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ORIGINAL ARTICLE

Enhancement of gold and silver recovery from discarded computer printed circuit boards by *Pseudomonas balearica* SAE1 using response surface methodology (RSM)

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Abstract

Two-step bioleaching was applied using a cyanogenic bacterium *Pseudomonas balearica* SAE1 to recover gold (Au) and silver (Ag) from the computer printed circuit boards (CPCBs) via central composite design of a response surface methodology (CCD-RSM). To enhance Au and Ag recovery, factors like pH level, pulp density, temperature and glycine concentration were optimized and their interactions were studied. CCD-RSM optimization resulted in 73.9 and 41.6% dissolution of Au and Ag, respectively, at initial pH 8.6, pulp density 5 g/L, temperature 31.2 °C, and glycine concentration 6.8 g/L, respectively. Two quadratic models were proposed by RSM which can be utilized as an efficient tool to predict Au and Ag recovery through bioleaching. The experimental results are in line with the predicted results, indicating reliability of RSM model in enhancing the Au and Ag recovery from CPCBs. The increased bioleaching yield of Au and Ag from discarded CPCBs has its importance in industrial e-waste recycling and safe disposal.

Keywords Bioleaching · e-waste · CCD-RSM · Precious metals · Safe disposal

Introduction

With the innovation and advancements in technology, industries are turning towards greater automation that resulted in increased use of electronic gadgets in our daily life (Kumar et al. 2017a). Simultaneously, development of advanced, reliable and faster electronics and computing technologies has led to a shorter life span of the electronic gadgets resulting in higher e-waste generation (Tansel 2017). The United Nations (UNs) estimates of global waste electrical and electronic equipment's (WEEEs) production were 14, 24, and 49 million tons in 1992, 2002, and 2012, respectively, and more than 50 million tons in 2017; and the number is growing at an exponential rate, i.e., approximately 10% every year (Kaya 2016. The e-waste is a rich source of metals and persistent organic pollutants (POP's), which makes it the hazardous material (Pradhan and Kumar 2014). The presence of significant quantities of metals especially precious metals such as Au and Ag in the e-waste makes its recycling worth economic; saving landfill space and lowers the burden on natural mines (Arshadi et al. 2016; Natarajan and Ting 2014).

Current practices of e-waste recycling is being carried using physico-mechanical, hydrometallurgical and pyrometallurgical processes (Kumar et al. 2017a). Pyrometallurgical treatment of e-waste includes smelting of metals and oxides, high temperature burning of plastics and electrochemical refining. Whereas, hydrometallurgical recovery of precious metals from e-waste is being carried using cyanide/non-cyanide (e.g., thiourea and ammonium thiosulphate) leaching and chemical (e.g., HNO_3, H_2SO_4 and aqua regia) leaching (Sun et al. 2017). However, these processes are expensive, energy intensive, generates large amount of spent acid, toxic gases, sludge, liquid waste, fumes of heavy metals (metals with low melting point like mercury, lead and cadmium), and may lead to formation of mixed halogenated dioxins and furans [especially, if the scrap contains plastic with brominated flame retardants (BFRs)] (Kaya 2016; Sahni et al. 2016). The hydrometallurgical/pyrometallurgical methods

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are rapid but low metal recovery rate, loss of precious metals during recovery, environmental impacts and high cost have push the development of alternative methods of metal recovery from e-waste (Kumar et al. 2017b; Priya and Hait 2017; Sun et al. 2017). In this context, bioleaching process recovers metals from e-waste and low-grade ores in an eco-friendly, cost economic and energy conservative way (Priya and Hait 2017). The microorganisms solubilize metals to their extractable form; recoverable from the solution (Brandl et al. 2001, 2008; Natarajan and Ting 2014, 2015; Pradhan and Kumar 2012). Bioleaching offers selective advantage of leaching metals which are not accessible by conventional methods as shown in Au and Cu mining (Agate 1996). Pradhan and Kumar (2012) studied bioleaching of e-waste using a mixed culture of *Chromobacterium violaceum* and *Pseudomonas aeruginosa* and reported 83, 73 and 8% of Cu, Au and Ag, respectively, compared to extraction through chemical leaching. Arshadi et al. (2016) reported extraction of 72% of Cu and 65 g/ton of Au from mobile phone PCBs using a cyanogenic bacteria *Bacillus megaterium*. Although bioleaching process is slow and time consuming yet increases the metal recovery due to higher extraction rate from low-grade ores/complex sources like e-waste where thermal and physico-chemical methods are not economical (Brandl et al. 2001). Other than this, the low energy requirement, minimum sludge generation, environmental compatibility and low cost of bioleaching process make it suitable from industrial point of view (Kumar et al. 2017b; Priya and Hait 2017). Bioleaching using cyanogenic microorganisms (*C. violaceum*; *B. megaterium*; *Pseudomonas* sp.) and acidophilic microorganisms (*Acidithiobacillus thiooxidans*; *Acidithiobacillus ferrooxidans*) are commonly reported (Willner and Fornalczyk 2013).

