. The supernatant was stored at 4°C for further measurement of metal concentrations. The metal recovery ability of the metal-absorbing bacterium *M. luteus* JCM1464 was evaluated and compared with that of isolated strains. The strain JCM1464 was cultivated in trypticase soy broth (TSB; Becton, Dickinson and Company, NJ, USA) instead of R2A medium.

The samples for the metal recovery test were diluted using 4% (w/v) HNO₃ to a detectable limit of <10 mg L⁻¹ and then passed through filters with a pore size of 0.2 μm (ADVANTEC Co. Ltd., Tokyo, Japan). The metal concentration was quantified using atomic absorbance spectrophotometry (AAS; ZA3300; Hitachi High-Tech Science Co., Ltd., Tokyo, Japan) following the manufacturer's instructions. Calibration curves were prepared by measuring the serially diluted standard solutions of each metal.

2.4 Development of metal recovery process without neutralisation in a laboratory setting

Simulated metal leachate (100 mg L⁻¹ for each metal) and 200 mL of 1–1.5 g-dry cell L⁻¹ bacterial cells (R2A medium) were prepared as described in section 2.3. Prepared cells of the first strain were harvested by centrifugation (6000 $\times g$, 10 min) and suspended in 100 mL simulated metal leachate. The metal recovery process was established using five bacterial strains sequentially. The first strain was suspended in the simulated metal leachate and incubated (30°C, 200 rpm) for a pre-determined period, as described in 2.3, and the cells were harvested. Two millilitres of the supernatant was collected to measure the metal concentrations. Then, the second strain was suspended in the supernatant, and metal recovery was performed. This cycle was repeated for all five strains. The metal

concentrations were quantified using AAS as described in section 2.3 to quantify the metal recovery abilities (q [mg g^{-1}]) of each strain following Equation (1).

$$q = (C_0 - C_e)/C_c \quad (1).$$

In this formula, C_0 : initial metal concentration [mg L^{-1}], C_e : metal concentration after metal recovery [mg L^{-1}], and C_c : bacterial dry cell concentration [g L^{-1}].

2.5 Application to high-concentration metal leachate

The metal recovery process was applied to a simulated metal leachate containing 1000 mg L^{-1} of five metal species to enhance its versatility. The sequential metal recovery process was conducted as described in section 2.4. Considering the high dilution ratio, the metal concentrations of the samples from the 1000 mg L^{-1} initial metal concentration were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (ICPE-9820; Shimadzu Co. Ltd., Kyoto, Japan). Sample preparation and metal quantification were conducted as described in section 2.3. To evaluate the effect of cation release, cation concentrations were also quantified using ICP-AES.

2.6 Statistical analysis

All experiments were conducted in triplicate, and the standard deviation was calculated. To evaluate the significance of differences, a two-tailed Student's t -test was conducted using Excel 2019 (Microsoft Co. Ltd., Redmond, WA, USA). A P-value < 0.05 indicated a significant difference.

3. Results and Discussion

3.1 Bacterial isolation

In hydrometallurgical e-waste recycling, the metal leaching process is primarily performed under strongly acidic conditions (pH 1–3), as described above. Therefore, the simulated metal leachate and bacterial screening conditions were set as pH 1.5 in this study. Forty-nine bacterial strains were successfully isolated using the pH shift culture. Significant metal recovery under pH 1.5 and 30°C was observed in four strains. Their 16S rRNA gene sequences were registered in the DDBJ as *Comamonas* sp. HTL (LC684024; DDBJ accession number), *Enterobacter* sp. MnA (LC684026), *Priestia* sp. Mn7 (LC684025), and *Pseudomonas* sp. MS3 (LC574007). The similarity of each isolate to the reference sequence registered in NCBI GenBank is shown in **Table 1**. When cultured at pH 7.0, isolated bacterial strains MnA, Mn7, and MS3 showed higher cell productivity (1.45, 1.15, and 1.08 g-dry cell L⁻¹, respectively) than the strain JCM1464 and previously known acid-tolerant bacteria under the experimental conditions (**Table 2**).

