

Bacterial Cyanide Generation in Presence of Metal Ions (Na^+ , Mg^{2+} , Fe^{2+} , Pb^{2+}) and Gold Bioleaching from Waste PCBs

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Abstract

As an alternative to use cyanide chemical for gold extraction, the use of a cyanogenic bacterium viz. *Chromobacterium violaceum* (*C. violaceum*) in YP medium has been investigated. The catalytic role of metal ions such as Na^+ , Mg^{2+} , Fe^{2+} and Pb^{2+} , besides the addition of Na_2HPO_4 , a nutrient, on the cyanide generation efficiency of the bacterium in this medium has been elucidated. While MgSO_4 and FeSO_4 in the medium were equally effective for the cyanide generation, presence of Na_2HPO_4 and $\text{Pb}(\text{NO}_3)_2$ was still better. In order to examine the effectiveness of *C. violaceum* in the YP media to generate cyanide ions, the dissolution of gold and copper from waste mobile phone printed circuit boards (PCBs), a good source of gold and copper in alkaline conditions was tested at 30°C at varying pH and metal ions. Gold leaching was found to be 11% in 8 days at pH 11.0 in presence of 4.0×10^{-3} mol/L MgSO_4 whereas, copper recovery was high (11.4%) at pH 10.0. Addition of 1.0×10^{-2} mol/L Na_2HPO_4 and 3.0×10^{-6} mol/L $\text{Pb}(\text{NO}_3)_2$ in the YP medium increased copper leaching to 30.3% and 38.1%, respectively at pH 10.0 in 8 days, but these were not effective for gold leaching.

Key words: *C. violaceum*, cyanide generation, bioleaching, printed circuit boards (PCBs), gold.

1. Introduction

Cyanidation process is widely used in the gold metallurgy to recover gold from the ores and concentrates, in spite of the fact that it is highly toxic and its impact on the environment, especially aquatic species and human being is well known. In the recent years, an alternate approach to the gold cyanidation process is being considered in which cyanide salts are intended to be replaced by a microorganism, particularly the cyanogenic bacteria that can produce cyanide ions and dissolve gold. So far several types of cyanogenic bacteria have been identified and reported, which include *Pseudomonas aeruginosa*, *P.fluorescens*, *Escherichia coli* and *Chromobacterium violaceum* (*C. violaceum*) (Moss *et al.*, 1981; Creczynski-Pasa *et al.*, 2004).

Amongst these the *C. violaceum* has largely been found to be more effective for the bio-dissolution of gold from different materials because of associated cyanide metabolic activities. In this study, the *C. violaceum* has been used in YP medium containing glycine as one of the components. It is a mesophilic, motile, gram-negative and facultative anaerobe (Moss *et al.*, 1981). The importance attached to *C. violaceum* as the cyanide generating bacteria stems from its characteristic features of HCN synthase in comparison to other cyanogenic bacteria. When the versatility of the energy metabolism of *C. violaceum* is compared with that of bacteria from other genus, it is generally found to be associated with six enzymes, whilst other bacteria lack one or more of these enzymes (Creczynski-Pasa *et al.*, 2004).

As regards the process of metal cyanidation using a bacterium such as *C. violaceum*, it is believed to follow the indirect leaching mechanism consisting of two stages. First, the conversion of glycine to hydrogen cyanide (leaching chemical) takes place by the associated enzymes under the metabolic activity of HCN synthase (*stage I*) (Knowles *et al.*, 1986), which is followed by the reaction of cyanide ions with the substrate to dissolve gold/ copper (*stage II*) as presented in Figure 1.

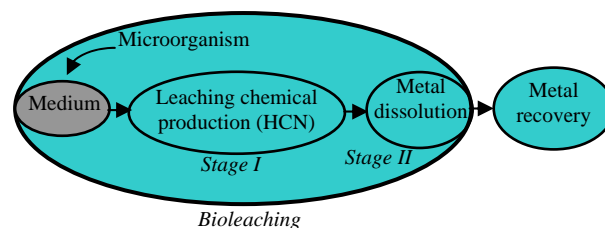


Figure 1. Schematic of metal cyanidation using a microorganism.