Cyanogenic microorganisms such as *C. violaceum*; *B. megaterium*; *Pseudomonas* sp. produce cyanide as secondary metabolite and mobilize many metals by forming metal-soluble complexes. These complexes have high chemical stability and water solubility (Brandl and Faramarzi 2006). There are a number of physiological and nutritional factors (pH, temperature, glycine concentration, pulp density and medium composition) which may affect the end-product of the process. Thus, optimization of the process to attain maximum recovery with minimum no. of experiments is required (Amiri et al. 2012). In this context, statistical methods are rapid and identify interactions among parameters. Response surface methodology (RSM) has been applied to model, analyze and study the relationship/interaction of the individual parameters (Amiri et al. 2011). There are few study on use of RSM for bioleaching of metals from e-waste (Arshadi and Mousavi 2014, 2015; Arshadi et al. 2016).

In this study, a pure culture of *Pseudomonas balearica* SAE1 (previously isolated from e-waste recycling facility in the author's laboratory) was employed to extract Au and Ag from discarded CPCBs. RSM was applied to optimize influential parameter like initial pH, pulp density, glycine concentration and temperature in order to enhance the simultaneous biorecovery of Au and Ag. The mutual effect of factors and their interactions during bioleaching were studied via CCD-RSM.

Materials and methods

Characterization of CPCBs

The pulverized CPCBs were obtained in zip lock bags from the storeroom of Exigo Recycling Pvt. Ltd., Panipat, Haryana, India. The particle size of CPCBs was ≤ 150 µm determined using standard test sieves as per IS 460: 1962. The compositional analysis of CPCBs was determined through aqua regia (HNO_3:HCl = 1:3) digestion method (Ilyas et al. 2007; Pradhan and Kumar 2012; Tuncuk et al. 2012). One gram of waste CPCBs was weighed and added to 100 mL aqua regia. Digestion was carried out by refluxing at 100 °C for a time period of 1 h and final volume was made up to 100 mL by the addition of deionized water. To obtain particle-free suspension, leachate/solution was first filtered using Whatman grade 1 filter followed by filtration through glass fiber filter (0.45-µm, PALL-GF-A/E-I). The dissolved metals ions were quantified using atomic absorption spectrophotometry (AAS) (AAnalyst 400, PerkinElmer) at 242.8 and 328.1 nm wavelengths for Au and Ag, respectively (Pradhan and Kumar 2012; Sahni et al. 2016). The finely ground CPCBs were sterilized in an autoclave at 121 °C, 15 psi for a time period of 30 min in autoclavable bags (HiMedia) and dried at room temperature (25 ± 3 °C) prior to bioleaching (Pradhan and Kumar 2012; Rozas et al. 2017).

Microorganism and culture conditions

A pure culture of *P. balearica* SAE1 (Accession No. KU053282) was taken from the author's laboratory, previously isolated from e-waste recycling facility, Haryana, India. The bacterial strain has ability to produce cyanide and showed a higher tolerance to e-waste toxicity, i.e., the EC_{50} = 325.7 g/L of PCBs (Kumar et al. 2017b). To culture *P. balearica* SAE1, 5 mL of inoculum was added to 100 mL Luria broth (LB) followed by incubation at 30 °C and 150 rpm in an incubator shaker (MaxQ 8000; Thermo Fisher Scientific).

Bioleaching

Bioleaching (two-step) was applied to solubilize Au and Ag of discarded CPCBs. In the first step, cells of *P. balearica* [5% (*v*/*v*) of inoculum having 2×10^8 CFU/mL] were inoculated to LB media in the absence of CPCBs in Erlenmeyer flasks of 250 mL capacity. The flaks were inoculated at respective temperature (as per CCD-RSM software) and 150 rpm for 48 h. After 48 h in the second step, sterilized CPCBs were added to LB medium followed by incubation at 150 rpm for additional time period of 7 days (Kumar et al. 2017b; Pradhan and Kumar 2012). After 7 days of bioleaching, the cell biomass and ground CPCBs were removed by filtration (Grade 1, Whatman filter paper) followed by 10-min centrifugation (Eppendorf, 5804 R) at 7000 rpm. To obtain particle-free suspension, leachate/supernatant was further passed through glass fiber filter of 0.45-µm size (PALL-GF-A/E-I) and were stored at 4 °C till further analysis. The metals dissolution was analyzed using AAS. LB medium containing CPCBs only was kept as control during bioleaching experiments.

RSM-based optimization

To achieve maximum recovery of Au and Ag, four factors, including pulp density, initial pH, temperature, and additive (glycine) concentration, were optimized by CCD-RSM. According to CCD, the total experimental run was as per Eq. (1) (Manikan et al. 2015):

$$2^k + 2K + n_0, \tag{1}$$

where 2^k is the factorial run, $2K$ is the axial run, n_0 is the number of central point replications, and K depicts the number of independent variables.

The behavior of the system was modeled using quadratic Eq. (2):

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j, \tag{2}$$

where Y is the predicted response, β_0 is the offset term, β_i is the linear effect, β_{ii} is the squared effect, and β_{ij} is the interaction effect (Manikan et al. 2015).

In this study, 30 experimental runs were performed as predicted by Design-Expert Software Version 10 (Stat-Ease, Inc., Minneapolis, Minnesota, USA) (Table 1). The experiments were performed in duplicates. Data procured from duplicate experimental runs were model fitted to determine the interaction/relationships of factors. The significance of interaction between variables and responses was determined. The quality fit and statistical significance of model was exhibited as the coefficient of determination (R^2) and F-test in the identical program. To validate the results of the mathematical model with experimental results, additional experiments were performed under optimal conditions generated by statistical approaches.

