Bioleaching of gold and copper from waste mobile phone PCBs by using a cyanogenic bacterium

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Abstract

This paper highlights the application of *Chromobacterium violaceum (C. violaceum)*, a cyanide generating bacterium to leach out gold and copper from the waste mobile PCB sample containing around 34.5% of copper and 0.0025% of gold in YP medium (yeast extract- 5g/L; polypeptone- 10g/L; glycine- 5g/L and $MgSO_4.7H_2O$ -1g/L). The bioleaching experiments were mostly carried out at 30^{o}C temperature and 15g/L pulp density while varying initial pH in the range 8 – 11 and shaking in an incubator at 150 rpm. Dissolution of gold and copper increased from 7.78% (0.225 ppm) to 10.8% (0.46 ppm) and 4.9 (419ppm) to 11.4% (879 ppm) in 8 days time with increase in pH from 8-11 and 8-10 respectively. The bioleaching of metals with *C. violaceum* was also investigated in presence of hydrogen peroxide as a source of oxygen in the system. *A*ddition of 0.004% (v/v) H_2O_2 solution increased the copper bioleaching to 23.54 % (1518 ppm) and 24.6% (1743 ppm) at pH 9.0 and 10, respectively in 8 days; improvement in leaching was insignificant for gold with 10.68% and 11.31% recovery at pH 9.0 and 11.0 under this condition. The results show that the bioleaching of gold and copper from the waste mobile PCBs with *C.violaceum* can be utilized to recycle such a waste material in environment friendly manner.

Key words: Bioleaching, Chromobacterium violaceum, recovery of gold and copper

Introduction

The waste printed circuit boards (PCBs) of used electrical and electronic equipments (WEE) are considered a valuable secondary resource because the amount of metals contained in these wastes is higher than that of most natural resources. The waste PCBs of mobile phones especially contains high amount of copper and a few precious metals such as gold, silver etc. The recovery of the precious and other metals from such a waste will inevitably ensure resource recycling and reduce environmental degradation generally associated with metal toxicity. Regarding the extraction of precious metals, the cyanidation process is being used in the gold metallurgy for a very long time to treat ores and concentrates (Marsden et al, 1960; Rose, 2010). For the processing of refractory ores such as those comprising of pyrite and arsenopyrite with gold and other precious metals like silver encapsulated within them or intimately connected with them, the bacterial oxidation (Iglesias et al, 1994) by *Acidithiobacillus ferrooxidans* has been successfully implemented in the process called biobeneficiation with high extraction of gold in the subsequent cyanidation step. The slow kinetics observed in the normal bio-oxidation of pyrite and arsenopyrite to release gold for leaching have been overcome by using large bioreactors on the industrial scale. With the success of biobeneficiation process for refractory ores, the new approach being explored as an alternative to the gold cyanidation process is to replace cyanide salts by a microorganisms, particularly the cyanogenic bacteria that can produce cyanide ions and dissolve gold directly. Amongst the known cyanogenic bacteria such as *Pseudomonas aeruginosa*, *P.fluorescens, Escherichia coli* and *Chromobacterium violaceum (C.violaceum)*, the use of *C. violaceum* has been found to be quite effective for the biorecovery of gold from different materials (Knowles et al., 2004). *C.*

violaceum is a mesophilic, gram-negative, motile and facultative anaerobe (Moss et al., 1981) and shows its association with maximum number of enzymes as compared to any other bacteria and therefore, rated as an effective cyanogenic species (Tania et al., 2004)

