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Biotechnological valorisation of Spent Automotive Catalytic Converters: Marine microorganisms in PGE leaching and recovery

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ABSTRACT

The recovery of Platinum Group Elements (PGE) from secondary sources, such as Spent Automotive Catalytic Converters (SACC), is crucial for sustainable resource management and reducing environmental impact. Marine microorganisms offer many promising biotechnological applications, but their use in PGE bioleaching and biorecovery has been poorly explored. This study investigates the potential of SACC-tolerant marine microorganisms for platinum (Pt), palladium (Pd), and rhodium (Rh) bioleaching and their ability to recover Pt(II) from a synthetic solution containing its chloride salt. SACC media enrichments led to the isolation of 21 strains from marine coastal sediments. Screening for pH and Pt tolerance identified two acid-tolerant strains and a Maximum Tolerance Concentration (MTC) of 800 mg/l to K_2PtCl_4. The best-performing strains, belonging to the *Microbacterium* genus, could solubilise 27.3 % Pt, 8 % Pd, and 6.6 % Rh at circumneutral pH (7.0). The same microorganisms were able to bio-recover up to 92 % of the available Pt(II). These findings provide new insights into exploiting marine microorganisms in mining applications for eco-friendly and efficient SACC management and recovery.

1. Introduction

Spent Automotive Catalytic Converters (SACC) represent one of the most important secondary raw materials as a source of Platinum Group Elements (PGE), Rare Earth Elements (REE), like cerium (Ce), zirconium (Zr), and other valuable resources [1]. In Europe, the estimated number of dismantled vehicles was 4.7 million in 2022 (https://ec.europa.eu/eurostat/statistics-explained/index.php/End-of-life_vehicle_statistics), suggesting a significant production rate of SACC. SACC are honeycomb-shaped ceramics: typically, silicates, like cordierite minerals, alkaline earth element-rich glasses, or other synthesis foams, coated with catalytic elements, such as PGE, mostly platinum (Pt), palladium (Pd), rhodium (Rh), as well as Ce and Zr oxides for pollutants abatement from combustion engines' exhausts [2]. PGE are the least abundant mineral resources on Earth, while representing a strategic resource being essential players in different industries' economy, notably, automotive, jewellery, and high-tech electronics [3]. Similarly to other elemental resources of paramount importance, such as REE, their primary production and mining contracts are relatively limited [4,5]. This exacerbate the demand for new processing methods and technologies, along with the introduction and implementation of regulations and standard procedures that facilitate sustainable apportionment from primary and secondary sources [6]. Recent determinations reported the sum of Pt, Pd, and Rh in a range of 1–3 kg/t [7] or 1–15 g per single piece

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of SACC [8]. These figures are orders of magnitude higher than their average abundance in geogenic materials [9]. Hence, accurate determination of PGEs in SACC is crucial for optimizing their recovery efficiency and recycling processes, enabling sustainable resource management associated with primary mining.

Biomining, which refers to technologies that utilise biological systems, mainly microorganisms, to facilitate the extraction and recovery of elements, represents an eco-friendly and economical option, even for PGE [10,11]. The (pyro-)hydrometallurgy for the recovery of valuable elements involves energy-demanding and environmentally unfavourable processes, such as cyanidation. Novel applications based on greener reagents like HCl, H_2O_2, and biogenic cyanide [8,12,13] can mitigate most risks, in fact, except for the vast amounts of wastewater and hazardous by-products, at still high costs [14,15]. Therefore, improving the environmental performance and efficiency of remediation and traditional metallurgy through biotechnology is vital. Biorecovery and bioleaching processes can substitute or hybridise into new or existing extractive processes [16,17]. Among eukaryotic microorganisms, various fungal taxa, including *Aspergillus niger* and species from the genera *Paecilomyces* and *Penicillium*, have demonstrated bioleaching capabilities from waste materials [18]. Concerning prokaryotes, different bacterial species belonging to the genus *Acidothiobacillus* are commonly employed in bioleaching processes due to their Fe- and S-oxidising properties. These microorganisms thrive in acidic environments, making them particularly effective at degrading minerals and enhancing the efficiency of elemental recovery from natural ores and anthropogenic materials [8]. This is because pH is one of the key parameters controlling elements' mobility and speciation, greatly influencing leaching yields and affecting the recovery processes and elements' (bio)availability [19,20]. More bacteria can use the otherwise unfavourable environment to store elements or promote precipitation reactions. Although exploiting the potential of marine biodiversity fully is far from possible [21], the largely unknown biosynthetic capabilities can provide new, interesting insights for mining applications, above all at circumneutral pH, and a variety of research purposes.

This study investigates the potential of marine extremophiles in SACC mining biotechnology, which holds promises and challenges in extracting valuable elemental resources. During biotechnological explorations, main obstacles to consider for the technology transfer are especially i) the accurate separation of different state phases, ii) studied system's local inhomogeneity when dealing with a small test portion, and iii) analytical issues, such as uncertainty of the measurement result with Pt in reduced forms (e.g., [22]), like in this case. With reference to NIST SRM 2557, SACC is hygroscopic and can contain organic matter adsorbed from automotive fuel. Homogeneity assessments of NIST SRM 2557 indicated that measurements performed on samples of at least 0.1 g may be related to the certified values. In this work, extremophile marine bacteria were tested for their ability to thrive at significant concentrations of PGE (Pt, Pd, and Rh) and other Potentially Toxic Elements (PTE) from SACC, such as the releasable Al, Cd, Ce, La, Ni, and Zn [9], focusing on the pH range and Solid-to-Liquid (S/L) ratio at which the system can operate effectively. We isolated and identified bacterial strains tolerant to SACC solids and leachable PTE in the solution. Strains came from sediments sampled in the Sarno River estuary, which is known for heavy pollution [23], where the significant presence of PTE [24] can exert a natural selection of PTE-tolerant bacteria as potential candidates for SACC treatment. Testing included strains' tolerance assays, including with K_2PtCl_4 and as a function of the pH, and preliminary bioleaching of SACC, using sonicated and non-sonicated test samples. Sonicated (SACC A) and non-sonicated (SACC B) samples were used in the enrichment strategy and during PGE bioleaching primarily to obtain better information on potential candidates for SACC treatment in real-world applications, while allowing us to define the necessity or not of such a mechanical pre-treatment to improve the final performances. The final aim was to identify the most effective strains for SACC bioleaching and Pt biorecovery through an integrated approach combining biotechnological methods, such as enriched-guided selection and microbial tolerance assays, with earth science techniques, including ICP-MS, offering new insights into exploiting marine microbes for mining applications. This study adds blue innovation to the recycling of End-of-Life products like SACC, whose sustainable management is critical with the advent of electric engines in the automotive sector.

