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Bioleaching of Gold and Silver from Waste Printed Circuit Boards by *Pseudomonas balearica* SAE1 Isolated from an e-Waste Recycling Facility

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Abstract Indigenous bacterial strain *Pseudomonas balearica* SAE1, tolerant to e-waste toxicity was isolated from an e-waste recycling facility Exigo Recycling Pvt. Ltd., India. Toxicity tolerance of bacterial strain was analyzed using crushed (particle size ≤150 µm) waste computer printed circuit boards (PCBs)/liter (L) of culture medium. The EC_{50} value for SAE1 was 325.7 g/L of the e-waste pulp density. Two-step bioleaching was then applied to achieve the dissolution of gold (Au) and silver (Ag) from the e-waste. To maximize precious metal dissolution, factors including pulp density, glycine concentration, pH level, and temperature were optimized. The optimization resulted in 68.5 and 33.8% of Au and Ag dissolution, respectively, at a pH of 9.0, a pulp density of 10 g/L, a temperature of 30 °C, and a glycine concentration of 5 g/L. This is the first study of Au and Ag bioleaching using indigenous e-waste bacteria and its analysis to determine e-waste toxicity tolerance.

Keywords Bioleaching · *Pseudomonas balearica* · Printed circuit boards · EC_{50} · Gold · Silver

Introduction

Over the past decade, an exponential increase in electronic waste (e-waste) generation caused by the high consumption of electronic devices and their early obsolescence has been observed [24]. Globally, 41.8 million tons of e-waste was generated in 2014, and the amount of e-waste has been predicted to rise to 50 million tons by 2018 [3]. The disposal of e-waste has raised environmental and health concerns due to the presence of hazardous and toxic components (i.e., lead, cadmium, mercury, beryllium, and polybrominated diphenyl ethers) [22, 33]. On the other hand, e-waste has been treated as a "secondary ore" in "urban mining" due to the presence of precious metals (e.g., Au, Ag, Pd) [11]. The concentration of precious metals in the e-waste of printed circuit boards (PCBs) is higher than that in primary ores; for example, the ore deposit of both Ag and Au is <10 g/ton compared to the PCB deposits of Ag and Au at 1000 and 250 g/ton, respectively [7, 16]. Thus, the interest in e-waste recycling has risen, especially with the aim of recovering precious metals from waste PCBs.

Several hydrometallurgical, pyrometallurgical, and biometallurgical (bioleaching) methods of metal recovery are currently practiced [24]. Both hydrometallurgical and pyrometallurgical methods of metals extraction are rapid and less time-consuming compared to bioleaching process. However, extensive energy requirement and investment cost, associated toxicity, and high metals loss during recovery from e-waste increases the redundancy of these methods. The considerable quantities of secondary byproducts are also generated, which limits their use [12, 22, 23, 37]. In contrast, bioleaching is an environmentally friendly and cost-effective technology that has been employed by various researchers to recover metals [5, 6, 31]. Bioleaching offers advantage of comparatively higher metal extraction rate from the low-grade ores/depleted/and complex resources, through the involvement of active bioagents [34]. In addition, low cost of bioleaching process is another major advantage; an important driver for the industry [32, 34]. Bioleaching

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lowers the demand for resources such as energy, ores, and landfill space, but its application is still in its infancy [36]. A variety of chemolithotrophic bacteria (e.g., *Acidithiobacillus thiooxidans, Acidithiobacillus ferrooxidans*) cyanogenic bacteria (e.g., *Chromobacterium violaceum, Pseudomonas* sp., and *Bacillus megaterium*) and fungi (e.g., *Aspergillus niger, Penicillium simplicissimum*) are known for their ability to mobilize metals from e-waste [5, 15, 20, 32]. Precious metal leaching has been mainly reported using cyanogenic bacteria, especially *Chromobacterium violaceum* [9, 30]. Cyanogenic bacteria, such as *Chromobacterium violaceum, Pseudomonas* sp., and *Bacillus megaterium* produces hydrogen cyanide (as a secondary metabolite) and form water-soluble metal cyanide complexes under alkaline conditions (pH 8–10; [9]), on reacting with metals containing solids such as waste PCBs [6, 13]. The present study aimed to simultaneously recover Au and Ag from PCBs using indigenous bacterial strains from an e-waste recycling facility and to access the e-waste toxicity on them.