Actually, the amount of cyanide generation by *C. violaceum* depends very much on the medium conditions in which it is grown. Mention may be made of previous investigations with different

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organic components which include the use of peptone 1% (wt/v), L-glutamate salts (Michaels *et al.*, 1965); peptone 1% (wt/v), 2 mmol/L -glycine or 0.5 mmol/L - methionine (Rodgers *et al.*, 1978) and several other organic components (Collins *et al.*, 1980). Besides the effect of organic components, some metal ions such as Na^+ , Mg^{2+} added into the medium at a low concentration can catalyze and enhance cyanide generation capacity of *C. violaceum*. Addition of Na_2HPO_4 in small amounts was also found to increase the amount of cyanide generated by such bacteria. In earlier researches addition of these metal ions on the metabolic activity of the bacteria was studied using media containing different organic components such as tryptone (10 g/L) and yeast extract (5 g/L) (Faramarzi *et al.*, 2004; Brandl Helmut *et al.*, 2008), polypeptone (10 g/L) and yeast extract (2 g/L) (Kita *et al.* 2005; Kita *et al.*, 2008; Lawson *et al.*, 1999). It is therefore very difficult to compare the effect of these metal ions on the cyanide generation capacity of *C. violaceum* in a particular medium.

As such the gold bioleaching process using a microorganism may be considered as one of the most eco-friendly options, but the dissolution rate may not be as fast as one can get from cyanide chemicals. Earlier researches (Kita *et al.*, 2005; Lawson *et al.*, 1999) have shown that the presence of some metal ions can improve the bacterial cyanidation process. The enhanced rate of gold dissolution was observed with cyanide solutions in presence of small amounts of $\text{Pb}(\text{NO}_3)_2$ which activated the gold surface leading to the high reactivity and faster leaching (Sandenbergh *et al.*, 2001; May *et al.*, 2005). However, effect of lead ions on the extent of cyanide generation by the cyanogenic microorganisms was not investigated. As regards the application of the cyanide generating bacteria for gold and copper leaching major stress was given to understand the dissolution behavior of metal powders (Brandl, Helmut *et al.*, 2005; Kita *et al.*, 2006; Smith *et al.*, 1985) in presence of various microbes and in a few cases the extraction of gold from ores (Lawson *et al.*, 1999). Recently, some efforts were made to treat secondary resources including the waste printed circuit boards PCBs by using cyanobacteria (Faramarzi *et al.*, 2004; Brand *et al.*, 2008). In view of the fact that waste mobile phone PCBs are rich source of gold, besides copper and other metals, the microbial recovery of gold by the cyanogenic bacteria may be worthwhile to pursue as a part of the overall objective to develop favorable recycling technology for waste PCBs. The residue from the bioleaching can then be treated by a suitable hydrometallurgical process to extract copper and other metals. Thus, the effect

of different metal ions including lead and Na_2HPO_4 on the cyanide generation of *C. violaceum* in a particular medium with the fixed organic components has been investigated in this paper. Potential use of the biogenic cyanide produced from *C. violaceum* under the effect of these metal ions to dissolve gold from the waste mobile phone PCBs has also been examined to establish an alternate process for their recycling.

2. Experimental

2.1. Microorganism and waste PCBs

C. violaceum was supplied by Korea Collection for Type Cultures (KCTC)- Biological Resource Center (BRC), Daejeon. Revival of *C. violaceum* in a medium comprising of 5 g/L polypeptone and 3 g/L beef extract in 100mL solution, was done at pH 6.8 and 30° C temperature in an incubator shaker at 150 rpm for over 2 days. To check the purity, the revived culture was incubated on agar plate for 24h at 37° C. Viable suspended cells were counted using drop plate method with series dilution on agar medium (beef extract 3.0 g/L, peptone 5 g/L, agar 15 g/L). The plates containing the pure culture were saved in refrigerator sealed with parafilm. A known volume of growing culture in the medium was inoculated aseptically for investigating the effect of different metal ions on the amount of cyanide generation. In this research, the growth media consisting of yeast extract (5 g/L), polypeptone (10 g/L) and glycine (5 g/L) designated as YP media, were used. The sample of waste mobile phone PCBs was cut to 1 mm x 1 mm size for bioleaching experiments by a scissor. The analysis of a typical sample was found to be around 34.5 (wt %) Cu and 0.025 (wt %) Au. The effect of different metal ions and Na_2HPO_4 on the cyanide production of *C. violaceum* in YP medium was studied. The concentrations of metal ions viz. NaCl, MgSO_4 and FeSO_4 that did not affect the cell population significantly were determined from the preliminary experiments conducted in their presence. By adding 0.17 mol/L NaCl, 4.0×10^{-3} mol/L MgSO_4 , 1.8×10^{-3} mol/L FeSO_4 and a mixture of these salts, besides varying the lead nitrate in the range 3.0×10^{-6} to 6.0×10^{-5} mol/L, the cyanide concentration in the inoculated medium was measured. The concentration of phosphate buffer varying in the range 1.0×10^{-2} to 3.0×10^{-2} mol/L was also examined into the medium containing *C. violaceum*. The metal content was analyzed by an atomic absorption spectrometer (AAS, AAnalyst 400, Perkin Elmer Inc.).