Apparatus

An incubator shaker (MaxQ 8000; Thermo Fisher Scientific) was used for shaking and setting temperature of the experimental flasks. Bacterial cell biomass was separated in a centrifuge (Eppendorf Centrifuge 5804 R) at 7000 rpm for 10 min. The dissolved metals ions were analyzed using AAS (AAnalyst 400, PerkinElmer). The pH of the medium was analyzed/set using a digital pH meter (Eutech pH Testr30; Thermo Fisher Scientific).

Results and discussion

Characterization of CPCBs

The results of metal content of CPCBs are presented in Table 2 which have been reported in our previous study (Kumar et al. 2017b). The amount of Cu (23.4 mg/g) was in majority. The precious metals Au (0.08 mg/g) and Ag (0.4 mg/g) were present in significant lower quantity. Xiang et al. (2010) and Liang et al. (2010); reported 0.0144 and 0.014 mg/g of Au and 0.22 and 0.03 mg/g of Ag, respectively, from waste PCBs (Printed Circuit Boards); lower than the quantity reported in our case. The differences in the metals concentration of waste PCBs are attributed to heterogeneity and complexity of e-waste (Sun et al. 2015). The heterogeneity in the metals content of e-waste depends upon origin and type of e-waste (Kumar et al. 2017b; Sun et al. 2015).

RSM-based optimization

Statistical evaluation

The, CCD of RSM was used to optimize different factors (pH, pulp density, temperature, and glycine concentration) effecting recovery of metals during bioleaching. The responses of 30 experimental runs (16 full factorial + 8 axial + 6 center points), along with predicted responses, are represented in Table 1. Statistical results of ANOVA suggested a quadratic model for Au and Ag recovery (Table 3). The significance of the model was depicted by “model F-values” of 8.03 (Au) and 26.9 (Ag) (Table 3). The values of Prob > F for the models that were less than 0.05 indicated that the models were statistically significant. The “adequate precision” measures the signal-to-noise ratio and should be > 4 (desirable) (Kumar et al. 2015). The ratio of 10.4 and 19.3 for model Au and Ag recovery indicates an adequate signal and can be used to navigate the design space defined by CCD (Amiri et al. 2012). A lower coefficient of variance (CV) for model Au (15.3%) and Ag (15.6%) recovery indicates that experiments conducted were specific and reliable (Kumar

Table 1 Experimental design with factors and levels suggested by CCD-RSM along with experimental and predicted results

Sr. no.	*A*	*B*	*C*	*D*	Experimental		Predicted	
					Au (%)	Ag (%)	Au (%)	Ag (%)
1	7.5	15	7.5	30	39.5	12.3	32.9	9.4
2	8	10	10	25	34.2	11.7	38.7	12.4
3	8	10	10	35	38.2	12.5	41.1	14.6
4	8.5	25	7.5	30	18.9	8.0	28.9	10.9
5	9	10	10	25	40.5	14.9	40.4	17.02
6	9	20	5	35	35.9	7.9	30.9	5.8
7	8	20	5	35	32.7	6.3	29.8	6.2
8	8.5	15	7.5	30	50.3	22.4	50.1	23.4
9	8.5	15	7.5	30	49.5	23.7	50.1	23.4
10	8	20	10	25	23.8	6.4	24.3	9.1
11	8	10	5	25	40.1	15.7	43.5	18.3
12	9	20	10	35	31.9	5.0	25.5	4.5
13	8.5	5	7.5	30	72.4	40.1	65.9	36.6
14	8.5	15	7.5	30	49.9	24.1	50.1	23.4
15	8	20	5	25	25.1	7.9	24.2	6.1
16	8	20	10	35	25.7	8.2	25.4	7.6
17	9	10	10	35	42.8	17.4	43.2	17.9
18	8	10	5	35	48.2	21.1	50.3	22.2
19	8.5	15	7.5	30	48.3	23.9	50.1	23.4
20	9	20	10	25	26.6	9.8	23.9	7.0
21	8.5	15	7.5	20	23.9	6.0	22.9	4.9
22	8.5	15	2.5	30	32.9	13.9	37.8	15.3
23	9	20	5	25	31.1	7.1	25	6.9
24	9.5	15	7.5	30	25.8	11.2	35.9	13.6
25	8.5	15	7.5	30	51.9	23.2	50.1	23.4
26	8.5	15	12.5	30	28.7	9.9	27.4	8.0
27	9	10	5	35	57.2	28.8	53.6	28.2
28	8.5	15	7.5	40	26.7	5.5	31.2	5.9
29	8.5	15	7.5	30	50.7	22.9	50.1	23.4
30	9	10	5	25	46.6	26.4	46.4	25.6

Table 2 E-waste (CPCBs) metal content analysis using aqua regia (HNO_3:HCl; 1:3) (Kumar et al. 2017b)

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

et al. 2015). According to Amiri et al. (2011), the quality of fit of the polynomial model equations (Eqs. 1 and 2) was expressed by the coefficient of determination (R^2). R^2 provided the proportion of the total variation in the response variable described by the predictors included in the model. The R^2 values for model Au and Ag recovery were 0.88 and 0.96, whereas adjusted R^2 values were 0.77 and 0.92, respectively. The higher R^2 values confirm that models are capable of representing the experimental system (Arshadi and Mousavi 2015). Figure 1a, b represents the actual versus predicted recovery percentage of Au and Ag from CPCBs. The actual responses were experimentally measured values for a particular run, whereas the predicted responses were suggested by software. The adjacency of points toward the 45° line confirms accuracy of the model to predict the responses (Amiri et al. 2011) Fig. 1c, d illustrates the normal probability plot of the quadratic model for Ag and Au recovery. The data of residuals fall on a straight line in the plot, which represents a normal distribution and thus supports the adequacy of the least-squares fit (Amiri et al. 2012). Three-dimensional (3D) response surface plots were used to study the interaction effects of different parameters on Ag and Au mobilization (Figs. 2, 3).