Acid-tolerant bacteria constitute an insufficiently utilised bioresource, except for the bioproduction of organic acids such as lactic acid and as a source of acid-tolerant enzyme (Juodeikiene *et al.* 2016). Acid-tolerant bacteria have higher biomass productivity and have shorter cultivation periods than those of acidophiles (Kurosawa *et al.* 1991, Sharma and Satyanarayana 2010). Accordingly, the isolated bacterial strains MnA, Mn7, and MS3 can be regarded as potentially beneficial bioresources with high cell productivity under neutral pH conditions.

Table 1 Similarity of the isolates to the reference sequence registered in NCBI GenBank.

Strain	Ref. type strain	Ref. Accession No.	Similarity (%)
HTL	<i>Comamonas testosteroni</i>	MK426827.1	99.99
MS3	<i>Pseudomonas laurentiana</i>	MG719526	99.93
Mn7	<i>Priestia aryabhattai</i>	MN653271.1	100.00
MnA	<i>Pseudomonas promysalinigenes</i>	CP077094.1	99.85

Table 2 Comparison of biomass productivity of acid-tolerant bacteria.

Strain name DDBJ Accession No.	Biomass [g L ⁻¹]	Reference
<i>Comamonas</i> sp. HTL LC684024	0.55	This study
<i>Enterobacter</i> sp. MnA LC684026	1.45	This study
<i>Priestia</i> sp. Mn7 LC684025	1.15	This study
<i>Pseudomonas</i> sp. MS3 LC574007	1.08	This study
<i>Micrococcus luteus</i> JCM1464 CABC00000000	0.54	This study
<i>Lactococcus lactis</i> MG1363	0.44	Ichinose <i>et al.</i> 2023
<i>Enterobacter aerogenes</i> HO-39	1.05	Yokoi <i>et al.</i> 1995

3.2 Metal recovery characteristics under pH 1.5

The metal recovery treatment periods of each strain were set as follows: HTL, 1.5 h; JCM1464, 0.5 h; MS3, 0.5 h; MnA, 3 h; and Mn7, 0.5 h. The reaction period was set as the time at which each strain recovered the significant and highest amount of total metal as shown in **Fig. S1** (Online resource 1). The bacterial metal recovery abilities under these conditions are listed in **Table 3**. The required reaction periods differed between strains.

Compared to the other isolates, strains HTL and MnA showed unique metal recovery behaviours. Strain HTL required 1.5 h to reach equilibrium conditions in Cu recovery (**Fig. S1**). Considering the redox potential (Walters 1998, Shimura and Yoshida 2011), Cu^{2+} is easily reduced to Cu compared to other tested metal ions and H^+ . Therefore, it seems that bacterial strains Mn7 and JCM1464 recovered reduced Cu, which is not affected by H^+ . The relatively long Cu recovery period of the strain HTL suggests that the strain has relatively few adsorption sites, which is suitable for the reduced metal. Further research is required to elucidate the metal condition on the cell surface.

Strain MnA recovered the highest amount of Li among the tested strains. In contrast with other heavy metals used in this study, Li has a monovalent ion. Its redox potential is significantly higher than that of other metals and H. Therefore, Li absorption on the anionic group of bacterial surface protein under acidic conditions is difficult. According to **Fig. S1 D**, the Li concentration in the supernatant was significantly decreased at 3 h. Bioaccumulation requires a longer reaction period to take up metal ions inside the cell than biosorption does (Chojnacka 2010, Tian *et al.* 2013). Therefore, the long reaction period (3 h) suggests that the strain MnA recovers Li by bioaccumulation and can perform the selective recovery of Li under pH 1.5 conditions. Only living cells can accumulate

metals. Hence, acid-tolerant bacteria are more suitable than normal bacteria (difficult to survive) and acidophiles (difficult to produce) for metal bioaccumulation under acidic conditions.