2. Materials and methods

2.1. Microbial media, SACC materials, and chemicals

Growth media used for bacteria isolation and experimental setup were: Marine Broth (MB) and Trypticasein Soy Broth (TSB) from Condalab (Madrid, Spain); International Streptomyces Project-2 Medium (ISP2): 4 g/l yeast extract, 10 g/l malt extract, and 4 g/l D-glucose; Mineral Salts Medium (MSM): 1 g/l glucose, 2 g/l NaNO3, 0.4 g/l MgSO4, 0.1 g/l $CaCl_2{\cdot}2\ H_2O$, 0.7 g/l K_2HPO_4, and 0.9 g/l Na2HPO4; modified Lysogeny Broth (LB mod): 10 g/l tryptone, 5 g/l yeast extract, 1 g/l $MgSO_4{\cdot}H_2O$, 2.0 g/l K_2HPO_4, 20 g/l NaCl, 6 g/l Tris-Base, 5 ml glycerol; M9 Minimum Medium: 0.1 g/l $MgSO_4$, 0.01 g/l $CaCl_2$, M9 salts (1X) (Difco™), Trace Elements solution (1X), supplemented with glucose 10 g/l and thiamine 0.33 g/l. All media were prepared in ultrapure water (Milli-Q Ultrapure Type 1 Water system, Millipore). For the solid media, 17 g/l of bacteriological agar (Condalab, Madrid, Spain) were added. Sodium hydroxide (NaOH) or hydrochloric acid (HCl) (Sigma Aldrich, Merck, USA) was used to adjust the pH during the experiments. Stock solution (21.17 g/l) of K_2PtCl_4 (98 % Sigma-Aldrich, California) was prepared in ultrapure water, filtered on a 0.22 μm filter, and stored in the dark at 4 °C.

The bulk SACC sample as in [9] was used. It comes from a private company serving the N-Italian collection basin of the corresponding waste category. In the present study, SACC A is obtained by sonicating and grounding the dried material into a fine sand (less than 0.2 mm), and its chemical composition is elsewhere (Table S1). SACC B is obtained through the same pre-treatment used for SACC A, except for sonication. The sonicated SACC A was used for liquid media enrichments since it is free from the excessive soot. SACC A and B were used to conduct comparative bioleaching experiments to check if sonication is a compelling sample pre-treatment.

2.2. Media enrichments and bacteria isolation

Marine sediments (surface layer, 0–3 cm) were collected in the coastal area facing the estuary of the Sarno River (40°43'38.0"N 14°27'54.0"E). The isolation was performed through media enrichment with increasing concentrations of SACC A. Exactly 100 ml of MSM were enriched with increasing concentrations of SACC A sample previously sterilised by autoclave (1, 10, 50 g/l) as the only carbon source. Subsequently, 10 % w/v of sediment was added and incubated at 20 °C, with constant shaking (120 rpm) for 7 days. The enriched bacterial community was amplified by transferring 1 ml culture into 100 ml of fresh MSM and incubated at 20 °C, with constant shaking (120 rpm) for 7 days. Each culture from all conditions were serially diluted up to 10^{-3} with sterilised seawater; thereafter, 100 μl of the dilution series were plated on four solid media (MSM, MB, LBmod, ISP2 agar plates), incubated at 20 °C, and monitored for 30 days to isolate single colonies. Colonies differing in morphology, colour, and size were selected and purified by repeated streaking and stored in glycerol stock (20 %) at −80 °C.

2.3. Taxonomic identification through 16S rRNA gene sequencing

Cell lysis was performed following different protocols according to the toughness of the bacterial strain. In detail, bacterial colonies were suspended in 200 μl TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), frozen at −20 °C for at least 1 h, and heated to 95 °C for 10 min. Then, an aliquot of the supernatant was used for PCR. When necessary,

mechanical breaking was obtained by vortexing the bacterial suspension with glass beads, or applying three freeze-thaw cycles to bacterial pellet (-70 °C in dried ice for 3 min and 95 °C for 2 min), before using glass beads [25], or three cycles with TissueLyser II (Qiagen) (1 min each at 25 Hz with glass beads). A 100 µl aliquot of the supernatant was used for further DNA extraction with GenElute™ Bacterial Genomic DNA Kits (Sigma-Aldrich).

PCR amplification of the 16S rRNA gene and ITS region was performed using the universal primers 27 F (5′-AGAGTTTGATCMTGGCTCAG-3′), and 1492 R (5′-GGTTACCTTGTTACGACTT-3′) for the first, and ITS1 Fw (5′-CTTGGTCATTTAGAGGAAGTAA −3′), and ITS4 Rv (5′-TCCTCCGCTTATTGATATGC −3′) for the Internal Transcribed Sequence (including ITS1, 5.8S, and ITS2 region) [26]. The reaction mix was obtained by combining 20 µl of XtraWhite Master Mix 2X (GeneSpin, Italy) with 1.5 µl of each primer (1 ng/µl) and different template concentrations according to the extraction process. The amplification steps included the initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 7 min. The 1.5 kb amplified 16S rRNA and the usually 500 bp ITS (ITS length is variable according to the strains [27]) fragments were run on 1 % agarose gel, and purified by a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel). The purified products were sequenced for both strands by a commercial sequencing service (Mix2Seq Service, Eurofins Genomics).

For the taxonomic identification, 16S rRNA sequences were compared by BLASTn tool with sequences deposited in NCBI GenBank (https://www.ncbi.nlm.nih.gov/) and EzBioCloud databases [28]. The identification was assessed at the genus level when the similarity percentage of the alignment was above 98 % and the e-value was below $1e^{-50}$. The nucleotide sequences of the strains from this study were deposited in the GenBank database under the accession numbers PV664838 to PV664857, and PV665017.

2.4. Tolerance assays: pH tolerance test and maximum tolerance concentration (MTC)

The assays were performed in triplicate, on 96-multiwell plates in 200 µl final volume. The plates were incubated at 20 °C under static conditions for 96 h, with a starting bacterial concentration of approximately 0.1–0.08 Optical Density (OD) (ca. 1.5 x 10^8 CFU ml^{-1}). The bacterial growth was measured through OD measurements at 600 nm (OD_{600}) every 24 h using a BioTek ELx800 Microplate reader (Agilent, USA).

In the pH tolerance test, the growth of the SACC-tolerant bacterial strains was monitored in TSB from pH 2.0–7.0. Each pH was appropriately reached by adding concentrated HCl or NaOH solutions in the medium.

Maximum Tolerance Concentration (MTC) assay was performed on the soluble salt of Pt(II) (K_2PtCl_4) at prefixed concentrations (2000, 1600, 800, 400, 200, 20, 2, 0.2, and 0 mg/l). TSB medium was used in most cases, and ISP2 was exclusive for strain H. The MTC assay was initially performed on a wide range of concentrations of K_2PtCl_4 because of the few data available in the literature, above all on marine bacteria. For this test, the stress induced by varied elemental concentrations was evaluated on the strains, assessing the growth percentage of each strain in the presence vs absence of Pt salt. For each strain, MTC was defined as the highest Pt concentration at which bacterial growth was not inhibited. Growth percentages were calculated using the OD_{600} values following the Eq. 1:

$$growth = \frac{OD\ treated\ sample}{OD\ untreated\ sample} * 100 \quad (1)$$

where "OD treated sample" corresponds to the OD_{600} after 96 h of growth at pH from 2.0 to 6.0, and "OD untreated sample" at pH 7.0 for the pH tolerance assay. For the MTC assay, "OD treated sample" corresponds to the OD_{600} after 96 h of growth with Pt soluble salt and "OD untreated sample" without it. There is confidence that OD readings are sufficient to determine the presence or absence of bacterial growth over a duration of 96 h.