It has been reported that higher concentrations of e-waste are toxic to bacteria [29, 30, 32], and metal bioleaching is strongly dependent on the metabolic activity and status of the cell [8]. Therefore, assessing the toxicity of e-waste is required to guarantee and prevent failure in the bioleaching process. Toxicity studies generate dose–response curves and provide estimates of the median concentration or 50% effective concentration (EC_{50}) and the threshold concentration of metal bioleaching. In this context, indigenous microorganisms are favorable over foreign microorganisms, as indigenous microbes have the physiological and metabolic machinery necessary to resist the contaminant due to natural selection [8]. Studies related to *Pseudomonas balearica* SAE1 to recover metals from e-waste and their toxicity assessment have not been conducted until now.

Materials and Methods

Source and Compositional Analysis of e-Waste

The e-waste of PCBs with particle sizes ≤150 µm (i.e., standard test sieves as per IS 460: 1962) was procured in zipper storage bags from the storehouse (i.e., a concrete room designed to store waste before its safe disposal) of Exigo Recycling Pvt. Ltd., Panipat, Haryana, India. The company collected waste PCBs from different states of India and then segregated, manually dismantled, pulverized, and recycled to recover precious (Au, Ad, Pd) and base metals (Cu). The waste leftover after physico-mechanically recycling of the metals was prepared for safe disposal at a treatment, storage, and disposal facility (TSDF). The metallic content of waste PCBs was determined using acid digestion with aqua regia (HNO_3: HCl = 1:3), a protocol used in several studies [19, 32, 40].

Isolation of Bacteria from e-Waste Recycling Facility

Bacteria tolerant to e-waste toxicity were isolated from e-waste refuse by inoculating an e-waste sample (1 g) in100 mL of Luria broth (LB) in 250-mL Erlenmeyer flasks. The flasks were incubated in an incubator shaker (Thermo Scientific MaxQ 8000) at 30 ± 2 °C and 150 rpm for 6 days. Flasks with 100 mL of LB inoculated with sterilized (by autoclaving) e-waste refuse were kept as controls. The bacterial population associated with the e-waste was enriched by transferring 5 mL (2×10^8 CFU/ mL) of the sample from this flask to second flasks with LB supplemented with 25 g/L of sterilized e-waste. The flasks were incubated at 30 ± 2 °C and 150 rpm for 6 days. The sequential enrichment (50, 75, 100 g/L of sterilized e-waste) was continued for 30 days. After 30 days, the sample from the last flask with 100 g/L of e-waste was serially diluted (0.85% NaCl) and spread on nutrient agar (NA) plates aseptically. Bacteria were subcultured using streak plate method to ensure the purity of culture.

Characterization and Phylogenetic Analysis of Bacterial Isolate SAE1

To identify bacterial isolate SAE1, actively growing cells in LB medium were harvested and their DNA extracted using a Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. Then, 16S rRNA gene amplification of the extracted DNA was conducted using universal primers 27F and 1492R. A PCR mixture of 20 µL volume was made, containing 50 ng of template genomic DNA (Estimated spectrophotometrically by A260 and A280 measurements; NanoDrop, Thermo Fisher Scientific, Inc., Massachusetts, USA), 5 pmol of forward and reverse primers and PCR master mix (Promega, USA). PCR reaction was performed with initial denaturation at 95 °C for 5 min followed by 35 cycles at 95 °C for 1 min, 51.8 °C for 1 min and 72 °C for 1 min and having a final extension at 72 °C for 7 min. The PCR product (1465 bp) was analyzed by agarose gel electrophoresis. The amplified product was then sent for sequencing to Xcelris Labs Limited, Gujarat, India. The obtained sequence was compared with pre-existing DNA sequences available on the GenBank database using the BLAST tool of the National Center for Biotechnology Information (NCBI), MD, USA. The sequences were aligned by ClustalW, and a phylogenetic tree was constructed using a neighbor-joining method with MEGA 6.0 [39]. The evolutionary distances were calculated using the Jukes and Cantor model and compared with that of the other microbial strains obtained from the GenBank database. A bootstrap analysis

of 1000 replicates [14] was used to evaluate the topology of the tree.