Strain JCM1464 adsorbs 59 mg g⁻¹ Cu and 17 mg g⁻¹ Mn under neutral pH conditions (Letnik *et al.* 2017, Pipiska *et al.* 2018). Although the strain recovered the highest amount of Mn under pH 1.5 among the tested strains, its metal adsorption ability might be negatively affected by abundant H⁺.

There have been few studies on base metal recovery under strongly acidic conditions, most of which were conducted using a single metal solution (**Table 4**). In this study, the solution contained five metal species, which is a more practical condition than a single metal solution. Therefore, accurate determination of the metal recovery ability of each strain was difficult. Compared to previous studies, the isolated strain Mn7 recovered a higher amount of Cu (3.28 mg g⁻¹) than *P. fluorescens* did. To our knowledge, bacterial Li recovery under strongly acidic conditions has not been reported. The Ni and Co recovery abilities of isolated strains were lower than those of normal bacteria reported in previous studies. Considering this result and growth ability under neutral pH conditions, strain Mn7 is expected to be particularly efficient for metal recovery under acidic conditions. This strain has relatively high metal recovery ability and settles down easily in suspension. Large-scale biomass production and efficient cell separation processes allow for the application of acid-tolerant bacteria and expand bioprocesses under acidic conditions.

Table 3 Metal recovery ability [mg g^{-1}] of each strain under optimal metal recovery period [h]

Strain (Reaction period [h])	Co	Cu	Li	Mn	Ni
HTL (1.5)	n.s.	3.11 ± 0.09	n.s.	n.s.	n.s.
MnA (3.0)	n.s.	2.89 ± 0.47	2.05 ± 0.14	n.s.	n.s.
Mn7 (0.5)	n.s.	3.28 ± 0.21	n.s.	1.42 ± 0.07	3.12 ± 0.24
MS3 (0.5)	n.s.	n.s.	n.s.	n.s.	1.65 ± 0.44
JCM1464 (0.5)	n.s.	1.98 ± 1.07	n.s.	3.25 ± 0.29	1.84 ± 0.21

“n.s.” indicates no significant recovery.

Table 4 Summary of previous studies on bacterial metal recovery under strongly acidic conditions

Strain	Recovery [mg g^{-1}]	pH	Initial metal conc. [mg L^{-1}]	Temp. (°C)	Reference
<i>Streptomyces roseorubens</i> SY	Ni 8	2.0	100	40	Long <i>et al.</i> 2018
<i>P. fluorescens</i>	Co 2.36 Cu 2.54 Ni 14.7	1.0	50	30	Lopez <i>et al.</i> 2000
<i>B. sphaericus</i>	Ni 2.59	1.0	50	40	Aryal 2015

3.3 Development of a metal recovery process without neutralisation in a laboratory setting

The reaction period and target metal of each strain was determined as shown in **Table 5** based on their metal recovery ability and selectivity (**Table 3**). All five metal species were successfully recovered by the bacteria under both 100 and 1000 mg L⁻¹ for each metal (**Figs. 2 and 3**). The total metal recovery ratios of the process from the simulated metal leachate are shown in **Table 6**. After the process, simulated metal leachate was still strongly acidic (pH 1.7), indicating that all the strains exhibited metal recovery ability under strongly acidic conditions. These results indicate the possibility of utilising acid-tolerant bacteria for base metal recovery without neutralisation.

Under 100 mg L⁻¹ initial metal concentrations conditions, the recovered metal amount and selectivity differed from the case of the single strains (**Table 3**) except for the first strain, HTL (**Fig. 2**). The strains MS3, MnA, and Mn7 recovered Co, and significant metal recovery was not observed in the strain JCM1464. Under high (1000 mg L⁻¹) metal concentration, the metal recovery characteristics of each strain differed from those displayed under the 100 mg L⁻¹ metal concentration (**Fig. 3**).