2.5. Bioleaching and biorecovery experiments

Triplicate experiments were conducted in appropriate medium containing 5 % SACC pulp density, under shaking at 150 rpm for seven days. The initial bacterial concentration was adjusted to OD_{600} 0.1, and incubation performed at either 28 °C (strains named AE and V) or 20 °C (all other strains). At the end of experiments, the solids, including SACC sample powder and microbial biomass, were separated from the liquid fraction (leachate) by centrifugation at 3600 g at 4°C for 15 min using a refrigerated benchtop centrifuge Sigma 3–18KS. The leachates and solids were stored separately in 50 ml tubes at −20 °C for inductively coupled plasma mass spectrometry (ICP-MS) analysis. Pt, Pd, and Rh bioleaching from SACC A and B samples was tested on a group of promising candidates among SACC-tolerant strains by analysing solids (residual precipitate from SACC A or B experiments, after appropriate digestion) and leachates from the experiments. The bioleaching efficiency was calculated on the corresponding solid. Moreover, it was referred to total analyte recovery from bulk SACC [9] and NIST SRM 2557 for comparison.

Biorecovery experiments were performed at 20 °C for 96 h on TSB or ISP2 enriched with 100 mg/l of K_2PtCl_4 (corresponding to approximately 47 mg/l of Pt in the solution). Microbial growth was set up as previously described. The leachates and solids (microbial biomass) were separated by centrifugation at 3600 g for 10 min at 4 °C and stored at −20 °C for analysis. The biorecovery (percentage of element recovery) was calculated considering the leachates and corresponding microbial biomass in the mass balance calculation. Assessment of bioleaching and biorecovery efficiency followed the method described in literature [29, 30].

2.6. Chemical analysis of solid and liquid samples

All leachates and digested solutions of solids were analysed after appropriate dilution on a Perkin Elmer ELAN DRC-e ICP-MS, at the "Bruno Capaccioni" Geochemistry Laboratory of the BiGeA Department. The measurements were checked using calibration blanks, procedural blanks, and a 50 µg/l internal standard (in 5 % HNO_3) for each analyte. Solids from biorecovery experiments (microbial biomass) were digested using aqua regia at a S/L ratio of 1:10 followed by 10 µl H_2O_2 (H_2O_2 solution 30 %, Carlo Erba) on a hot plate until the solution was clear. Solids from bioleaching experiments (SACC sample powder and microbial biomass) were digested in the same way, considering that the pseudo-total digestion can dissolve most elements of interest, likely attacking the catalytic wash coat of car catalyst waste and leaving behind its ceramic substrate resistant to strong acid attack. Ultrapure water was used for blanks and dilution of digested samples and standard solutions. Samples and procedural blanks were prepared in the same way for instrument tuning and subtraction of blanks and control samples. In bioleaching experiments (see main text and Table S3), the percentage of elemental removal provided in the main text was calculated using the following Eq. 2:

$$R = \frac{Ci - Cf}{Ci} * 100 \quad (2)$$

where R is the removal percentage, C_i is the initial concentration of the element in the solid, and C_f is the final concentration of the same element in the leachate.

2.7. Data analysis

All biological experiments were performed in triplicate, if not otherwise stated. Data elaboration accompanied by basic descriptive statistics and, when possible, ANOVA. Graphs were generated using GraphPad Prism ver. 9.5.1 for macOS (GraphPad Software, San Diego, CA, USA, www.graphpad.com) and MS Excel for WindowsOS.

3. Results and discussions

3.1. Geochemical characterization of samples and media

As an anthropogenic material and unlike geogenic ones, SACC include elements unusually enriched (Table S1). ICP-MS analysis of TSB and ISP2 used for microbial growth in bioleaching experiments showed that they contain Al, Fe, Mg, and Zn at noticeable concentrations. The low concentrations of PGE in the media, TSB and ISP2, may be related to external contamination. These figures, albeit considered in the mass balance calculation, are negligible given the expected ranges of elemental concentration in SACC, which differ, at least, by one order of magnitude. In other words, during bioleaching and biorecovery experiments PGE are in the order of tens ppm. Table 1 reports the chemical concentrations determined in media used and observed variability. This is consistent with the element solubility of SACC in deionized water, of which selected data are also shown in Table 1 reported from [9]. We have no data on NIST SRM 2557 leaching in deionized water, but we can expect higher Pb and Ni releases because SRM is a pre-1993 sample of SACC and slightly differs from current SACC, especially for Pb and Ni (Table S1). This can denote the change before restrictions on heavy fuel burning, so the experiments on SACC of the present study can be more representative of current SACC treatment compared to the use of available standard reference materials.

Interestingly, the culture media alone leached PGE from the SACC samples under controlled conditions without influence of biological systems at circumneutral pH. Figure S1 shows Pt, Pd, and Rh leaching of SACC A and B subjected to five parts of culturing media (TSB and ISP2). Leaching efficiency is low, but it can be relevant to Pt with TSB in SACC B. This might relate to the presence of elemental PGE as loose particles in the soot of SACC B (not sonicated sample), as reported elsewhere [9].

3.2. Sarno sediments host bacterial genera tolerant to SACC

Estuarine marine sediments at the mouth of the Sarno River are characterized by the presence of PTE and organic contaminants [31,32], favouring the natural selection of microorganisms able to tolerate various types of contaminants. Besides high PGE concentrations, the chemical analysis of SACC samples (Table S1) showed high concentrations of PTE, such as Ni, Pb, and Zn, increasing the potential for selecting strains capable of using or tolerating such elements at significant concentrations. Therefore, Sarno sediments were dissolved in media enriched with SACC samples to improve the selection of strains of interest. This process allowed the isolation of 21 marine strains (based on different morphology, colour, and size) able to grow in the presence of SACC A (Table 2). Most of the strains were isolated on plates including 10 g/l of SACC A, except strains AE and H isolated on plates containing SACC A at the concentration of 50 g/l. After morphological analysis, 16S rRNA and ITS gene sequencing identified eight genera, seven belonging to the Bacteria kingdom and one, *Pichia* sp., belonging to the Fungi kingdom. Interestingly, there are no reports in the literature regarding the relationship between most of the genera identified in this study and PGE. The available information mainly related to PTE-microbe interactions, thus the PTE bioremediation potential [33].

Table 1
ICP-MS analytical determinations of TSB and ISP2 culturing media ($n = 3$). Values are in μg/l, and intermediate precision is expressed as standard deviation (SD) in parentheses. For comparison, SACC W is the leaching data of SACC subjected to deionized water leaching (Funari et al., 2024). The confidence interval is within 10 % or as recommended for SACC-type SRM.