Determination of e-Waste Toxicity Tolerance Levels and Dose–Response Analysis

The bacterial isolate SAE1 along with *Chromobacterium violaceum* (MTCC 2656) was tested for its toxicity tolerance at 10, 100, 200, 300, 400, and 500 g/L pulp density of e-waste. *Chromobacterium violaceum* used in the present study was procured from the Institute of Microbial Technology in Chandigarh, India. Toxicity tolerance was measured by bacterial growth at respective pulp density in terms of the colony-forming unit (CFU) count method. The Clinical and Laboratory Standards Institute guidelines for minimum inhibitory concentration testing were followed using Luria–Bertani (LB) broth to determine e-waste toxicity [10]. The experiments were performed in a 250-mL Erlenmeyer flask containing 100 mL LB medium. The flasks were inoculated with 1% (*v*/*v*) of inoculum containing 1.4×10^5 CFU/mL, along with the respective concentration of sterile e-waste. Flasks containing medium and bacterial cells without e-waste were kept as controls. All flasks (in duplicates sets) were incubated at 30 °C and 150 rpm for 24 h. After 24 h, samples from each flask were taken following enumeration of bacteria by serial dilution and spread plate methods.

For the dose–response curve, percent inhibition response was calculated as shown in Eq. (1):

$$\% \text{ IR} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100 \quad (1)$$

where *IR* is the inhibition response, *Control* is bacterial growth in the absence of e-waste, and *Test* is bacterial growth in the presence of e-waste.

Nonlinear regression was performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, California). The dose–response curve was determined using log (agonist) versus normalized response-variable slope procedure in GraphPad Prism 6 [25]. EC_{50} value (statistically derived estimate about the concentration of a substance resulting in 50% reduction of growth in a specified time period) was estimated based on dose–response data.

Two-Step Bioleaching

A two-step bioleaching process was employed for solubilization of metals from e-waste [6, 29, 32]. In the process, bacterial culture [5% (*v*/*v*) containing 2×10^8 CFU/mL] was inoculated into sterile LB medium in the absence of e-waste for 48 h. After 48 h e-waste sterilized by autoclaving [35] was added to culture flasks and incubated for a time period of 7 days, then the flasks were analyzed for the presence of metal ions.

Optimization of Experimental Parameters

It was reported that bioleaching of metals from waste PCBs using cyanogenic microorganisms depends on initial pH [9, 29], pulp density [1], temperature, and glycine concentration (precursor) [6, 13]. Therefore, the aforementioned factors were tested on variable ranges of pulp density (10, 50, and 100 g/L), glycine concentration (2.5, 5.0, 7.5, and 10.0 g/L), temperature (25, 30, 35, and 40 °C), and initial pH (7, 8, and 9) for maximum metal mobilization from waste PCBs in the present study.

Analytical Methods

Samples were filtered using Whatman grade 1 filter paper and then centrifuged (Eppendorf Centrifuge 5804 R; Eppendorf India, Ambattur, Chennai, India) at 6300 rcf for 10 min to remove solid particles (e-waste) and cell biomass. The supernatant/leachate was collected and analyzed for the presence of metal ions using atomic absorption spectrometry (PerkinElmer AAnalyst 400; PerkinElmer, Inc., Waltham, Massachusetts) on respective wavelengths. Prior to analysis, leachate was passed through a 0.45-µm glass fiber filter (PALL-GF-A/E-I) to ensure particle-free suspension [32]. The final pH of the supernatant/leachate was analyzed by portable digital pH meter (Eutech pH Testr30; Thermo Fisher Scientific, Inc., Massachusetts, USA).

Statistical Analysis

All the experiments were conducted under the statistical framework of duplicate experiments along with appropriate control. Experimental data were statistically interpreted with two-way analysis of variance (ANOVA). The statistical significance was determined at a probability level of $P < 0.05$ using GraphPad Prism version 6 (GraphPad Software, Inc., La Jolla, California).