One possible reason for these differences is the effect of bacterial components remaining in the supernatant-like soluble extracellular polymeric substances and small-molecule compounds excluded from the cell (Mwandira *et al.* 2020a). In our study, these components might have remained in the simulated waste metal leachate, affecting the metal recovery ability of the strain in the next cycle. Another possible underlying reason is the release of cations from the cells. Bacteria release cations, such as Ca, K, Mg, and Na, as a response to the adsorption or accumulation of metal ions (Huang *et al.* 2018). In this study, all tested strains except the strain HTL released some cations to simulated

metal leachate (**Table 7**). In particular, the concentration of K^+ continuously increased. The strain HTL adsorbed Mg^{2+} in addition to the tested base metals. The cations released or adsorbed by bacteria might alter the metal concentration, electric charge balance, and effects on the metal recovery characteristics of the subsequent strain.

Compared to the low metal concentration conditions (**Fig. 2**), the metal recovery abilities of strains HTL, JCM 1464, and Mn7 were increased under the high metal concentration condition (**Fig. 3**). Among them, strain JCM1464 showed the highest total metal recovery ability (179.38 mg g^{-1}). One of the possible underlying reasons is competition between metal ions and H^+ . *M. luteus* cells have been shown to adsorb metals on anionic functional groups on the cell surface (Letnik *et al.* 2017, Pipiska *et al.* 2018). Under pH 1.5 conditions, H^+ was 17.1~19.8 times more abundant than the metal ions under the 100 mg L^{-1} metal concentration condition, suggesting that competition between metals and abundant H^+ occurred and metal adsorption sites were occupied by H^+ . In contrast, under 1000 mg L^{-1} metal concentrations conditions, adsorbed metal amounts increased following the adsorption equilibrium. However, this strain requires expensive TSB medium, and its growth ability was the lowest in this study. Therefore, strain Mn7 (91.95 mg g^{-1}) was more suitable for application in metal recovery processes under high metal concentrations. Furthermore, the strain Mn7 has a large cell size and precipitates easily when stirring is stopped. A settling tank, such as those used in wastewater treatment, can potentially be used to recover the biomass.

In contrast, strains MnA and MS3 did not recover metals in significant amounts under high metal concentration conditions. This result suggests that the metal recovery capacity was saturated, and the metal concentration change of the supernatant did not exceed the measurement error of ICP-AES. Therefore, these strains are better suited for

recovery processes in leachate with lower metal concentrations. In addition, the recovered amounts of these strains were thought to be significantly smaller than the initial metal concentrations and were strongly affected by dilution error. According to previous studies, the metal concentration and pH of the e-waste leachate varies in the order of 10~1000 mg L⁻¹, depending on the experimental conditions and waste composition (Isildar *et al.* 2016, Demarco *et al.* 2019, Xu *et al.* 2022). Flexible metal recovery processes that can deal with e-waste with various components are expected to be established by identifying bacterial strains with different metal recovery characteristics.

Table 5 Order, reaction period, and target metals of bacterial strains in the sequential metal recovery process

Order	Strain	Reaction period [h]	Target metals
1	<i>Comamonas</i> sp. HTL	1.5	Cu
2	<i>Micrococcus luteus</i> JCM1464	0.5	Cu, Mn, Ni
3	<i>Pseudomonas</i> sp. MS3	0.5	Ni
4	<i>Enterobacter</i> sp. MnA	3.0	Cu, Li
5	<i>Priestia</i> sp. Mn7	0.5	Cu, Mn, Ni