	TSB	ISP2	SACC W
Al	< 1	15000	9000
Fe	< 1	48000	3000
Mg	313000	1248000	300000
Pb	< 1	< 1	1000
Ni	< 1	913	1000
Zn	9000	118000	3000
Ce	< 1	< 1	3500
La	< 1	< 1	6000
Pt	43(±58)	0.23(±0.21)	50(±6)
Pd	23(±21)	0.4(±0.36)	< 1
Rh	1.2(±2.08)	< 1	< 1

Ten isolated strains are included in the genus *Acinetobacter*, which alongside the genera *Shewanella,* and *Pseudomonas*, belongs to the Phylum Pseudomonadota, Class Gammaproteobacteria, a large and heterogeneous group of microbes. *Acinetobacter,* despite its pathogenic potential [34], is also known to contribute to the mineralization of various aromatic compounds [35], and reduction of PTE [36,37]. Among the isolates, one colony was affiliated with the genus *Shewanella* and one with the genus *Pseudomonas. Shewanella* is a genus with high yield and a highly adaptable metabolism, able to thrive in diverse environments [38]. It belongs to the group of dissimilatory metal-reducing bacteria capable to use a wide range of elements, including Pt and Pd, as terminal electron acceptors in respiration [39,40]. Its metal-reducing proteins are also used for nanoparticle biosynthesis [41]. This makes *Shewanella* a genus frequently used for water and soil purification, microbial fuel cells, and bio-metallurgical processes. *Pseudomonas* spp. are well known for their metabolic diversity, and some species can degrade polycyclic aromatic hydrocarbons [42].

Among the SACC-tolerant strains, four belonged to the genus *Microbacterium*, two to *Rhodococcus,* one to *Tsukamurella*, and one to *Pseudonocardia* sp. These four genera fit into the Actinomycetota (or Actinobacteria) Phylum, Class Actinomycetia. *Microbacterium* spp. are members of a genus widespread in many diverse habitats, marine included [43]. They present extracellularly secreted enzymes and proteins that found application in the biosynthesis of precious elements, Au and Ag, nanoparticles [44] or bio-emulsifier production for remediation purposes [45,46]. *Rhodococcus*, *Nocardia*, and *Tsukamurella* are a subgroup of related genera rich in mycolic acid, and some strains are even lysozyme resistant [47]. *Rhodococcus* can live in a large variety of environments and possesses great commercial potential. Of particular interest is its ability to produce mineral nanostructures, degrade a wide range of organic compounds, and resist high concentrations of PTE, offering, so far, the possibility to efficiently recover valuable resources and obtain high value-added products [48]. The enzymatic toolkit of *Tsukamurella* and *Pseudonocardia* can degrade and metabolize a wide range of substances, including PTE [49] and hydrocarbons [50,51]. All the isolated strains, especially those belonging to the actinomycete class, can represent an uncharacterized source of natural products (metallophores, biosurfactants) that can serve as useful tools in the biomining and bioremediation sectors.

3.3. Acid- and Pt-tolerant strains among the SACC-tolerant Sarno isolates

The SACC natural pH lies around 8.0 for gasoline catalysts and 4.0–6.0 pH for diesel ones. The mineralogy of the ceramic support, which can be of different origins, further influences the pH of the leachates [52]. In a bio-hydrometallurgical approach, it is convenient to have microorganisms tolerant to a wide range of pH at choice. SACC (undifferentiated) naturally tend to acidify the pH of water at around pH 5.0, but satisfactory PGE leaching is usually obtained at pH 2.0 in the traditional routes. The capacity of the 21 isolated strains to tolerate acidic conditions was evaluated by pH tolerance test (Fig. 1). As expected for marine bacteria, SACC-tolerant bacterial strains resulted in an

Table 2
List of 21 SACC-tolerant marine strains isolated from the Sarno sediments displaying the Strain ID, the concentration of SACC (g/l) from which the colony was isolated, the isolation medium, the genus identified by 16S rRNA sequencing, the Accession Number (AN) available on GenBank from 15/12/2025, and the sequence similarity (%) obtained by blast on NCBI and EzBioCloud.

Strain ID	SACC concentration (g/l)	Isolation medium	Identification by 16S rRNA	Accession Number (AN)	Sequence similarity (%)	
					NCBI	EzBioCloud
Phylum Pseudomonadota						
Class Gammaproteobacteria (Gram negative)						
AC	10	MSM	*Acinetobacter* sp.	PV664838	99.7	99.3
AD	10	MSM	*Acinetobacter* sp.	PV664839	99.3	98.1
AA	10	ISP2	*Acinetobacter* sp.	PV664840	99.9	98.9
B	10	ISP2	*Acinetobacter* sp.	PV664841	99.7	99.0
C	10	ISP2	*Acinetobacter* sp.	PV664842	99.7	99.1
E	10	MB	*Acinetobacter* sp.	PV664843	99.8	99.0
F	10	MSM	*Acinetobacter* sp.	PV664844	99.8	98.6
I	10	ISP2	*Acinetobacter* sp.	PV664845	99.7	98.8
O1	10	ISP2	*Acinetobacter* sp.	PV664846	99.7	99.4
U	10	MB	*Acinetobacter* sp.	PV664847	99.7	99.3
L	10	MB	*Shewanella* sp.	PV664848	99.7	99.6
N1	10	MB	*Pseudomonas* sp.	PV664849	99.6	98.4
Phylum Actinomycetota						
Class Actinomycetia (Gram positive)						
T2	10	MB	*Microbacterium* sp.	PV664850	99.3	99.0
R1	10	ISP2	*Microbacterium* sp.	PV664851	99.8	98.4
R2	10	ISP2	*Microbacterium* sp.	PV664852	99.5	98.0
AG	10	LB mod	*Microbacterium* sp.	PV664853	99.7	98.7
Y	10	ISP2	*Rhodococcus* sp.	PV664854	99.8	98.8
AH	10	LB mod	*Rhodococcus* sp.	PV664855	99.7	99.0
					NCBI	EzBioCloud
AE	50	ISP2	*Tsukamurella* sp.	PV664856	99.6	99.5
PZ	10	LB mod	*Pseudonocardia* sp.	PV664857	99.4	98.4
Phylum Ascomycota						
Class Saccharomycetes (yeast)						
H	50	LB mod	*Pichia* sp.	PV665017	100	

average tolerance range between pH 6.0–7.0. Fig. 1 shows that most strains (17 out of 21) can easily tolerate a pH value of 5.0, which can be a suitable pH for SACC A and B treatment. At pH 4.0, growth was observed for *Pichia* sp. H and *Tsukamurella* sp. AE. Moreover, *Pichia* sp. H was the only one tolerating the entire pH window tested, including pH 3.0 and 2.0. According to the growth performances of the 21 strains, *Shewanella* sp. L resulted the fastest growing strain, while *Pseudonocardia* sp. PZ growth was the slowest (Fig. 1, pH 7.0).