Results and Discussion

Compositional Analysis of e-Waste (PCBs)

Metals compositional analysis of e-waste dust was performed, and results are presented in Table 1. The concentration of copper (Cu 23.4 mg/g) and iron (Fe 22.2 mg/g) was in bulk. However, precious and base metals were determined as Au (0.08 mg/g), Ag (0.4 mg/g), Ni (2.0 mg/g), Co (1.1 mg/g), Cr (0.9 mg/g), and Zn (0.7 mg/g). The present study depicts a significantly lower metals concentration than those reported previously from waste PCBs [2, 29, 32]. The low concentration is because the waste used in the present study was subjected for physio-mechanical recovery of

Table 1 E-waste (PCBs) metal content analysis using aqua regia (HNO_3: HCl; 1:3)

Metals	Concentration (mg/g)
Cu	23.4 ± 1.9
Fe	22.2 ± 1.7
Ni	2.0 ± 0.42
Co	1.1 ± 0.2
Cr	0.9 ± 0.09
Zn	0.7 ± 0.02
Ag	0.4 ± 0.04
Au	0.08 ± 0.01

This is a partial composition of PCBs, indicating major metals

metals at industry before procurement, whereas other studies that reported higher metals composition used virgin waste from industry. The precious metals concentration in the present study was higher compared to Xiang et al. and Liang et al. who reported 0.0144 mg/g and 0.014 mg/g of Au and 0.22 mg/g and 0.03 mg/g of Ag from waste PCBs, respectively [27, 41]. Heterogeneity was observed in comparison with metal ions of those reported by various researchers [4, 41, 42], which may be attributed to the origin, nature, industrial waste processing of e-waste material, and type of analytical methods used.

Characterization of e-Waste Associated Bacteria

Three morphologically different bacterial strains were isolated from the original sample of e-waste on nutrient agar medium. However, sequential enrichment leads to the selection of single bacterial strain SAE1 at 100 g/L pulp density of e-waste and was used in the bioleaching study. No growth was observed in the control flasks containing sterilized e-waste. Bacterial colonies were circular, entire, flat, and mucoid with yellowish pigmentation. The bacterial cell was Gram-negative and rod shape in nature. The identity of bacterial isolate SAE1 was confirmed by 16S rRNA gene sequencing. The results of BLASTN analysis of the 16S rRNA sequence showed the highest homology (100%) with *Pseudomonas balearica* when compared with the available sequence in the National Center for Biotechnology Information (NCBI) GenBank database. The sequence obtained was deposited in the NCBI GenBank database (Accession no. KU053282). The phylogenetic analysis was performed using a neighbor-joining method with 1000 bootstrap sampling values, and a tree was constructed (tree not shown). It was observed that bacterial isolate SAE1 showed a 100% similarity index with species of genus *Pseudomonas*. Different *Pseudomonas* species, for example, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* have been reported for their bioleaching potential from e-waste [30, 32]. Other than e-waste bioleaching, many *Pseudomonas* species were reported for their inorganic phosphate solubilization [28] and rare earth metals leaching capabilities [17].

Determination of e-Waste Toxicity Tolerant Capability of SAE1

The toxicity was accessed by reduced number of CFU/mL of *Pseudomonas balearica* SAE1 along with *C. violaceum* to that of control. The percent inhibition response was calculated and shown in Fig. 1a. *Pseudomonas balearica* SAE1 was capable of growing at e-waste pulp density of 500 g/L, whereas *C. violaceum* was completely inhibited at e-waste pulp density of 200 g/L in 24 h. The toxicity of e-waste is attributed to the presence of heavy metals and other toxic pollutants such as polybrominated diphenyl ethers [22, 33]. To get a better idea of e-waste toxicity, a quantitative dose–response curve was plotted in Fig. 1b, c. The EC_{50} value for the bacterial strain SAE1 and *C. violaceum* was 325.7 g/L ($LogEC_{50} = 2.5$) and 83.70 g/L ($LogEC_{50} = 1.9$), respectively. The 95% confidence intervals were Log 2.5 to 2.6 (315.1–346.7 g/L) for SAE1 and Log 1.8 to 2.0 (68.7–102.0 g/L) for *C. violaceum*. However, these values may vary depending on bacterial species and type of e-waste used. According to Chen et al. EC_{20} and EC_{50} values provide estimates about the feasibility of the bioleaching process [8]. Therefore, EC_{20} was determined by using the GraphPad QuickCalcs online tool (http://graphpad.com/quickcalcs/ECanything1/). The EC_{20} value of *P. balearica* SAE1 (149.6 g/L) was higher than *C. violaceum* (45.8 g/L). E-waste at approximately 10 g/L pulp density was nontoxic to *P. balearica* SAE1 without any percent inhibition in this case; therefore, it can be used for bioleaching. However, the results of the dose–response analysis clearly suggest the technological feasibility and viable operation range of metals bioleaching from e-waste using *P. balearica* SAE1.