Table 3 ANOVA for quadratic model of Au and Ag (%) recovery

Response	Source	Sum of squares	*df*	Mean square	*F*-value	*p*-value
Au recovery (%)	Model	3865.6	14	276.1	8	0.0001
	A-pH	12.5	1	12.5	0.3	0.5
	B-pulp density	2053	1	2053	59.6	0.0001
	C-glycine concentration	158.9	1	158.9	4.6	0.04
	D-temperature	105.1	1	105.0	3.1	0.1
	AB	4.2	1	4.1	0.1	0.7
	AC	1.3	1	1.3	0.1	0.8
	AD	0.2	1	0.1	0.01	0.9
	BC	24.3	1	24.3	0.7	0.4
	BD	1.6	1	1.6	0.1	0.8
	CD	19.4	1	19.4	0.5	0.4
	A^2	420.9	1	420.9	12.2	0.003
	B^2	11.9	1	11.9	0.3	0.5
	C^2	523.1	1	523.1	15.2	0.001
	D^2	909.9	1	909	26.4	0.0001
	Residual	515.9	15	34.4		
	Pure error	6.9	5	1.3		
	Corr. total	4381.5	29			
	$R^2 = 0.88$					
Ag recovery (%)	Model	2102.9	14	150.2	26.9	0.0001
	A-pH	26.9	1	26.9	4.82	0.04
	B-pulp density	990.5	1	990.5	177.3	0.0001
	C-glycine concentration	78.5	1	78.5	14.2	0.001
	D-temperature	1.5	1	1.5	0.2	0.6
	AB	41.3	1	41.3	7.4	0.01
	AC	7.1	1	7.1	1.2	0.3
	AD	1.7	1	1.7	0.3	0.6
	BC	80.3	1	80.3	14.3	0.001
	BD	13.9	1	13.9	2.5	0.1
	CD	2.7	1	2.7	0.5	0.4
	A^2	242.2	1	242.2	43.3	0.0001
	B^2	0.2	1	0.2	0.1	0.8
	C^2	234.8	1	234.8	42.1	0.0001
	D^2	546.9	1	546.9	97.9	0.0001
	Residual	83.7	15	5.5		
	Pure error	2.2	5	0.45		
	Corr. total	2186.7	29			
	$R^2 = 0.96$					

Pure error: It is the normal variation in response, which appears when a run is repeated at identical conditions. It can be estimated by replicate experiments. The more replicate experiments will provide better estimates of the pure error

Corrected total: The total sum of square (SS) corrected for the mean (calculated by taking sum of the squared distances of each individual response value from its overall average)

Residual: It measures the experimental errors

Model fitting

CCD of RSM suggested a quadratic model for recovery of Au and Ag on the basis of observed responses of 30 experimental runs. The quadratic polynomial equation in terms of coded factors is as follows in Eqs. (3) and (4):

$$
\begin{aligned}
Y_{\mathrm{Au}} = {} & 50.08 + 0.72A - 9.25B - 2.57C + 2.09D - 0.50\mathrm{AB} - 0.29\mathrm{AC} \\
& + 0.09\mathrm{AD} + 1.23\mathrm{BC} - 0.32\mathrm{BD} - 1.10\mathrm{CD} - 3.92A^2 \\
& - 0.66B^2 - 4.37C^2 - 5.75D^2.
\end{aligned}
\tag{3}
$$