In general, it is possible to observe a longer lag phase for all the bacterial strains at pH 5.0 (Fig. 1), compared to higher pH. This trend was even more marked at pH 4.0, where *Tsukamurella* sp. showed an acclimation phase until 72 h. However, *Tsukamurella* sp. is a strict aerobic genus growing at the liquid-air interface, therefore the spectrophotometric measurement of OD_{600} can only qualitatively evaluate the growth for these flocs-forming strains. The growth of the two acid tolerant strains in the presence of pH stress was not drastically affected even at the lowest pH values. *Pichia* sp. H showed a growth percentage of 64 ± 11 at pH 2.0 compared to pH 7.0, which represents the optimal growth. *Tsukamurella* sp. AE at pH 4.0 showed a growth of 64 ± 16 % compared to the control (Fig. S2). So far, the lower pH tolerated by the two strains has not reduced their growth by more than 40 %. The genus *Pichia* sp. is known from the literature to tolerate a wide range of pH, including the more acidic ones [53–55]. For its robustness to a plethora of harsh conditions, like salinity, temperature, and pH, it is a well-assessed model for metabolic engineering and a possible candidate for bio-hydrometallurgical applications. *Tsukamurella* sp. AE and *Pichia* sp. H strains tolerance to acidic conditions substantiated the choice of using 50 g/l SACC in serial enrichments.

Element cytotoxicity is an important variable to consider when working with bio-based treatments for anthropogenic materials like SACC. Platinum is well known to exert toxic effects, but mainly dependent on its speciation [56]. The soluble salt forms, i.e., K_2PtCl_4, can be dangerous not only in animal systems, but they can also be genotoxic in bacteria [57]. An exploratory bioassay was conducted to determine the bacterial tolerance to Pt in its soluble salt form of K_2PtCl_4. The tolerance assay highlighted that the strains isolated from Sarno River sediments exhibited an MTC value of 200 mg/l for seven out of 21 strains, with a growth percentage ranging from 48 % to 112 %. The other 14 strains had an MTC value of 20 mg/l, with a growth percentage in the presence of Pt ranging from 79 % to 138 % compared to the control sample (Fig. 2). Low concentrations of K_2PtCl_4 enhanced microbial growth of a few strains belonging to *Acinetobacter* and *Rhodococcus* spp. In non-microbial models, some PTE, including Pt, can stimulate biological activity at low concentrations [58]. This phenomenon, known as hormesis, describes a dose-response effect elicited by a harmful substance in which low concentrations are beneficial while higher concentrations are inhibitory. The obtained MTC of 200 mg/l for K_2PtCl_4 demonstrates a high level of tolerance to Pt in the tested strains. While MTC values can vary depending on Pt salts used, previous studies have reported values such as 0.75 mg/l for $H_2PtCl_6{\cdot}6\ H_2O$ [59], 366 mg/l for $PtCl_4$ [60], and 5 mg/l for doubly negatively charged $PtCl_6$ [61], highlighting the influence of Pt chemical speciation and experimental conditions.

A second MTC assay was conducted on a narrower range of K_2PtCl_4 concentrations (1600, 800, 400, and 200 mg/l) for the seven strains tolerating 200 mg/l. The results confirmed the MTC value of *Pseudonocardia* sp. PZ and set the MTC value at 400 mg/l for *Rhodococcus* sp. Y and *Pseudomonas* sp. N1. The highest tolerance to Pt(II) was reached by *Pichia* sp. H, *Microbacterium* spp. T2 and AG, and *Rhodococcus* sp. AH, with an MTC value of 800 mg/l for K_2PtCl_4, exhibiting growth percentages from 79 to 100 (Fig. 3).

3.4. Bioleaching ability toward PGE like Pt, Pd, and Rh

Based on the tolerance to SACC (g/l) (Table 2), pH growth range and performance (Fig. 1), and K_2PtCl_4 tolerance (Figs. 2–3) of the Sarno river isolates, eight strains were selected as best candidates for the bioleaching test on SACC A and B samples. In detail, *Pichia* sp. H and

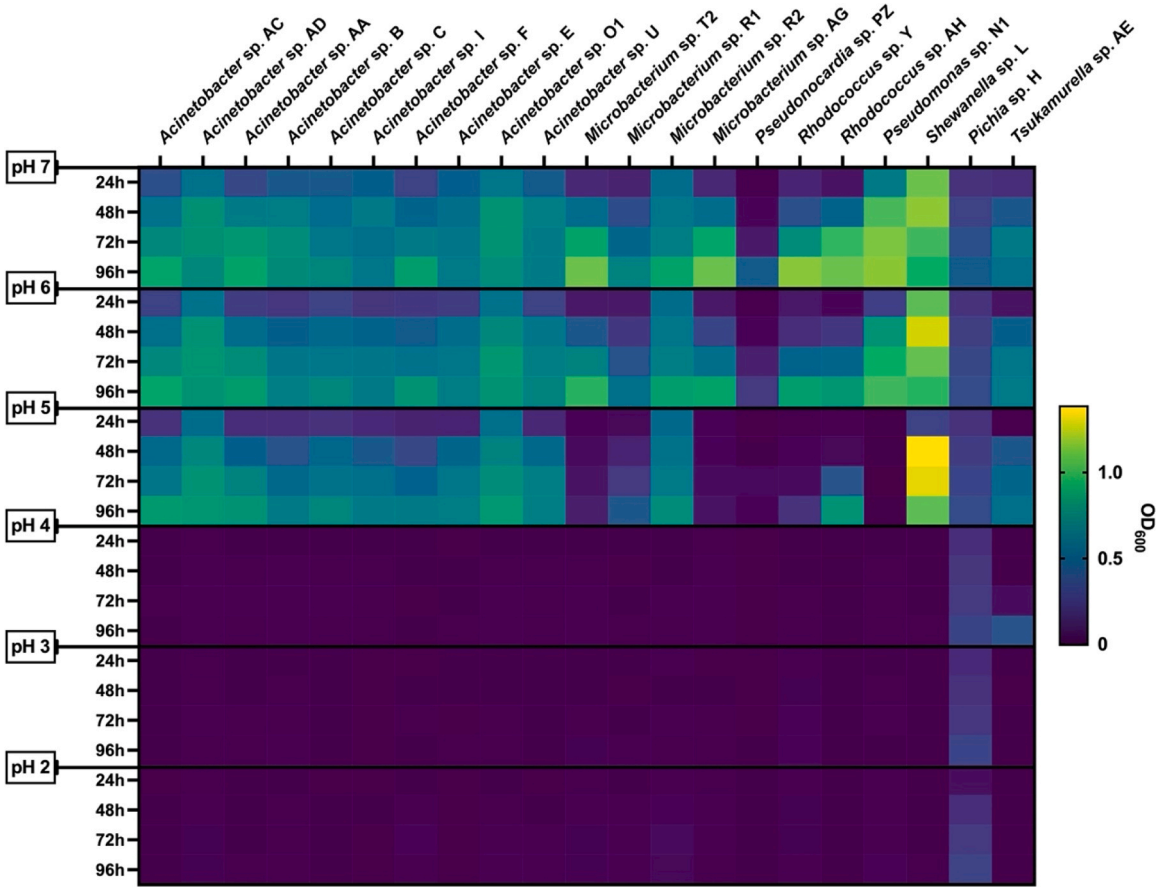

Fig. 1. Heatmap of the bacterial growth (OD 600 nm) along a 96 h time course indicative of the pH tolerance of the 21 SACC-tolerant strains. Bacterial growth was evaluated at pH 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0).