Cell density was set at 2 g L⁻¹

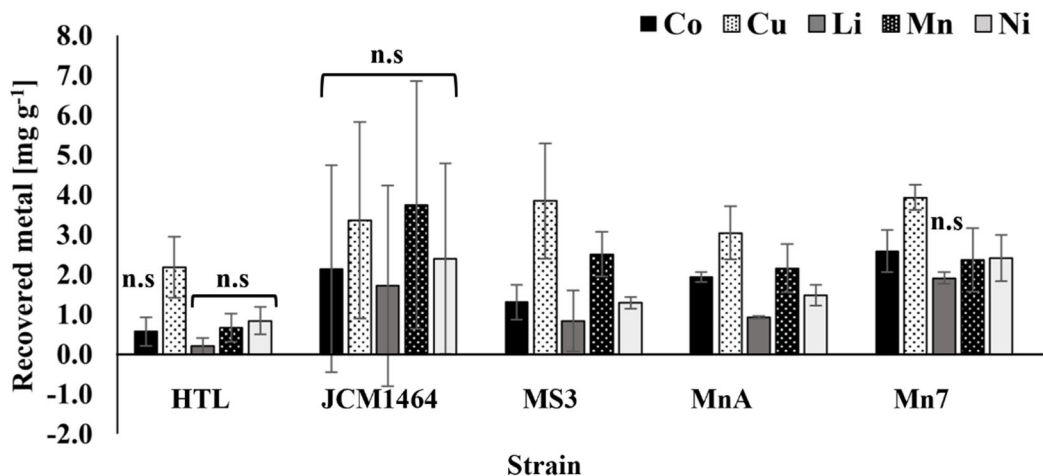


Fig. 2 Metal recovery ability and selectivity of each strain in the sequential metal recovery process at a 100 mg L⁻¹ initial metal concentration

“n.s.” indicates no significant recovery ability

Table 6 Total metal recovery rate of the metal recovery process

a) Initial metal concentration 100 mg L⁻¹

	Co	Cu	Li	Mn	Ni	Total
Initial metal [mg L⁻¹]	108.83	105.75	100.26	97.15	101.41	513.40
Recovered metal [mg L⁻¹]	9.74	22.45	9.94	5.33	13.37	60.83
Recovery rate (%)	8.95	21.23	9.91	5.49	13.18	11.85

b) Initial metal concentration 1000 mg L⁻¹

	Co	Cu	Li	Mn	Ni	Total
Initial metal [mg L⁻¹]	1112.65	982.08	848.74	1007.96	962.31	4913.74
Recovered metal [mg L⁻¹]	80.45	66.97	63.80	58.96	73.51	343.68
Recovery rate (%)	7.23	6.82	7.52	5.85	7.64	6.99

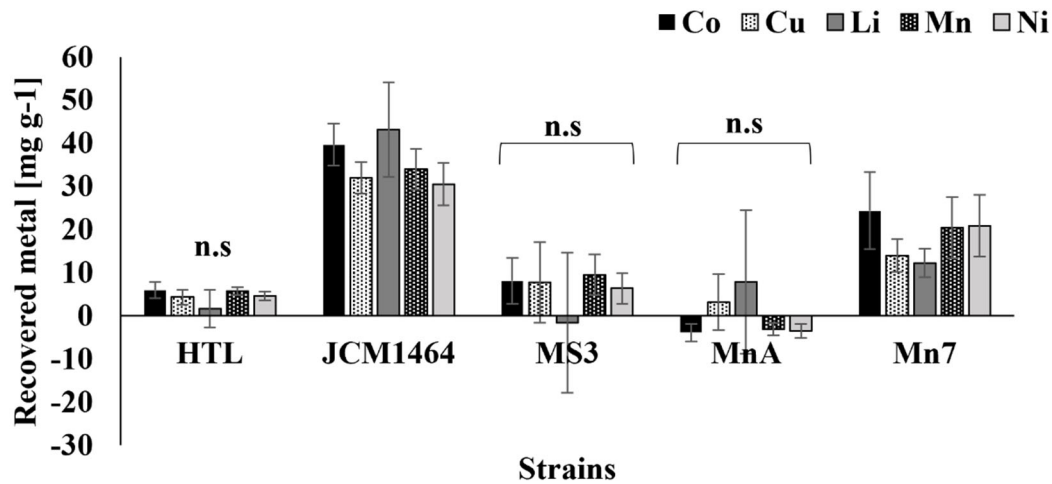


Fig. 3 Metal recovery ability and selectivity of each strain in the sequential metal recovery process at a 1000 mg L⁻¹ initial metal concentration