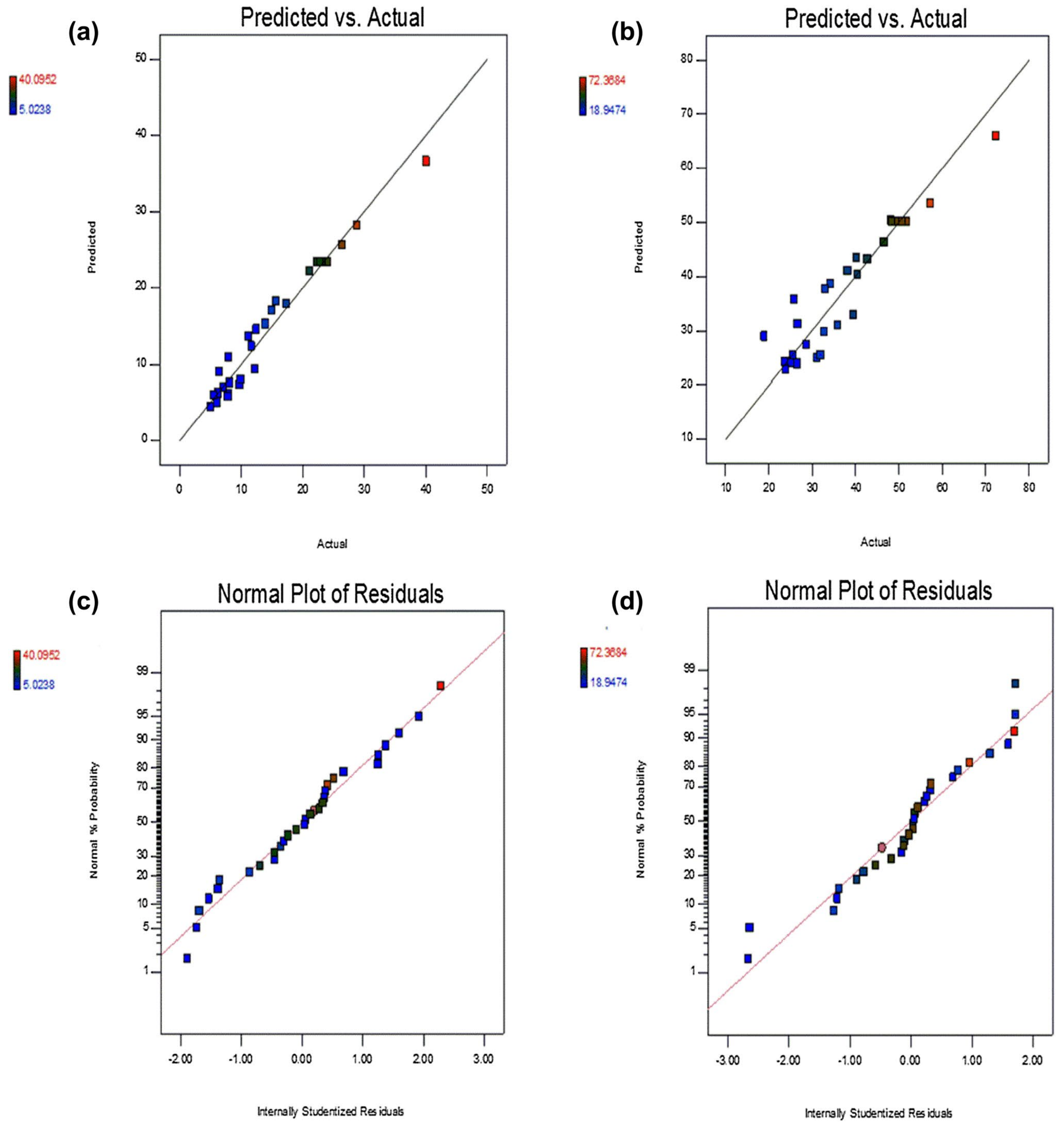

Fig. 1 Predicted vs. actual plots for: **a** Ag bioleaching (%); **b** Au bioleaching (%) and normal probability plots for: **c** Ag bioleaching (%); **d** Au bioleaching (%)

$$\begin{aligned} Y_{\mathrm{Ag}} = {} & 23.34 + 1.06A - 6.43B - 1.81C + 0.25D - 1.61\mathrm{AB} - 0.67\mathrm{AC} \\ & - 0.33\mathrm{AD} + 2.24\mathrm{BC} - 0.93\mathrm{BD} - 0.42\mathrm{CD} - 2.97A^2 \\ & + 0.10B^2 - 2.93C^2 - 4.47D^2. \end{aligned} \tag{4}$$

A, B, C, and D represent the initial pH, pulp density (g/L), glycine concentration (g/L), and temperature (°C), where multiple between-two variables show their interactions with each other. The model for Au ($p < 0.001$) and Ag ($p < 0.001$) recovery was statistically significant at high confidence level. Model terms with $p < 0.05$, including individual variables A, B, and C; quadratic variables A^2, C^2, and D^2; and interaction variables AB and BC, were considered as significant variables in the case of Au and Ag recovery