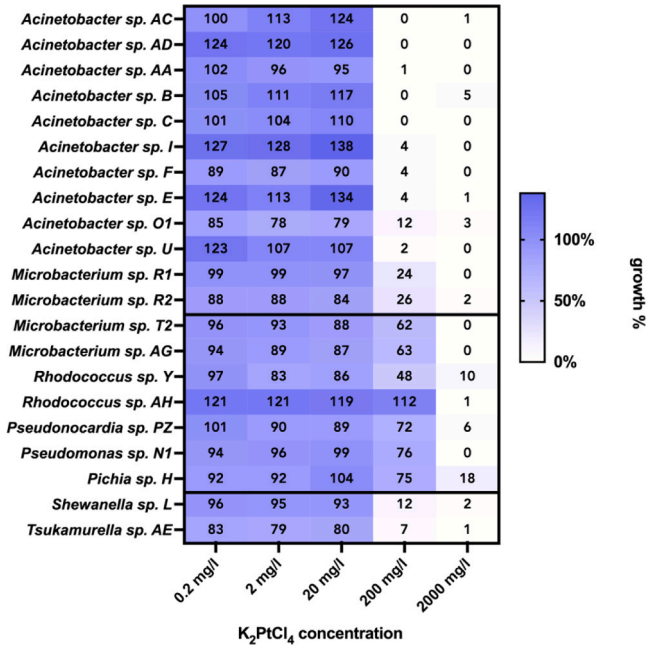

Fig. 2. Bacterial growth percentage at concentrations of K_2PtCl_4 from 0.2 to 2000 mg/l for the 21 SACC-tolerant strains, after 96 h of growth.

Tsukamurella sp. AE were selected as the only isolates obtained from 50 g/l SACC waste selective enrichments and, consistently, the only tolerant to more acidic pH. The strain *Pichia* sp. H was also selected, together with *Microbacterium* spp. T2 and AG, *Rhodococcus* spp. Y and AH, and *Pseudomonas* sp. N1, for their tolerance to 200 mg/l K_2PtCl_4 and growth ability (at 5–6 pH). Growth performances of the strains were also considered as fast-growing bacteria offer important advantages in bio-based reactor processes. *Shewanella* sp. L was selected as exhibiting the highest growth rate and a dissimilatory metal-reducing metabolism; on the contrary, *Pseudonocardia* sp. PZ was excluded due to its low growth rate.

PGE leaching yield was estimated for Pt, Pd, and Rh in the SACC samples, considering also possible differences between SACC A and B. ICP-MS analysis of TSB and ISP2 media used for bacterial growth showed that their PGE concentrations are very low (Table S2). Table 3 reports the analytical recovery of mass fractions from the strains' leachates at circumneutral pH. Interestingly, the tested bioleaching conditions resulted in maxima of 69 μg Pt with *Microbacterium* sp. T2, 12 μg Pd with *Microbacterium* sp. AG, and 1 μg Rh with *Microbacterium* spp. T2 and AG, and *Tsukamurella* sp. AE. PGE bioleaching was more effective from SACC A than SACC B. Obstacles in leaching arose particularly using SACC B. Likely, this is due to an enhanced soot content (and toxicity) of SACC B compared to SACC A [9] that can impede microbial activities.

Fig. 4 shows the leaching percentages derived from the mass of corresponding SACC A and B recovered (pseudo-total digestion of separated solids). In SACC A, the highest Pt-bioleaching efficiency for *Microbacterium* sp. T2 equalled 27.3 %, while Pd and Rh were at 8 and 6.6 %, respectively, in the presence of *Microbacterium* sp. AG. In SACC B, maximum Pt-bioleaching efficiency was 5 % in the presence of

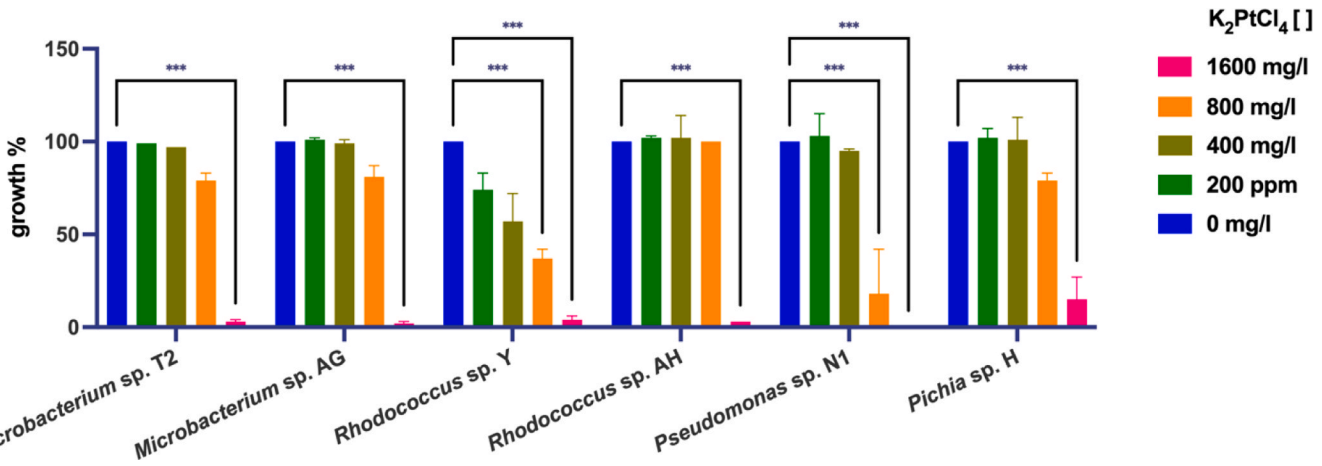

Fig. 3. Bacterial growth percentage at concentrations of K_2PtCl_4 from 200 to 1600 mg/l for the six more tolerant strains, after 96 h of growth. Statistical significance $p = 0.001$ is reported on the graph as ***.

Table 3
Pt, Pd, and Rh mass fractions in μg (as max-min values of analytical recovery). Min-max values of PGE mass fractions are obtained from strain's leachates of SACC A and B at circumneutral pH (in 20 ml test volume).