As such the gold bioleaching process using a microorganism may be considered as one of the most ecofriendly options. The advantages associated with the bioprocessing particularly the requirement of low capital cost, low energy consumption, and minimal chemical/ consumable needed, make it attractive for extracting gold from different resources including waste mobile phone PCBs. Earlier, for the gold leaching using cyanide generating bacteria , major stress was given to understand the dissolution behavior of metal powders (Smith et al, 1985; Kita et al., 2005, 2006) in presence of various microbes and in a few cases the extraction of gold from ores (Lawson et al, 1999). The treatment of secondary resources was also reported by Brandl et al. (2008) including the waste printed circuit boards [Faramarzi. 2004] of waste electric and electronic equipment (WEEE) using cyanobacteria. Initial study on the bioleaching of gold and copper from the waste mobile phone PCBs by using a cyanide generating microbe was recently carried out in the Investigators Laboratory (Chi et al., 2010). Under the bacterial activity of C. violaceum, glycine is converted to cyanide (Knowles et al., 1986) by using β-cyanoalanine synthase (Rodgers, 1978, Macadam and Knowles, 1984) that can react favourably with gold and copper at room temperature (298K) as follows (Rees et al., 1999):

$$4Au + 8CN^- + O_2 + 2H_2O = 4Au(CN)_2^- + 4OH^-, \Delta G^0 = -392.234 kJ/mol \quad (1)$$

$$4Cu + 8CN^- + O_2 + 2H_2O = 4Cu(CN)_2^- + 4OH^-, \Delta G^0 = -322.027 kJ/mol \quad (2)$$

$$Cu(CN)_2^- + CN^- + O_2 + 2H_2O = Cu(CN)_3^{2-} + 4OH^-, \Delta G^0 = -228.585 kJ/mol \quad (3)$$

$$Cu(CN)_3^{-2} + CN^- + O_2 + 2H_2O = Cu(CN)_4^{3-} + 4OH^-, \Delta G^0 = \text{-163.323kJ/mol} \quad (4)$$

In the reactions above, oxygen plays the role of an oxidant and cyanide ion as the complexing agent. In the cyanidation using C. violaceum the gold and copper dissolution depends on a number of factors, including cyanide concentration, dissolved oxygen (DO), pH, particle size, mixing and temperature, of which DO plays a very important role. Furthermore, during the growth phase of C. violaceum DO is consumed for the respiration which inhibits the gold and copper dissolution (Kita et al. 2005, 2008). In view of this, the bioleaching of gold and copper from the waste mobile phone PCBs has been investigated by using C. violaceum in absence and in presence of H_2O_2 as a source of oxygen to supplement the DO level and results are presented in this paper.

Materials and methods

C. violaceum used in this study was supplied by Korean Collection for Type Cultures (KCTC), Biological Resource Center (BRC) located in Korea Research Institute of Bioscience and Biotechnology (KRIBB) Daejeon. Revival of *C.violaceum* in a medium comprising of 5g/L polypeptone and 3g/L beef extract in 100mL solution was done at 6.8 pH and 30^0C temperature in an incubator shaker at 150 rpm for over 2 days. Bacterial cell was calculated based on the viable suspended cell counts using drop plate method with series dilution on agar medium (containing beef extract 3.0g/L, peptone 5g/L, agar 15g/L). Plates were incubated for 24h at 37^oC. The plates containing the pure culture were saved in refrigerator sealed with parafilm at low temperature.

Waste mobile phones including different models were supplied by the recycling companies in Korea. To be used in the bioleaching experiments, the sample of PCBs of waste mobile phone was prepared by cutting in to pieces. Firstly, mobile phone was manually dismantled to separate cover and printed circuit boards (PCBs). After that PCBs was dismantled by hand to remove iron and non-metal components and then cut to 1mm x 1mm size by scissor. The copper and gold from the solid samples were dissolved in aqua regia and the metal contents in the solutions were determined by Atomic Absorption spectrometer (AAS PerkinElmer 400). The total metal content in the PCBs was found to be ~58 %, in which copper analyzed was typically 34.52% along with 0.0025% gold.