2.2. Bioleaching

All experiments were carried out in sterilized Erlenmeyer flask of 250 mL capacity containing 200 mL of YP media. A 1 mL growing culture of *C. violaceum* under log phase was added to the flask aseptically. The desired amounts of phosphate buffer solution and metal ions were then transferred to the flask. The pH of the solution was adjusted by 2.0 mol/L sodium hydroxide solution. The flask with the inoculated set was placed in an incubator shaker maintained at 30 °C and was incubated for specified period. The supernatant sample was drawn every 24 h and the cell count, pH and amount of cyanide generated were estimated. For each inoculated set, a control experiment without metal ions was also carried out. There was no air supply maintained during the experiments. Bacterial population was determined by counting the colonies followed by cells count on agar plate incubated at 37 °C for 24h which was correlated with the optical density. The morphological feature of *C. violaceum* was observed under a biological microscope (Olympus BX 51) and cell population was confirmed by the counting chamber.

In order to examine the bio-dissolution of gold and copper from the waste mobile phone PCBs, a known amount of sample (1 mm x 1 mm size) was taken into a 250 mL flask containing 200 mL media inoculated with 2% active culture of *C. violaceum*. The flasks were incubated at 30 °C and 15 g/L pulp density in an orbital motion incubator with shaking at 150 rpm while maintaining the pH in the range 8.0 - 11.0. The supernatant was drawn at an interval of 24h for bacterial cell count, and measuring the pH, dissolved oxygen (DO), and cyanide concentration. After completing the bioleaching the solution was filtered over Whatman 42 filter paper and clear solution was analyzed for gold and copper, and the percentage of metals leached was calculated (leaching efficiency, %). The residue obtained in each experiment was dissolved in aqua regia and the metal in the leached and aqua regia solution was analyzed by AAS to compute the material balance. The concentration of cyanide generated by bacteria during the process was determined colorimetrically by UV/VIS spectrometer (UV-1601PC, Shimadzu) at 580.5 nm using pyridine and barbituric acid (Clescert *et al.*, 1998). For this 5 mL supernatant sample was drawn from the experimental flask and pH was adjusted to 11.0 by 4 mol/L NaOH solution before filtration. To the 10 mL of the clear solution 4 mL of NaH₂PO₄ (138 g/L) was added and the mixture was allowed to stand for 3 min followed by the addition of 2 mL chloramines-T (10 g/L in distilled water). After 5 min a 5 mL solution of pyridine-barbituric

acid mixture (amount - 75 mL : 15g in 1 L) was added to develop the stable violet colour. The cyanide concentration was then analyzed colorimetrically at 580.5 nm against a standard solution. Dissolved oxygen (DO) and pH were determined everyday by DO meter (HQ40d, Hach) and pH meter (Orion- 720A), respectively. Data given in the text are the average value of duplicate set of experiments with an accuracy of $\pm 3.0\%$.

3. Results and discussion

3.1. Cyanide generation by *C. violaceum*

a. Bacterial cell population and cyanide generation in presence of metal ions

The bacterial cell population was determined in presence of different metal ions at 30 °C and initial pH of 7.4. Results given in Figure 2(a) show that the growth of *C. violaceum* attained the log phase after 2 days and maximum cell count was achieved in 5 days irrespective of the addition of metal ions. The decrease in cell population after 5 days of incubation may be attributed to the decrease in cell multiplication once the optimum cell growth in a medium is attained due to a short stationary phase typically observed for the cyanobacteria. This also reflects on the decreasing metabolic activity of such microbes with time (Knowles and Bunch, 1986). The cell count was found to be high of 12.3×10^8 cells/mL with a very little fall in the bacterial population (11.3×10^8 cells/mL) till 7 days when no metal was present in the YP medium. The addition of 0.17 mol/L NaCl, 4.0×10^{-3} mol/L MgSO₄, 1.8×10^{-3} mol/L FeSO₄ separately and a mixture of these salts into the medium, adversely affected the cell population. The cell population in the medium containing NaCl and the mixture of the metal salts was much lower than that observed in presence of magnesium sulfate and ferrous sulfate. This may be attributed to the toxicity and inhibitory effect on bacterial growth because of presence of Cl⁻ ions and also high amount of metal ions. While Fe²⁺ is precipitated at pH 7.4 or higher, adsorption of a number of bacterial cells led to the decrease in the cell population in presence of ferrous sulfate.