	μg Pt (*min-max*)	μg Pd (*min-max*)	μg Rh (*min-max*)
ISP2 medium			
H, leachate from SACC A	0.3–1	0.1–1	0–0.4
H, leachate from SACC B	0.3–1	0–0.1	0–0
TSB medium			
T2, leachate from SACC A	30–69	1–10	0.3–1
T2, leachate from SACC B	7–17	0.5–1	0–0.2
AH, leachate from SACC A	2–17	2–3	0–0.3
AH, leachate from SACC B	20–23	1–2	0.1–0.2
N1, leachate from SACC A	9–22	2–6	0–0.6
N1, leachate from SACC B	7–13	0.7–1	0–0.1
L, leachate from SACC A	4–40	0.1–4	0–0.7
L, leachate from SACC B	14–22	1–2	0–1
AG, leachate from SACC A	35–48	5–12	0.5–1
AG, leachate from SACC B	15–41	0.6–3	0–0.4
Y, leachate from SACC A	19–50	4–10	0.1–0.4
Y, leachate from SACC B	28–50	0.2–3	0–0.2
AE, leachate from SACC A	16–18	4–7	0.6–1
AE, leachate from SACC B	5–9	0–1	0–0.1

Rhodococcus sp. Y, while Pd and Rh bioleaching can be considered insignificant. Some factors, such as small test portions (20 ml), the system's local inhomogeneity, and the wide range of expected concentrations are the main drivers of observed variability (Table S2). In general, SACC bioleaching performed well with strains of *Microbacterium* spp., T2 and AG, and *Rhodococcus* sp. Y according to PGE achievable bioleaching.

The bioleaching performance of SACC A vs SACC B also indicates that removing the soot particles may enhance bacterial leaching from SACC. Bioleaching performances at circumneutral pH are very limited when recovery yields are calculated based on leachates (measured in this study) and total solids (NIST SRM 2557 and SACC A from Funari et al., 2024) concentrations (Table S3). However, we demonstrated SACC bioleaching capabilities of strains that thrive at circumneutral pH and tolerate slightly acidic conditions under feasible and biologically favourable conditions. Such conditions are far from conventional hydrometallurgical settings. Moreover, using pseudo-total digestion data of SACC A and B solids, as shown in Fig. 4, would allow a more accurate appraisal of leaching performance for the tested strains, hypothesising that they can combine with traditional hydrometallurgy (at some processing phases, e.g., before thermal treatments or after strong acid attacks). The 20 ml volume is used for a lab-scale experiment, and there is a potential performance decline with upscaling. Bioleaching studies must be practiced on a bigger scale for industrial rollout.

3.5. Pt biorecovery ability

The Pt bio-recovery potential was investigated on a synthetic Pt-rich solution with 0.2 mM K_2PtCl_4 salt, corresponding to 47 mg/l of Pt(II).

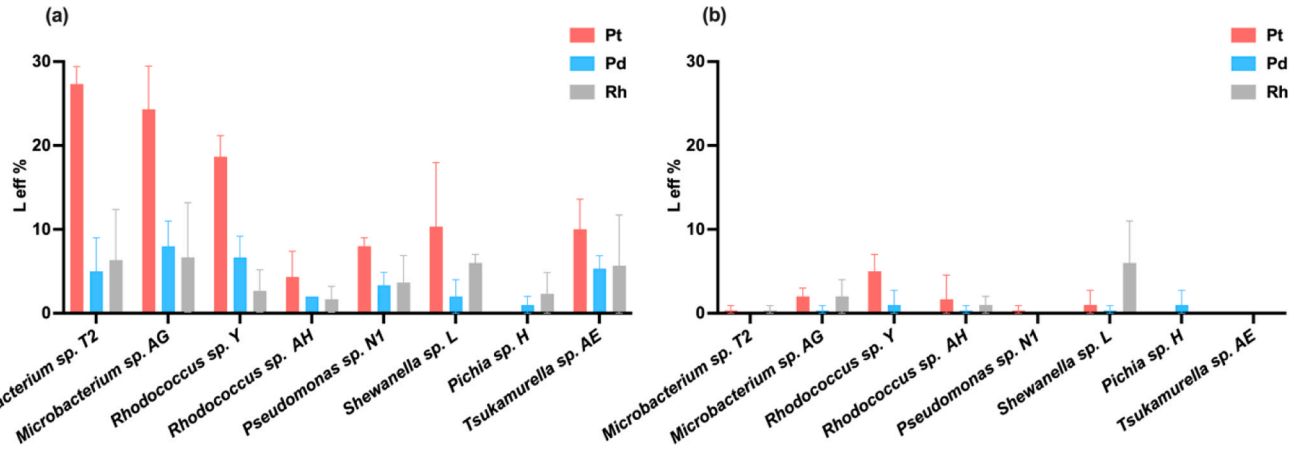

Fig. 4. Bioleaching efficiency (L eff %) of PGE from SACC A (a) and SACC B (b) operated by the strains isolated for SACC treatment purposes. Calculation is based on Eq. 2 with pseudo-total digestion data for solids.

For the strain's selection, it was primarily considered the impact of the physicochemical stressor. Four strains, *Pichia* sp. H, *Microbacterium* spp. T2 and AG, and *Rhodococcus* sp. AH, were used for their 800 mg/l MTC with K_2PtCl_4 and their optimal growth performances at alkaline-neutral pH (Fig. 1). At neutral pH the Pt salt is totally dissolved in bioavailable Pt(II), and the bacterial membrane more negatively charged with deprotonated functional groups enhancing the electrostatic attraction of cations [62]. Recovery percentage was calculated by analysing Pt in the supernatant and solids (Table S4). Platinum biorecovery was achieved by all four tested strains (Fig. 5), with *Pichia* sp. H showing less than 50 % recovery, and up to 92 % recovery for *Microbacterium* spp., AG and T2, and *Rhodococcus* sp. AH. Significant PGE concentrations were determined in the solid phase, i.e., comprising the separated biomass with any precipitate, confirming the active biorecovery potential of these strains. Based on the results of such targeted experiment, we hypothesise that biosorption, bioaccumulation, or bioreduction are the most probable mechanisms of Pt-microbe interaction.