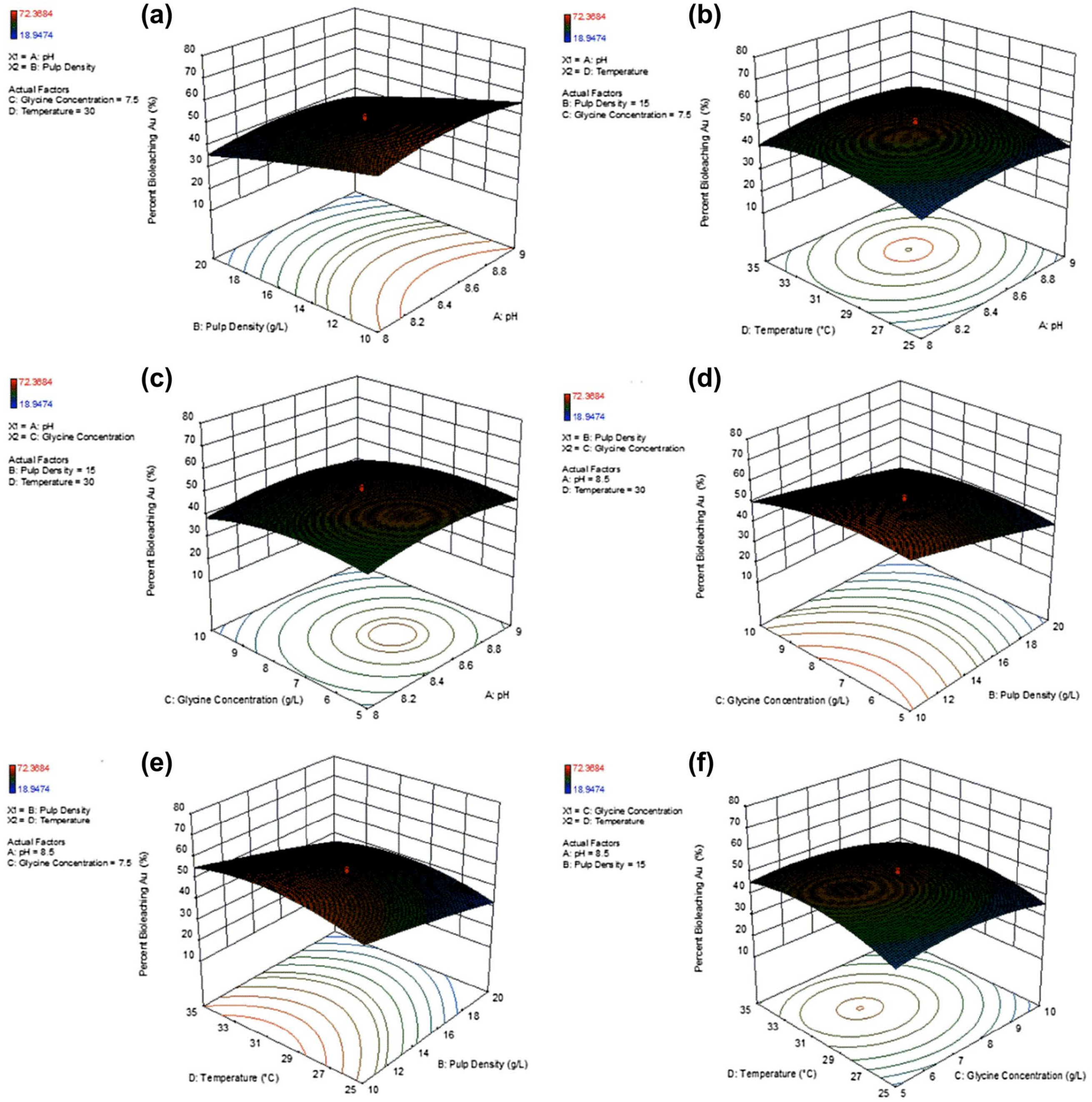

Fig. 2 Response surface plot (3D) for recovery of Au by *P. balearica* SAE1 showing effect of interaction of parameters: **a** pulp density and pH; **b** temperature and pH; **c** glycine concentration and pH; **d** glycine concentration and pulp density; **e** temperature and pulp density; **f** temperature and glycine concentration

(Table 3). These terms (individual, interaction, and quadratic variables) play an important role in obtaining the optimum conditions for Au and Ag recovery.

Response plots

Au recovery Au recovery was carried out using two-step bioleaching process. During two-step bioleaching process gold reacts with biogenic cyanide and form dicyanoaurate, a water-soluble complex (Faramarzi et al. 2004). Brandl et al. (2008) studied bioleaching of Au, Ag and platinum (Pt) from metal containing solid waste using cyanogenic bacteria. They reported 68.5% mobilization of Au as dicyanoaurate from the total Au added. Jujun et al. (2014) evaluated biorecovery of precious metals from waste PCBs using *Pseudomonas chlororaphis* and reported 8.2% of gold solu-