All metal biodissolution experiments were carried out in sterilized Erlenmeyer flask of 250 mL capacity containing 200mL of YP media (5g/L yeast extract, 10g/L polypeptone and 5g/L glycine) and 1g/L $MgSO_4.7H_2O$ at 15g/L pulp density. A 1mL growing culture of *C. violaceum* under log phase was added to the flask asceptically. The flasks were incubated at 30^0 C in an orbital motion shaker at 150 rpm. To investigate the effect of pH on the gold and copper dissolution, pH of medium was adjusted in the range 8 – 11 by the addition of 2M NaOH solution. In order to increase the dissolved oxygen (DO) concentration H_2O_2 solution in the concentration range 0.00 – 0.005% (v/v) was added into the medium after the first day (24h) of the leaching as the dissolved oxygen level observed to decrease to a minimal value in this period. Effect of hydrogen peroxide concentration on the metal dissolution was investigated by adding 1mL H_2O_2 solution to the medium at every 1h interval till the termination of the experiments. After completing the bioleaching the solution was filtered over Whatman 42 filter paper and the leach liquor was analyzed for gold and copper to calculate the extent of metals leached out. The residue obtained in each experiment was dissolved in aqua regia and the solution was analyzed for the metals of interest by AAS to compute the material balance. The concentration of cyanide generated by bacteria during the process was determined colorimetrically (Clesceri et al., 1998) by UV/VIS spectrometer (1601, Shimadzu) at 580.5nm using pyridine and barbituric acid. Dissolved oxygen (DO) and pH were determined everyday by DO meter (HQ40d, Hach) and pH meter (Orion- 720A), respectively

Results and discussion

Initially, experiments were performed to estimate the amount of cyanide generated by C. violaceum at different pH in YP media at 30^0 C and results are presented in Fig.1. It is apparent from the data that cyanide generation was high of 68 ppm at pH 9.o in 5 days which dropped to 56 ppm in 7 days. The cyanide generation was lower at still higher pH. The change in DO concentration with time in YP media is presented in Fig. 2 which shows that almost entire dissolved oxygen initially present (6.8 ppm) was consumed by bacteria in 24 h for its respiration and thereafter the DO level remained at the low level (0.06 ppm). The bacterial count was also determined (data not given) under this condition and was found to be $4.18x10^9$ cells/ mL in 5 days time at pH 9.0, which decreased to the lower value of $8.7x10^8$ cells/ mL at pH 11.0. The pattern of gold and copper dissolved (concentration - ppm) and leaching efficiency from the waste PCBs sample at different pH is given in Fig. 3 at 30^oC temperature. The concentration of