Optimization of Experimental Parameters

Influence of Pulp Density

The effect of increasing pulp density on metal recovery is shown in Fig. 2a. It was observed that *P. balearica* SAE1 was able to mobilize maximum of 56.4 and 30.5% of Au and Ag, respectively, at 10 g/L pulp density. Further increase in pulp density from 10 to 100 g/L resulted in significantly decreased recovery of metals. The Au and Ag mobilization at 100 g/L pulp density was 8.3 and 1.8%, respectively. The low metals mobilization at higher pulp density may be due to toxic effects of the e-waste, which have inhibited the bacterial metabolism [20], thereby resulting in reduced growth and poor lixiviant production. A similar study by Pradhan and Kumar, reported 69.3% mobilization of Au at 10 g/L pulp density and was decreased to 20.28% at 100 g/L pulp

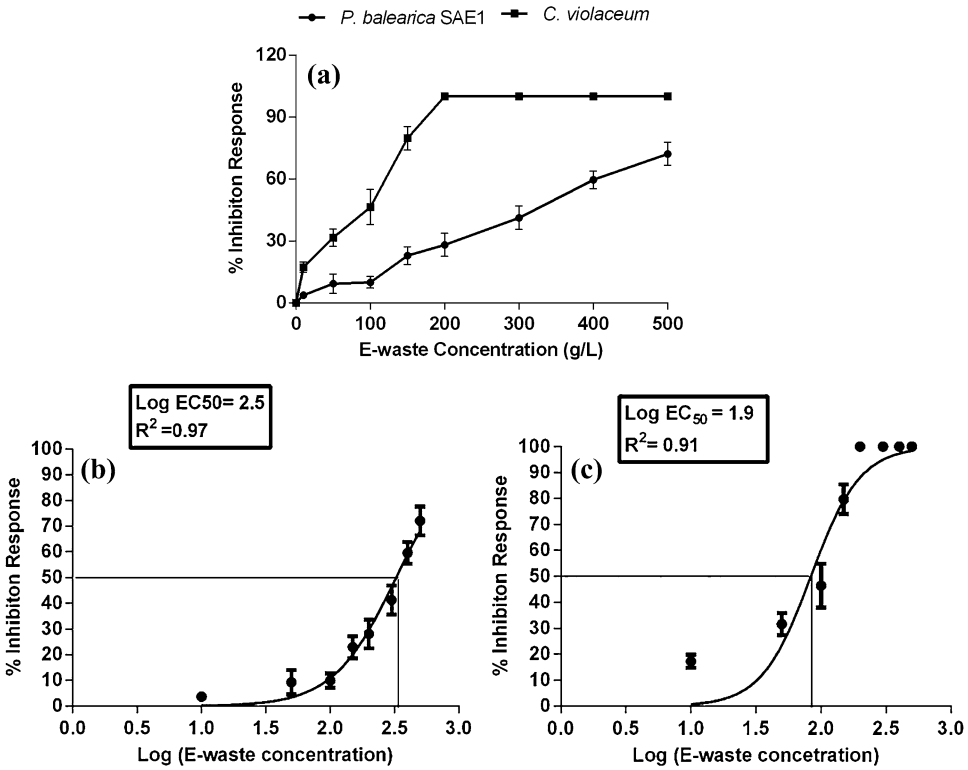

Fig. 1 **a** Percent inhibition response of *P. balearica* SAE1 and *C. violaceum*; **b** dose response curve for *P. balearica* SAE1; **c** dose response curve for *C. violaceum*. The dose–response curve was determined using equation "*log (agonist) versus normalized response–variable slope*" procedure in GraphPad Prism 6