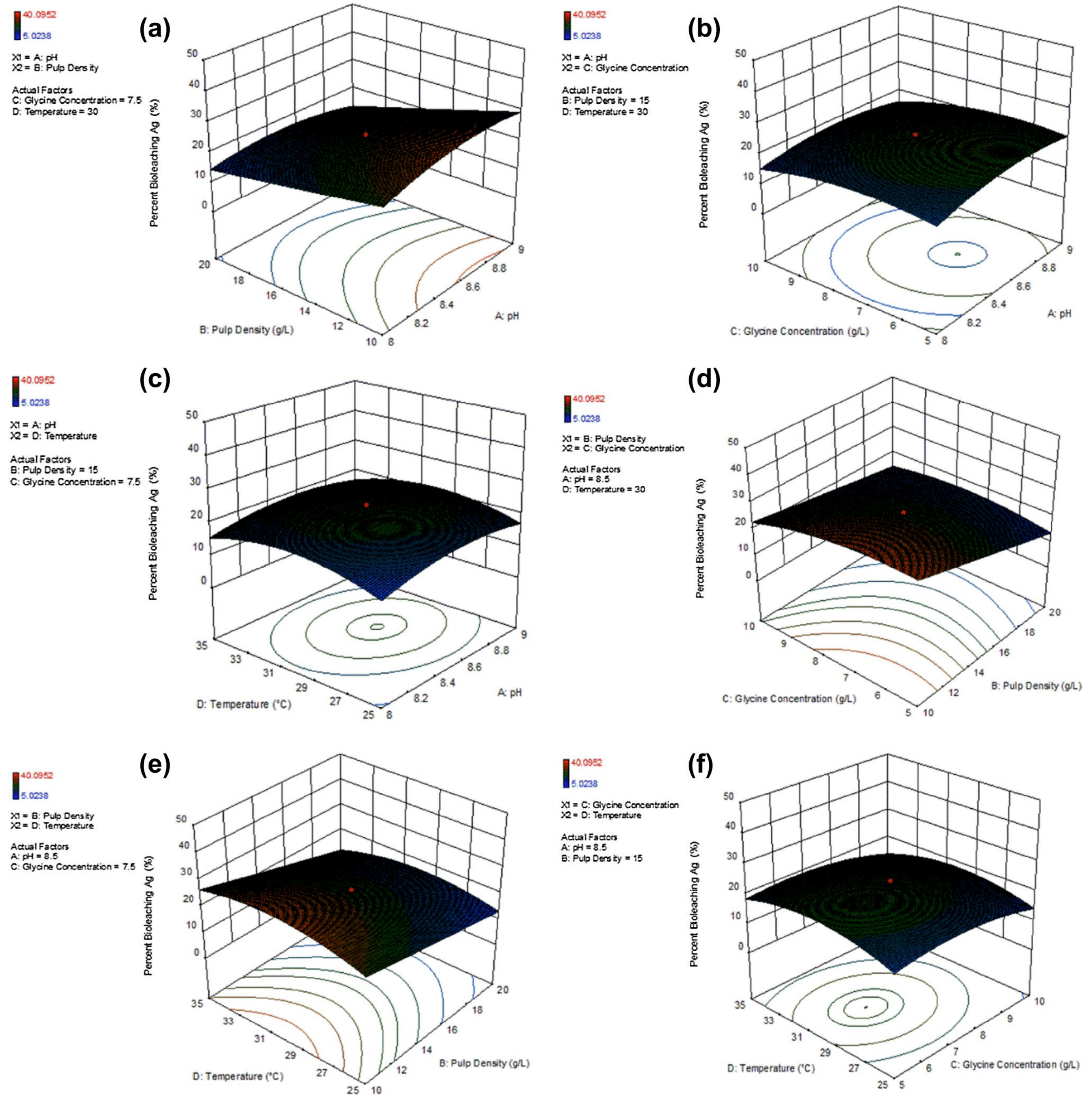

Fig. 3 Response surface plot (3D) for Ag recovery by *P. balearica* SAE1 showing effect of interaction of parameters: **a** pulp density and pH; **b** glycine concentration and pH; **c** temperature and pH; **d** glycine concentration and pulp density; **e** temperature and pulp density; **f** temperature and glycine concentration

bilization. In another study, Işıldar et al. (2016) performed pretreatment of e-waste by mixture of *A. ferrivorans* and *A. thiooxidans*; subsequently 44% of Au was recovered using *P. putida*. Pradhan and Kumar (2012) reported 73% of Au recovery from e-waste (PCBs) using a mixed culture of *C. violaceum* and *P. aeruginosa*.

Figure 2a illustrates the interaction effect of pH and pulp density on Au recovery. The maximum Au recovery was observed at high pH (> 8 to < 9) and low pulp density (10 g/L). The higher Au recovery at pH > 8 is due to the pKa of cyanide. At pKa = 9.3 and pH value higher than 9.3; the dominant form of cyanide is CN^-. At pH 7, the dominant form of cyanide is HCN gas, which is volatile and may lose out from the solution (Arshadi et al. 2016). According to Faramarzi et al. (2004), Au forms a water-soluble complex known as dicyanoaurate when it reacts with cyanide. The dissolution of Au involves an electrochemical process in which the anodic reaction is gold oxidation whilst the cathodic reaction is oxygen reduction, as shown in Eqs. (5) and (6) (Akcil et al. 2015). The overall reaction can be represented as given in Eq. (7).

$$\text{Cathode reaction: } 4\text{Au} + 8\text{CN}^- \rightarrow 4\text{Au(CN)}_2^- + 4\text{e}^-. \tag{5}$$

$$\text{Anode reaction: } \text{O}_2 + 2\text{H}_2\text{O} + 4\text{e} \rightarrow 4\text{OH}^-. \tag{6}$$

$$\text{Overall: } 4\text{Au} + 8\text{CN}^- + \text{O}_2 + 2\text{H}_2\text{O} \ = 4\text{Au(CN)}_2^- + 4\text{OH}^-. \tag{7}$$

It was observed that increase in pulp density > 10 g/L resulted in decreased Au recovery. At higher pulp density, the environmental toxicity increases, but consequently bacterial activity decreases (Natarajan and Ting 2014). Pradhan and Kumar (2012) also reported that increasing the pulp density; metals recovery decreased. Figure 2b represents the effect of pH and temperature on Au recovery. Figure 2c shows the importance of glycine concentration on Au recovery. An increase in glycine concentration from 5 to 7.5 g/L resulted in an increase in Au mobilization. A further increase in glycine concentration > 7.5 g/L caused the Au mobilization to decrease due to the toxic effect of glycine on bacterial growth (Shin et al. 2013). According to Arshadi et al. (2016), higher amount of glycine is more effective on Au recovery. Bacterial cyanide production can be enhanced by increasing the glycine concentration (a direct precursor of cyanide) till it is not toxic to bacteria. Shin et al. (2013) reported maximum amount of biogenic cyanide production at glycine concentration of 5 g/L using *C. violaceum*. Figure 2b, c exhibit circular contours which mean the factors did not show any interaction and have an independent effect on Au mobilization. Figure 2d illustrates the effect of the glycine concentration and pulp density. The maximum recovery of Au was observed at a glycine concentration of 6–7 g/L (approximately) and low pulp density of 10 g/L. Figure 2e shows the effect of temperature and pulp density. The curves of contour show interaction between the parameters, but the interaction is not statistically significant as per Table 3. The maximum recovery of Au was observed at a temperature of approximately 32 °C and low pulp density. Figure 2f exhibits circular contours and the maximum mobilization of Au was at the center of the concentric circle. Overall, CCD-RSM optimization enhanced Au recovery up to 72.4% at a pulp density of 5 g/L.