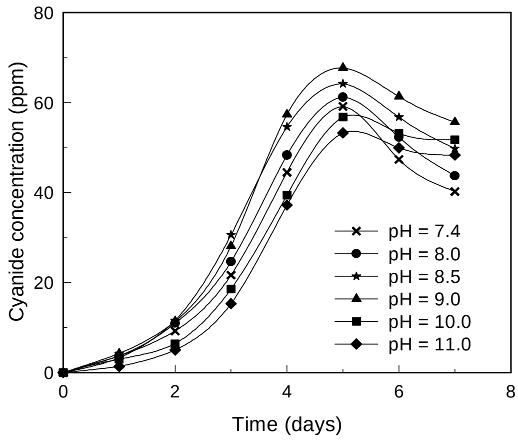

Figure 1. Effect of pH on cyanide generation in YP medium at 30^oC

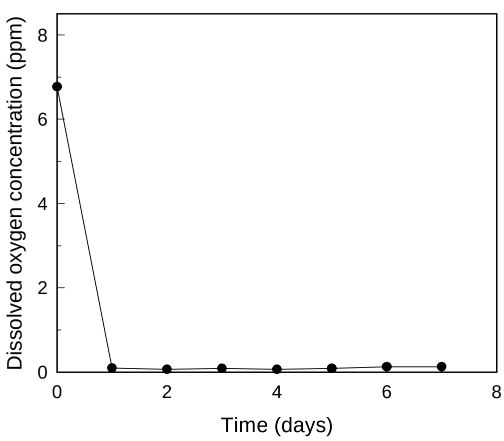

Figure 2. Change of DO concentration in the YP medium at 30^oC

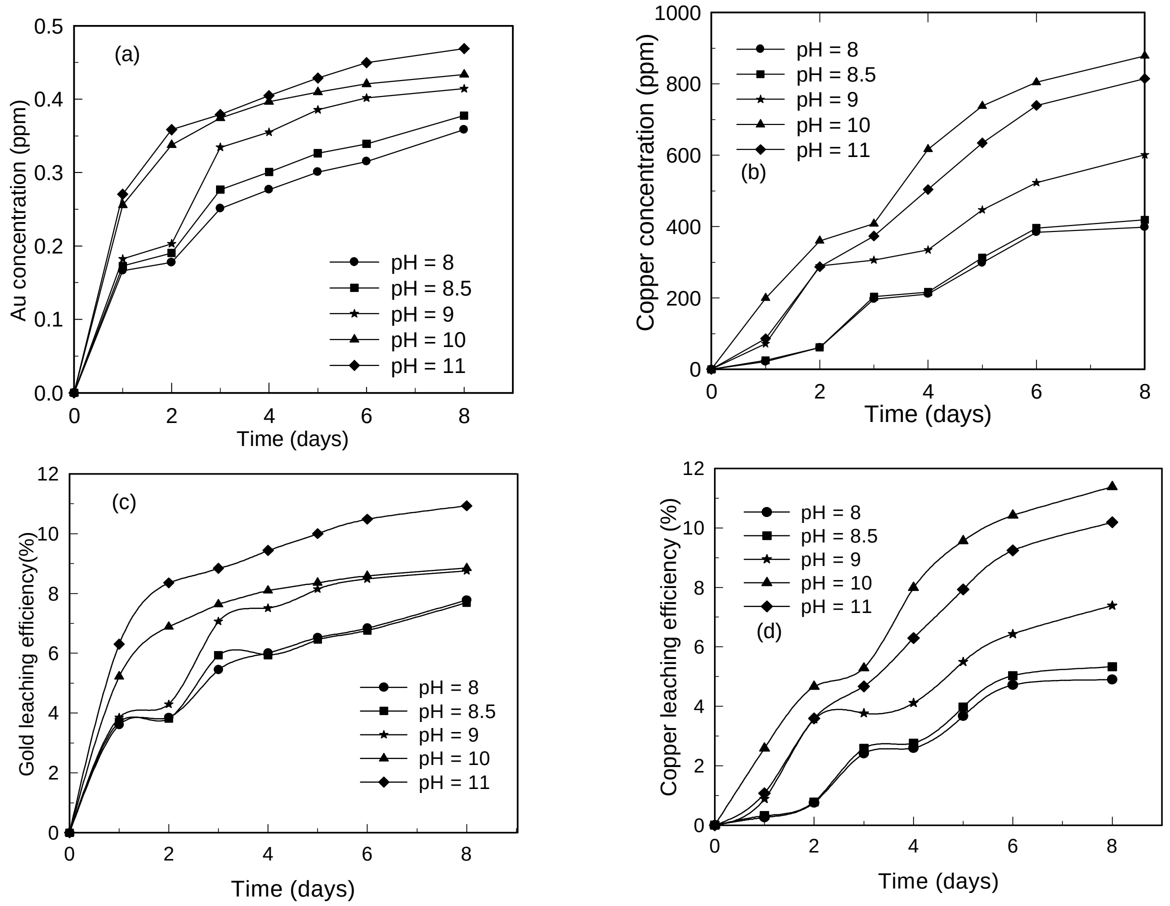

Figure 3. Effect of pH on gold and copper leaching in YP medium at 30^{o}C

gold (Figure 3a) and copper (Figure 3b) in the solution was found to be 0.46 ppm and 879 ppm at pH 11.0 and 10.0 in 8days respectively, whereas, lowest concentration of the two metals was found to be 0.35 ppm and 419 ppm at pH 8.0. This may be correlated to the metal leaching efficiency which was found to increase from 7.78% to 10.9% for gold at pH 11.0 (Figure 3c) and 4.9% to 11.4% for copper at pH 10.0 (Figure 3d). It may also be mentioned that gold dissolution was faster in the initial two days and then became slow as compared to copper which show higher disolution rate after 3 days of leaching in the entire pH range that was investigated.