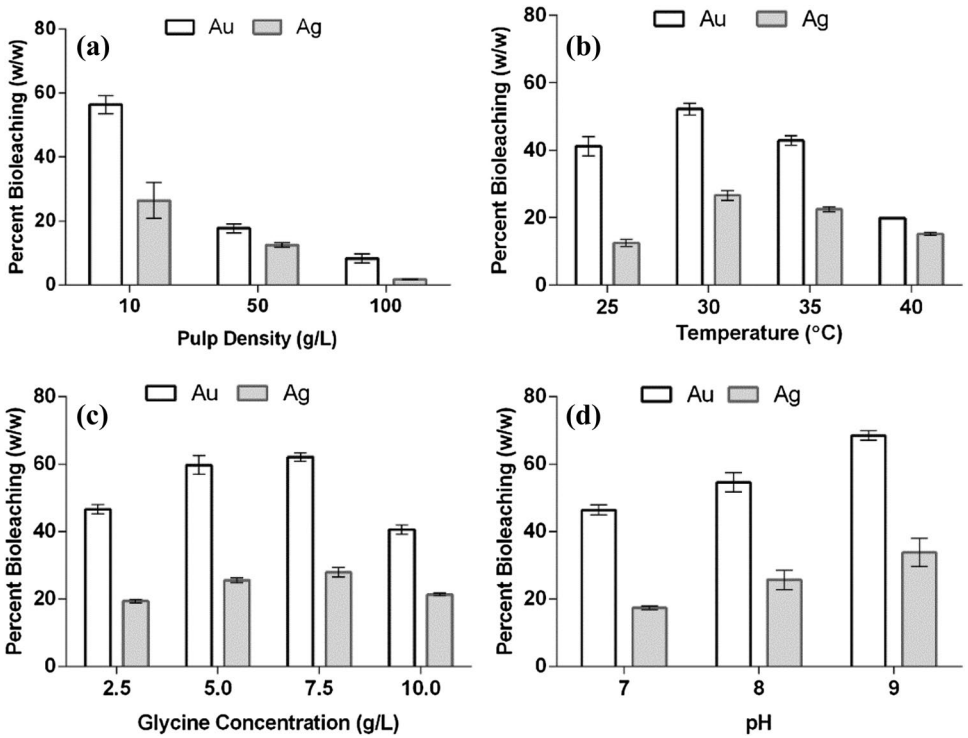

Fig. 2 OFAT optimization for enhancement of precious metals recovery during bioleaching of e-waste: **a** influence of pulp density, **b** influence of temperature, **c** influence of glycine concentration, **d** influence of pH

density using *C. violaceum* [32]. The total Au present in the e-waste of Pradhan and Kumar, was 0.084% (*w*/*w*) [31]. Natarajan and Ting, reported higher mobilization of Au (11.3%) and Cu (86.2%) from pretreated (with nitric acid) electronic scrap material at low pulp density (5 g/L) using *C. violaceum* [30]. The percentage of bioleaching was calculated from 0.024 and 3.04% of total Au and Cu, respectively, present in the pretreated e-waste of Natarajan and Ting [30].

However, metals bioleaching at low pulp density is a potential challenge in implementing the process economically.

Influence of Temperature

Researchers reported the influence of temperature on cyanide production using *Pseudomonas chloroaphis* and *C. violaceum*. The suitable temperature for cyanide production ranged from 25 to 35 °C [36, 43]. Therefore, the present study selected a range of temperatures from 25 to 40 °C. The results were presented in Fig. 2b. The highest recovery of Au and Ag was 56.2 and 26.6%, respectively, and appeared at 30 °C, pH 8.0, and pulp density 10 g/L. The moderate temperature (30 °C) offers the advantage of low energy requirement during industrial-scale application, thereby potentially reducing the cost of the process.