Silver recovery Ag leaching by *P. balearica* SAE1 was enhanced up to 40% using CCD of RSM. Figure 3a illustrates the interaction effect of pH and pulp density on Ag mobilization. The curves of the contour line show the effect of parameters on Ag recovery by *P. balearica* SAE1. Ag mobilization decreased as the pulp density increased from 10 to 20 g/L, whereas Ag mobilization increased as the pH increased from 8 to 9. The maximum Ag mobilization was obtained at low pulp density (10 g/L) and high pH (approximately 8.8). Our results are in reasonable agreement with Arshadi et al. (2016), who reported higher recovery of Au at alkaline pH (10) and low pulp density from mobile phone PCBs using cyanogenic bacterial strain *B. megaterium*. Figure 3b shows the interaction effect of glycine concentration and pH on Ag recovery from discarded CPCBs. It was observed that interaction between glycine concentration and pH showed a blurred significance (p = 0.3). Figure 3c illustrates the effect of temperature and pH on Ag recovery. In Table 3, it was observed that the interaction effect of temperature and pH was not significant ($p = 0.6$). Figure 3b, c exhibits circular contour during Ag mobilization, indicative of the independence of the factors toward the response. The maximum Ag mobilization falls at the center of the concentric circle. Figure 3d shows the effect of glycine and pulp density on Ag mobilization. The maximum Ag mobilization was observed at low glycine concentration (approximately 6.5 g/L) and pulp density (10 g/L). Further, increase in glycine concentration and pulp density lead to a decrease in Ag mobilization. This is because higher amounts of glycine and e-waste pulp density become toxic to bacterial cells and inhibit their metabolic activity, thereby resulting in poor lixiviant production and low metals recovery (Shin et al. 2013). The interaction effect of glycine and pH on Ag recovery shows an appreciable level of significance (p = 0.001). The contours of Fig. 3d were elliptical curves that indicate the interaction among the parameters. Figure 3e illustrates the effect of temperature and pulp density on Ag mobilization. It was observed that an increase in temperature from 25 to 32 °C increased the Ag leaching. This may correspond to the fact that maximum biogenic cyanide production occurs at a temperature range of 25–35 °C (Jujun et al. 2014). Figure 3e exhibits a circular contour, which indicates no interaction of temperature and glycine concentrations on Ag recovery. Overall, pulp density was the most influential parameter in Ag recovery from discarded CPCBs with a negative effect, which means Ag mobilization decreased as pulp density increased. Thus, the results provide estimates of optimized ranges of factors influencing the recovery of Au and Ag from e-waste and will be useful to industry for scale-up studies.

Model validation

To confirm the validity of models, independent bioleaching experiments (duplicates) were performed under optimal conditions as predicted by the software. According to the software numerical optimization, the maximum predicted

Table 4 Point prediction and experimental recovery of precious metals (Au and Ag) under statistical optimal conditions for model validation and accuracy

Response	Predicted response		Actual response	
	(%)	(mg/kg)	(%)	(mg/kg)
Au recovery	67.6	54	73.9	59
Ag recovery	39.2	156	41.6	166

recovery of Au and Ag was 67.6 and 39.2%, respectively (Table 4). The optimum conditions for maximum Au and Ag recovery were initial pH 8.6, pulp density 5 g/L, temperature 31.2 °C, and glycine concentration of 6.8 g/L, respectively. The optimum conditions obtained were in agreement with the results of the 3D plots. The confirmatory results are presented in Table 4, which show that the obtained responses for Au (73.9%) and Ag (41.6%) recovery were in good agreement with predicted responses. Control experiments without bacteria were also performed in parallel and showed no mobilization of Au and Ag, hence validating the accuracy of the models.

Conclusion

Two-step bioleaching process was optimized to leach Au and Ag from discarded CPCBs using CCD-RSM by a cyanogenic bacterium *P. balearica* SAE1. Two quadratic models were proposed by RSM which can be utilized as an efficient tool to predict Au and Ag recovery through bioleaching. The maximum recovery occurred at initial pH 8.6, pulp density 5 g/L, temperature 31.2 °C, and glycine concentration 6.8 g/L, which led to extraction of 73.9% of Au and 41.6% of Ag, respectively. The glycine concentration and pulp density have a significant effect on bioleaching of Au and Ag by *P. balearica*. The high yield of bioleaching process by *P. balearica* makes it suitable for industrial application to extract precious metals from e-waste. The safe disposal of residual e-waste after resource recovery is still a major cause of concern.

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Compliance with ethical standards

Conflict of interest No conflicts of interest.

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