The dissolution of metals may be explained by looking at the Eh-pH diagrammes of gold and copper (Marsden and House, 1960, Alonso-Gonzalez et al., 2010). Different copper cyanide species are formed at different pH. The $Cu(CN)_2^-$ is stable at pH less than 9 while $Cu(CN)_3^-$ and $Cu(CN)_4^{3-}$ will be generated at pH above 9.0. The concentration of $Cu(CN)_2^-$ thus decreases at higher pH and copper dissolves mainly as $Cu(CN)_3^{2-}$ and $Cu(CN)_4^{3-}$. At higher pH the higher amount of copper dissolution was therefore observed than that at lower pH in presence of limited amount of cyanide generated in the present system.. As regards gold, the $Au(CN)_2^-$ is formed at higher pH even with low oxidation potential or the low DO values, although high amount of free cyanide and higher DO is reported to favour gold dissolution in general (Mardsen and House, 1960).

Based on the above results, it was considered worthwhile to provide increased DO concentration by adding hydrogen peroxide during the bioleaching in presence of C. violaceum. However, the addition of H_2O_2 may affect the survival and the growth of bacteria as the higher dose is reported to harm the C. violaceum (Kita et al, 2008). The effect of hydrogen peroxide concentration on the DO level in YP medium was investigated and its effect on the cell population was also recorded. Data given in Figure 4 show that DO level was maximum of 22 ppm when 0.005% (v/v) H_2O_2 was added in the solution as comapred to 12 ppm DO concentration with 0.004% hydrogen peroxide. However, the results on

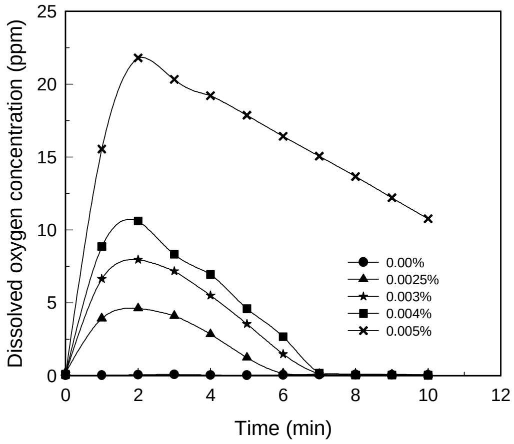

Figure 4. Increase of DO concentration in YP medium with various H_2O_2 concentration at 9.0pH

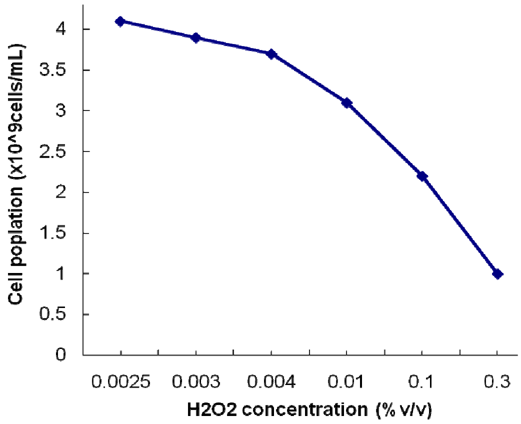

Figure 5. Effect of H_2O_2 concentration on bacteria cell count in YP medium at pH 9.0

bacterial population given in Fig. 5 show that the hydrogen peroxide concentration beyond 0.004% in the media has adversely affected the cell count. With increase in H_2O_2 concentration from 0.0025% to 0.004%, the cell population was only marginally affected; it decreased from $4.1x10^9$ to $3.8x10^9$ cells /mL The addition of 0.005% H_2O_2, in the YP medium containing the bacteria the cell count decreased substantially.