Optimal sorption of elements occurs at intermediate to high (5−8) pH, depending on the bacterial species [63–66], being mostly untested for PGE. Typically, governing parameters are pH, salt concentrations, the impact of physicochemical stressors, and elemental speciation [67]. It is also worth mentioning that mixed cultures are more employed than

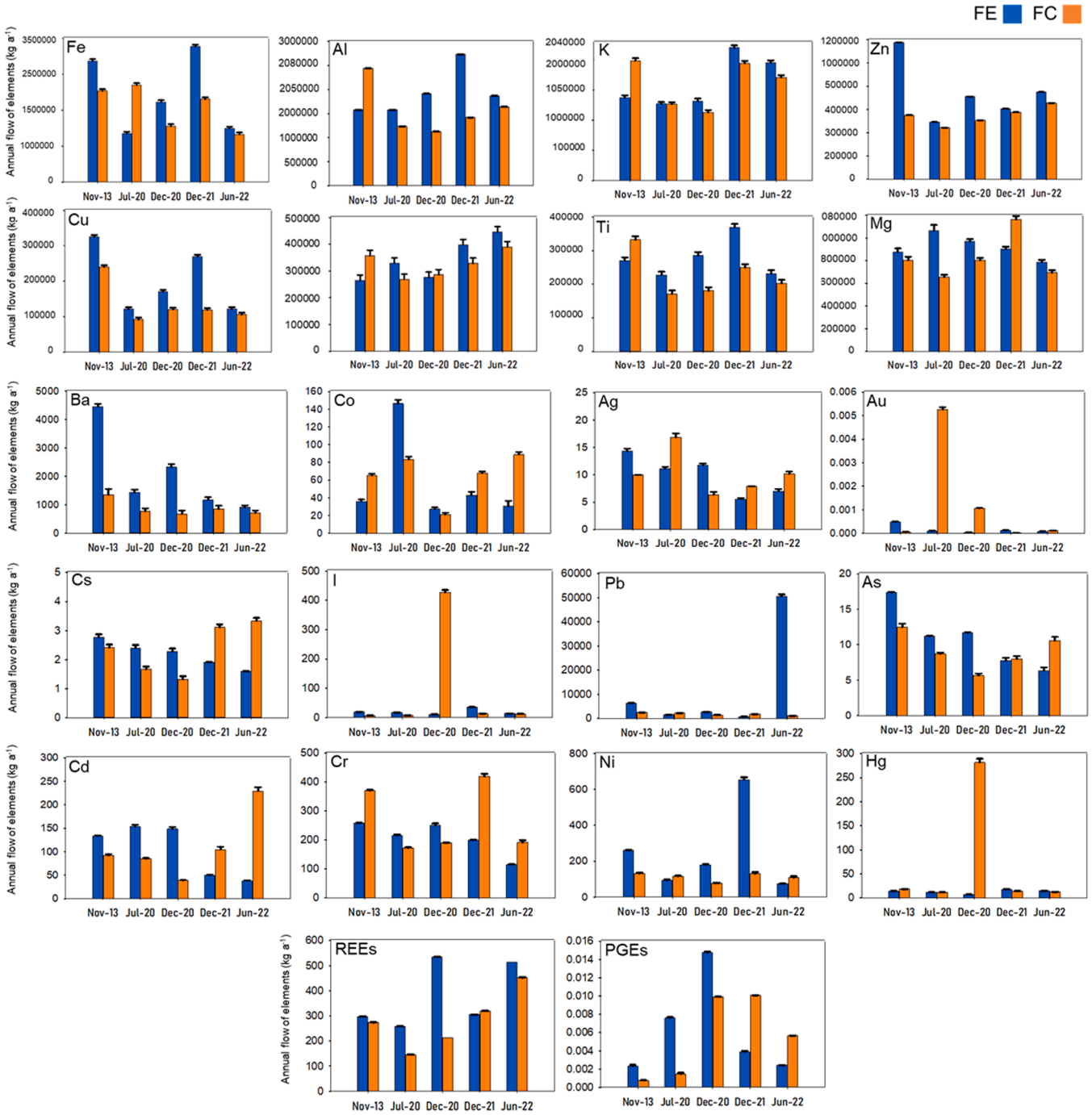

Fig. 5. Pt Biorecovery percentages of about 47 mg/l Pt in solution from culturing broth, after 96 h contact time. Biorecovery is given as percent difference between element concentration in the solid (microbial biomass and any associated precipitates) and the initial concentration of soluble Pt. The variation of biorecovery percentages as error bars of duplicated experiments for each strain.

pure colonies because their synergistic effects can improve survival and activities, naturally. In biofilms, complex communities have nanowires and multistep electron hopping (in which electrons jump from cell to cell towards the mineral), which have been suggested as direct reducers [68]. Pt recovery under neutral pH and in a clean matrix will be different from practice: industrial process streams have challenging conditions, such as, high salt concentrations, low pH (pH < 3.0), and elevated ammonium levels, which generally favour PGE refinery processes [39]. Assessing bacteria for their biomining potential should tailor to the element or group of elements wanted, and include the ability to exploit pH, temperature, PTE concentrations, and synergies of colonies of different species.

High growth rates and cell densities (of cells reacting with Pt) will increase the metal recovery capacity in a biologically driven reactor technology [67]. In this study, *Microbacterium* sp. AG and T2, and *Rhodococcus* sp. AH proved to be the most promising strains for SACC biotreatment based on measurable performances. Future projects will focus on the understanding of PGE-microbe interaction mechanisms operated by the best-performing strains found here, by means of an accurate determination (e.g., FTIR/XPS data) of biomolecules and cellular compartments involved in PGE binding. Table S5 synthesises our strain selection approach for the identification of the best candidates, with scores assigned based on empirical observations.

4. Conclusions

Examples of bio-mediated processes in hydrometallurgy have been widely developed in recent years. However, the use of marine microorganisms for such purposes is limited and represents a novel approach for PGE recovery. This study pioneers bioleaching and biorecovery of PGE (Pt, Pd, and Rh) from SACC powders with customized and scalable operative conditions in a controlled environment. A highly polluted marine area, such as the Sarno River, provided the site for the isolation of microorganisms capable to resist SACC powders, up to a concentration of 50 g/l. Since PGE have the highest leaching yield at acidic pH, acid-tolerant microorganisms are more suitable for hydrometallurgical applications. Interestingly, in this study two acid-resistant microorganisms were isolated, *Pichia* sp. H and *Tsukamurella* sp. AE. Considering that neutrophilic microorganisms have developed alternative mechanisms to interact with ionic species of elements, we found that *Microbacterium* spp. T2 and AG, and *Rhodococcus* sp. AH, are the best and promising for this purpose, incorporating most available Pt from simulated Pt-rich solutions with K_2PtCl_4 salt. Moreover, *Microbacterium* sp. T2 and AG, and *Rhodococcus* sp. Y and AH were the best bioleaching strains, using the SACC with low soot content. Therefore, a sonication pretreatment should be required to apply these marine bacteria in primary bioleaching processes and their ability investigated also for alkaline extraction. Moreover, because strains' ability to incorporate PGE is remarkable their bioaccumulation efficiency can be finely exploited in secondary processing of enriched waters, and their efficient recovery from spent converters is a valuable secondary resource that supports environmental and economic sustainability. In large-scale applications, critical factors like microbial stability, cost control, and compatibility with existing industrial processes must be considered. Nonetheless, these solutions should be developed because they are sustainable compared to traditional processes that can have a negative effect on the ecosystem.

CRediT authorship contribution statement

Conceptualization: V.F. Methodology: V.F., L.V., D.C., C.G., G.A.V., P.T., E.D., T.C.M. Writing — original draft preparation: L.V. Writing — review and editing: L.V., D.C., P.T., C.G., G.A.V., S.B., G.V., F.P.E, G.D.S, J.G., E.D., V.F. Supervision: V.F., D.d.P., T.C.M., E.D.

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2025.118729.

Data availability

Data will be made available on request.

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