“n.s.” indicates no significant recovery ability

Table 7 Released cation concentrations [mg L⁻¹] during metal recovery under 1000 mg L⁻¹

¹ initial metal concentration conditions

Strain	Ca	K	Mg	Na
HTL	n.s	n.s	-5.10	n.s
JCM1464	n.s	56.68	n.s	105.45
MS3	n.s	28.47	7.07	n.s
MnA	n.s	17.42	n.s	n.s
Mn7	4.47	43.53	7.15	n.s

“n.s” indicates no significant release

“-” express absorb

3.4 Features, limitations, and further improvement of the process

The highest metal recovery ability was 21.23% (Cu) in this process. This is not as high as that with previous chemical processes such as precipitation and solvent extractions, which can recover more than 90% metals (Liu *et al.* 2021, Han *et al.* 2023). An integrated chemical recycling process of e-waste was established, combining multistage extraction of Co, Mn, and Ni using organic solvents and precipitation of Li with Na₂CO₃ (Han *et al.* 2023). More than 96% of all metal species were recovered using this method from LiB leachate. However, these complicated chemical processes require huge amounts of organic solvent and neutraliser, as well as high reaction temperature. They also require a large reactor volume for the addition of solvent or neutraliser, and this generates large quantities of wastewater (Chen *et al.* 2015, Han *et al.* 2023). Although the metal recovery ability was lower than that of the conventional processes, the proposed process decreased the consumption of neutraliser and did not entail a change in working volume during the process. Furthermore, our process is preferable in terms of environmental friendliness because it does not require high reaction temperature and organic solvents, and it utilises a bioresource that is not conventionally applied to industry.

To enhance the efficiency of metal recovery, further studies are needed to isolate novel acid-tolerant bacteria with high metal recovery ability, high biomass productivity, and ease of separation from leachate. In addition, genetic and proteomic engineering can also be effective tools for enhancing metal recovery efficiency.

Bacterial metal recovery abilities have been enhanced by the overexpression of metal-binding proteins on the cell surface (Hui *et al.* 2018). The production of a hybrid metal adsorbent using bacterial metal-binding protein and inorganic scaffold material is another possible strategy (Mwandira *et al.* 2020b). Metal-binding proteins from acid-

tolerant bacteria are promising for such efficient hybrid metal adsorbent functions under acidic conditions. Furthermore, the development of a metal desorption process and bacterial cell harvesting and reuse can aid in the development of sustainable integrated base metal recycling processes.

Considering the facilitation of industrial utilization of acid-tolerant bacteria, the clarification of metal recovery mechanisms is necessary. The SEM image indicated that the strain Mn7 formed an extracellular matrix after metal recovery (**Fig. S2**, Online resource 1). Further genetic and morphological studies will contribute additional insights into bacterial metal recovery mechanisms under strongly acidic conditions.

4. Conclusions

In this study, we proposed the utilisation of acid-tolerant bacteria for base metal recovery under acidic conditions. Acid-tolerant bacteria with metal recovery ability under acidic conditions were isolated and utilised to establish a laboratory-based process of metal recovery from simulated waste metal leachate under pH 1.5 conditions. Our process is expected to solve the pH disparity between upstream (leaching) and downstream (recovery) processes of base metal recycling from e-waste. Bypassing the neutralisation process contributes to a reduction in neutraliser consumption and wastewater production compared to chemical processes and biosorption under a neutral pH. This study is the first step towards developing a base metal recovery bioprocess under acidic conditions, although metal recovery efficiency should be enhanced. It is expected to contribute to the establishment of integrated base metal recycling bioprocesses without neutralisation. Furthermore, this study promotes the novel industrial application of acid-tolerant bacteria, a conventionally unutilised bioresource.

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