Influence of Additive Concentration

Cyanide producing ability of cyanogenic microorganisms is favored by the addition of glycine [2, 6, 36]. Thus, different concentrations of glycine (2.5, 5.0, 7.5, and 10.0 g/L) were added to the experimental flasks to investigate their effects on recovery of Au and Ag. It was observed that increase in glycine concentration from 2.5 to 7.5 g/L have increased the metals leaching capabilities of bacterial strain SAE1. However, a further increase in glycine concentration from 7.5 to 10.0 g/L resulted in decreased metals mobilization. This is due to inhibitory effects of glycine on bacteria at a concentration above 7.5 g/L. According to Faramarzi et al. glycine concentration greater than optimum was toxic, which led to reduced bacterial growth and poor metals mobilization [13]. Işıldar et al. and Shin et al. reported inhibitory effects of glycine above 7.5 g/L using *P. fluorescens* and *Pseudomonas putida* during the bioleaching of metals from e-waste [21, 38]. The maximum leaching of Au (62.1%) and Ag (27.9%) was at glycine concentration of 7.5 g/L (Fig. 2c). Metals bioleaching at glycine concentrations 5 and 7.5 g/L did not show any significant differences. Therefore, glycine concentration 5 g/L was taken as optimum for further experiments.

Influence of pH

The pKa of HCN is 9.3, alkaline pH increases the availability of aqueous cyanide ions (CN^-) and thereby enhances the metal mobilization efficiency. However, optimum bacterial growth occurs at physiological pH range of 7–8 [29]. An equilibrium reaction of bacterial cyanide production can be represented as per Eq. (2) [30]:

$$HCN \leftrightarrow H^+ + CN^- \quad (2)$$

Therefore, metal leaching efficiency of *P. balearica* SAE1 was investigated on a range of pH from 7 to 9. From the results of Fig. 2d, higher metals leaching (Au 68.5%; Ag 33.8%) was observed at culture conditions of pH 9 after 7 days, whereas low metals mobilization was observed at pH 7. This is because at low pH (7) equilibrium shifts to produce more HCN gas, which is volatile and less water-soluble. At alkaline pH (9), the equilibrium shifts toward aqueous cyanide ions (CN^-), making it highly available for metals solubilization/complexation [30]. Our study reported significantly higher recovery of Au at pH 9 than the studies of Arshadi and Mousavi, Chi et al. and Natarajan and Ting [2, 9, 30]. Though cyanide has been used in extraction of Ag from industrial mine and metallurgical waste. Ag leaching using alkaline cyanidation process provides optimal conditions and highest recovery of this metals [18]. Hence, alkalophilic cyanogenic microorganisms have a great application in Au and Ag leaching from mine tailings. There are few reports of silver leaching at alkaline pH using cyanogenic microorganisms from metals containing solid waste [6, 21].

Bioleaching Assay Under Optimized Conditions

The two-step bioleaching process was optimized for recovery of Au and Ag. Results of Fig. 3 present the metals dissolution profile of Au and Ag during the two-step bioleaching process. The leaching of metals increased up to the seventh day; thereafter, no mobilization was observed. The maximum Au (68.5%) and Ag (33.8%) were mobilized at pulp density 10 g/L, glycine concentration of 5 g/L, pH 9, temperature 30 °C, rpm 150, and waste PCBs of particle size ≤150 µm. Cyanogenic microorganisms such as *Pseudomonas* species and *C. violaceum* produce HCN as secondary metabolite [21, 30], which form water-soluble complex (i.e., dicyanoaurate with Au) [13]. The dissolution of Au can be represented as shown in Eq. (3) [36]:

$$4Au + 8CN^- + O_2 + 2H_2O = 4Au(CN)_2^- + 4OH^- \quad (3)$$

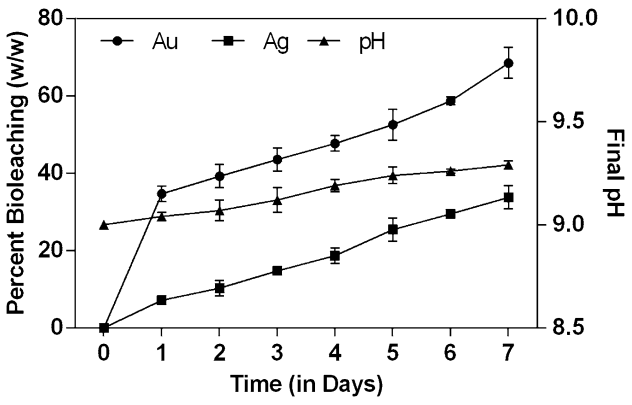

Fig. 3 Percentage bioleaching of precious metals (primary axis) and change in final pH (secondary axis) under OFAT optimized conditions by *P. balearica* SAE1