In contrast to the effect of metal ions on cell population, the addition of a little amount of metal ions can increase cyanide generation by *C. violaceum* (Figure 2(b)) because of catalytic effect enhancing the enzymatic process (Lawson *et al.*, 1999).

As can be seen in the figure, Fe²⁺ and Mg²⁺ were most effective to generate cyanide with the concentration of ~61 and 59 mg/L, respectively in 5 days time at initial pH of 7.4. The cyanide

concentration with the mixed metal ions was found to be almost similar (about 36 mg/L) in 7 days to that observed in absence of these additives. Therefore, the YP medium with Mg^{2+} was chosen

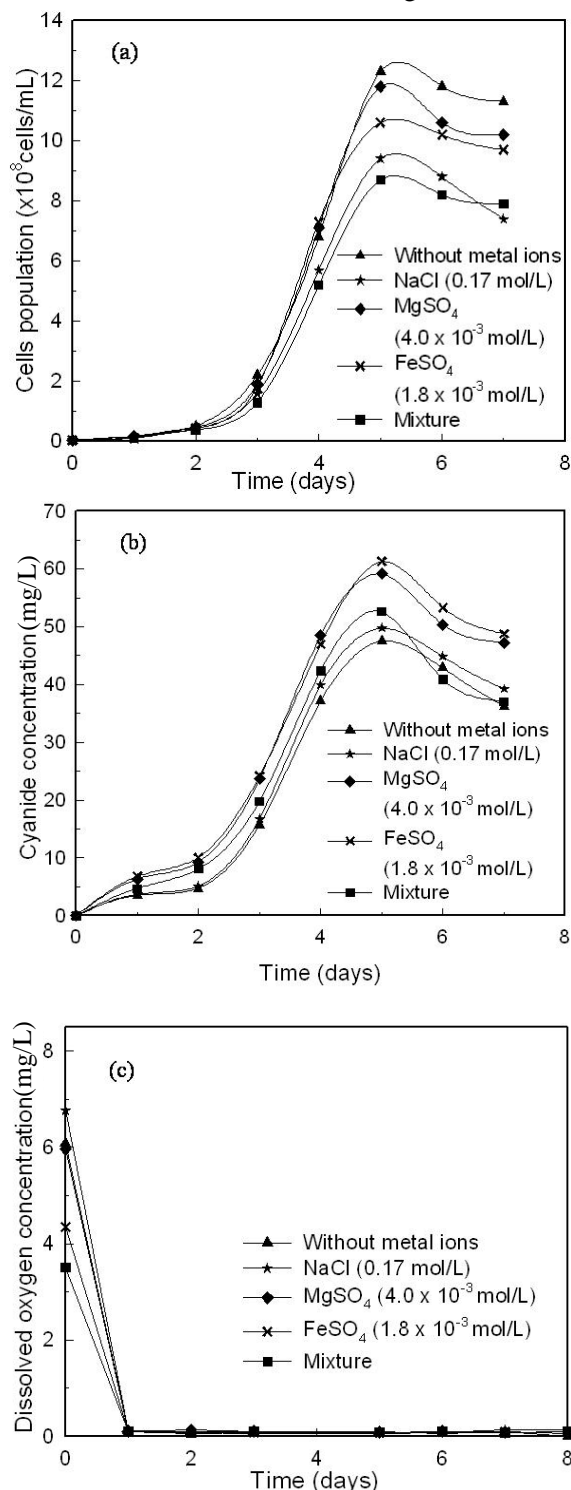


Figure 2. Effect of metal ions on (a) cell population of *C. violaceum* (b) cyanide generation, and (c) consumption of DO in YP medium (temperature 30°C, initial pH 7.4).

for the subsequent experiments. As air was not supplied into the medium, the dissolved oxygen concentration decreased from 6.8 to 0.1 mg/L quickly after the first day of incubation (Figure 2

(c)). However, this decrease in DO level has increased the cell population as well as cyanide concentration in the solution with time till 5 days and thereafter they showed decreasing trend. The consumption of dissolved oxygen is generally associated with the growth phase and bacterial respiration (Kita *et al.*, 2008).