The effect of hydrogen peroxide concentration on the leachability of gold and copper from the waste PCBs sample at various pH was examined in presence of C. violaceum and data at pH 10.0 are presented in Fig. 6. The addition of hydrogen peroxide was found to be very effective for copper and was not that effective for gold. The highest gold as well as copper dissolution at pH 10.0 was observed with 0.004% H_2O_2.; the recovery being 11.21% for gold and 24.6% for copper in 8 days. At hydrogen peroxide concentration of 0,005%, the leaching of the two metals was lower because of decreased cell population under this condition.

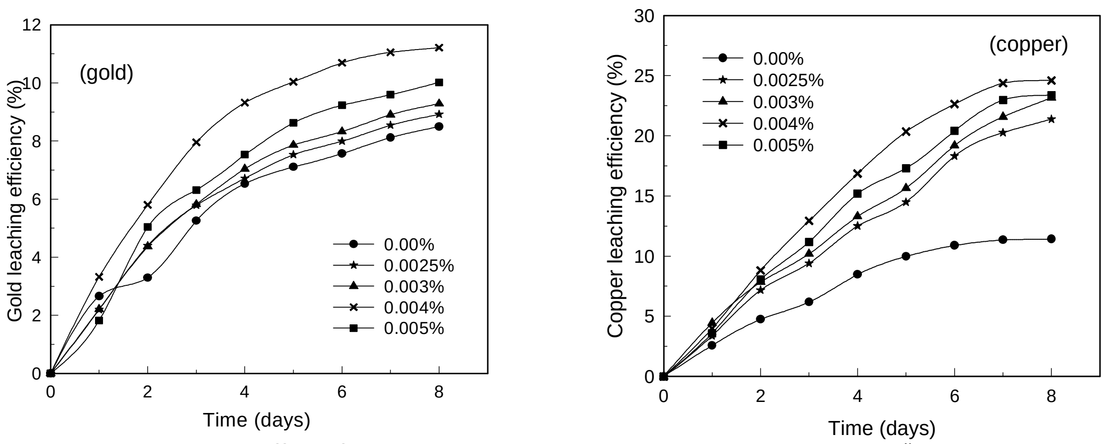

Figure 6. Effect of H_2O_2 concentration on bioleaching at pH 10 and 30^oC

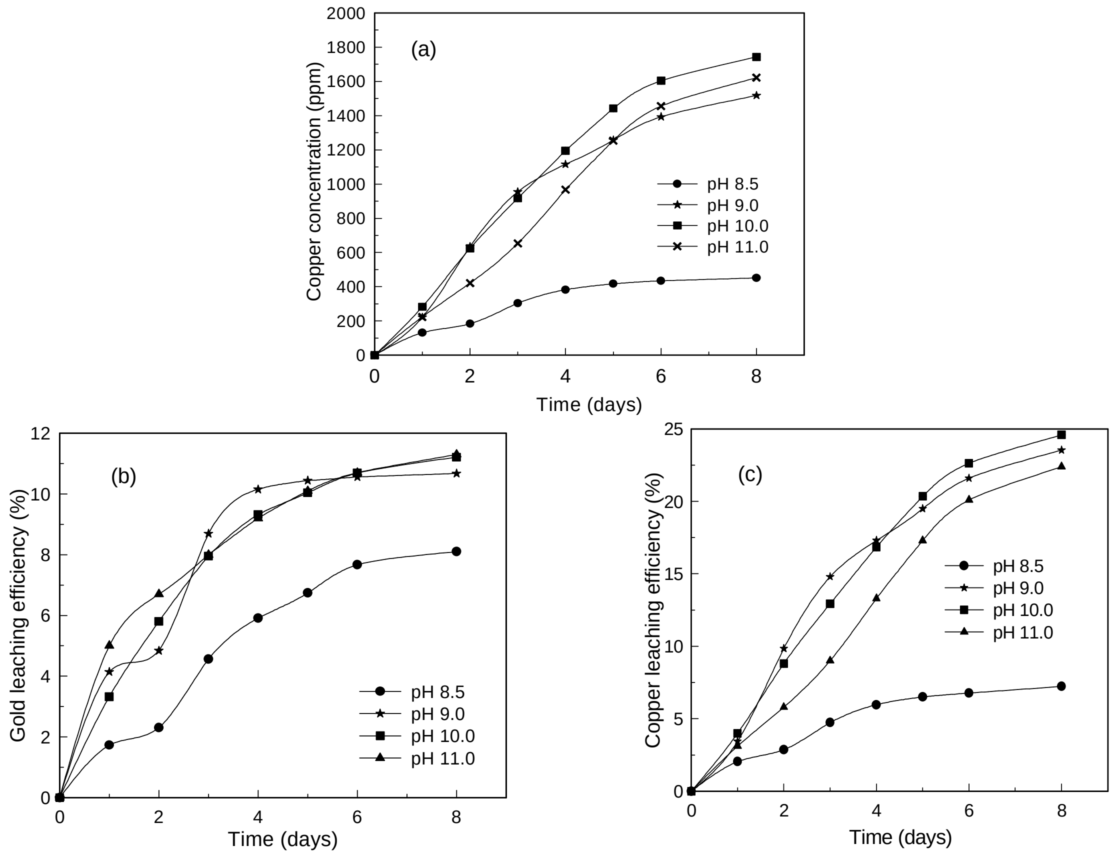

Figure 7 . Effect of pH in presence of 0.004% (v/v) H_2O_2 on gold and copper leaching in YP medium at 30^oC

In order to understand the effect of hydrogen peroxide during bioleaching of metals, the experiments were also conducted at varying pH and results are plotted in Fig. 7. The amount of copper leached out increased from 7.23% (463 ppm) to 24.6% (1734 ppm) with increase in pH from 8.5 to 10.0 after 8 days leaching when 0.004% H_2O_2 was added in the solution. The hydrogen peroxide thus appears to improve the recovery of copper because of increased DO concentration during the bioleaching. However, the gold recovery was not that much improved when H_2O_2 was added during the leaching in contrast to the leaching of copper. The gold leaching increased only marginally from 8.1% to 11.32% with increase in pH from 8.5 to 11.0.; the leaching efficiency of gold was nearly same (11.21%) at