Ag cyanidation follows very similar reaction and was reported to be mobilized as dicyanoargentate during cyanogenic bioleaching by various researchers [6, 13]. The present study observed higher Ag mobilization (33.8%; Fig. 3) as compared to the previous studies [32, 36], which showed 12.1 and 7% of Ag mobilization from e-waste using cyanogenic bacterial strains. Brandl et al. reported 5% mobilization of Ag as dicyanoargentate from jewelry waste using cyanogenic bacterial strain *Pseudomonas plecoglossicida* [6]. The higher mobilization of Ag may be attributed to heterogeneous nature of e-waste. This is because the previously compared studies [2, 32, 36] reported higher amounts of Cu (10.00, 12.06, and 90.43%, respectively) in their e-waste, which interferes with precious metals leaching by consuming cyanide produced during cyanogenic bioleaching, resulting in poor metal mobilization. In the present study, the amount of Cu is less (2.4%), making cyanide available for precious metals, which resulted in the higher mobilization of Ag and Au. Although the elevated concentration of dicyanoargentate was toxic to bacteria [6], the even higher e-waste toxicity tolerance ability of *P. balearica* SAE1 provided the selective advantage to the bacterial strain. The presence of cyanide was tested at Jeedimetla Effluent Treatment Ltd. Hyderabad, India. The results showed a positive cyanide test for the bacterial strain *P. balearica* SAE1. Further, proper controls were undertaken in the study, which did not show the presence of cyanide as well as leaching of Au and Ag, respectively.

The change in pH was also observed during bioleaching of metals (Fig. 3). In the absence of e-waste, the pH slightly decreased from 9.0 to 8.7 in 48 h (data not shown). This may be attributed to bacterial conversion of organic compounds such as glycine into glyoxylic, cyanoformic, and oxamic acids [26]. However, after addition of waste PCBs, pH increased gradually from 8.7 to 9.2 until the seventh day of incubation (Fig. 3). The increased pH corresponds to the reaction of HCN with metals present in e-waste; thus, the formation of metals-cyanide complexes and similar findings have been reported by Sahni et al. during bioleaching of metals from subscriber identity module waste using *C. violaceum* [37]. Arshadi and Mousavi, reported a similar trend of pH change during Au and Cu extraction from waste PCBs using cyanogenic bacterium *Bacillus megaterium* [2]. Because alkaline conditions allow for the easier recovery of metals compared to acidic conditions, pH is a significant parameter in the bioleaching process. In this study, simultaneous recovery of higher amounts of Au and Ag from waste PCBs at alkaline pH (9.0) by *P. balearica* SAE1 may be promising for industrial applications.

Conclusion

Indigenous bacterial strain *P. balearica* SAE1 was isolated from an e-waste recycling facility. Toxicity assessment study showed higher tolerance of SAE1 to e-waste toxicity, i.e., $EC_{50} = 325.7$ g PCBs/L of the culture medium. *Pseudomonas balearica* SAE1 was used for the bioleaching of Au and Ag from waste printed circuit boards. Precious metals (Au and Ag) dissolution was enhanced by optimization of parameters. *Pseudomonas balearica* SAE1 was able to leach 68.5 and 33.8% of Au and Ag under optimized conditions. The higher tolerance of *P. balearica* SAE1 to e-waste toxicity with efficient metal mobilization ability, confirms its suitability for industrial bioleaching operations of recovering precious metals from e-waste. These efforts will surely pave the way toward conservation of primary/natural resources, prevent environmental degradation, and significantly contribute to the transition to a circular economy.

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Compliance with Ethical Standards

Conflict of interest Author declares no conflict of interest.

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