pH 10.0 in presence of H_2O_2.. As discussed above (Fig. 3d), the gold bioleaching in absence of hydrogen peroxide was observed to be 10.8% at pH 11.0 in 8 days. It may thus be concluded that the addition of hydrogen peroxide to increase the DO concentration has improved mostly the copper dissolution with a little improvement in the gold leaching. This may be attributed to the galvanic interaction of the two metals particularly in presence of relatively lower cyanide concentration available in the solution with the metabolic activity of *C. violaceum.*

Conclusion

The copper and gold from the waste mobile phone PCBs can be recovered by the cyanide generated in the YP media by *C. violaceum*. The dissolved oxygen plays a definite role during the bioleaching of metals since the DO level decreases significantly within 24 h because of respiration of the bacteria at 30^{0}C. In order to supplement the DO level during the leaching with *C. violaceum*, hydrogen peroxide was added in the solution at a definite interval and the results show that the its concentration upto 0.004% increased the DO level without affecting the bacteria significantly. The gold recovery in presence of hydrogen peroxide as a source of oxygen increased only marginally from 10.8% to 11.31% at pH 11.0 as compared to copper which showed improved leaching from 11.4% to 24.6% at pH 10.0. The reason for preferred copper leaching than that of gold may be the result of low amount of cyanide generation by *C.violaceum* along with the high metal content in the sample which consumed most of the cyanide produced in the system with higher DO level.

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