The presence of different metal ions in the medium had no effect on the oxygen consumption during the growth of the bacteria. Thus, the role of dissolved oxygen concentration on cyanide generation of *C. violaceum* was not very significant after 24h and it was the sustained bacterial metabolism that improved the cyanide generation. During the growth of *C. violaceum* in YP medium when no metal ions were present, the pH of solution dropped quickly from 7.4 to 6.3 after one day and thereafter it increased to 8.1 in 6 days. The presence of metal ions particularly Mg^{2+} , and a mixture of the salts had shown almost similar trend with respect to increase in pH, which was found to be nearly 8.5 in 7 days. The decrease in pH of the medium is attributed to the acidification process that occurs to convert the organic particularly glycine to form carboxylic acid such as glyoxylic, cyanoformic and oxamic. It is for this reason that cyanide generated in first two days was not high (Figure 2(b)). The increase in pH after this point was accompanied by increased generation of cyanide through the conversion of intermediate products such as carboxylic acids, from the second day onwards and the cyanide reached to the highest concentration in 5 days. A similar kind of behaviour was observed earlier also (Smith *et al.*, 1985).

In view of improved dissolution of gold powder by *C. violaceum* in presence of lead ions (Sandenbergh *et al.*, 2001; May *et al.*, 2005), effect of lead nitrate on cyanide generation in YP media was also investigated. With a little difference in cyanide generation in presence of Mg^{2+} and Fe^{2+} , 4.0×10^{-3} mol/L of MgSO_4 was added into the medium and results are presented in Figure 3.

It is apparent that the presence of lead in the medium affected the amount of cyanide produced by *C. violaceum*. With 3.0×10^{-6} mol/L (~1 mg/L) $\text{Pb}(\text{NO}_3)_2$ into the YP medium higher amount of cyanide (66 mg/L in 5 days and 59 mg/L in 7 days) was generated. The lead nitrate concentration above 3.0×10^{-6} mol/L inhibited the bacterial cyanide generation. As observed below, the cyanide concentration was however, found to be a little higher (69 mg/L in 5 days) with 1.0×10^{-2} mol/L Na_2HPO_4 even without any lead nitrate. Incidentally, the presence of a similar amount of lead nitrate is reported to improve the gold

recovery in the cyanidation (chemical) process (Sandenbergh *et al.*, 2001).

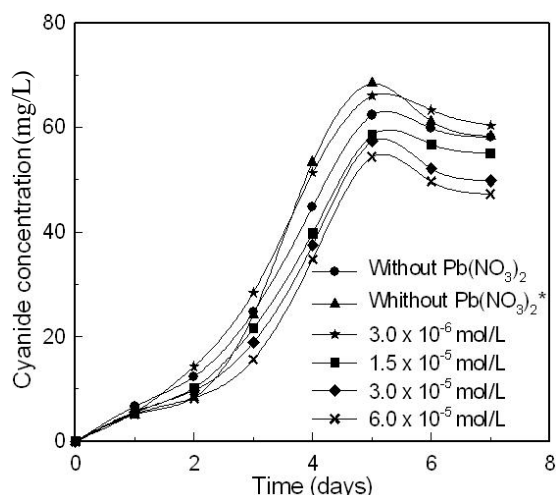


Figure 3. Effect of lead nitrate on cyanide generation by *C. violaceum* in the YP medium (4.0×10^{-3} mol/L MgSO_4 , temperature 30°C , initial pH 7.4).

(*)YP media with 4.0×10^{-3} mol/L MgSO_4 and 1.0×10^{-2} mol/L Na_2HPO_4 .

b. Effect of Na_2HPO_4

The phosphate is reported to increase the cyanogenesis process of *C. violaceum* (Rodgers *et al.*, 1978; Lawson *et al.*, 1999). Its effect on the cyanide generation in YP medium was investigated in presence of 4.0×10^{-3} mol/L MgSO_4 . As can be seen from Figure 4 the addition of phosphate buffer affected the efficiency of cyanide generation. The cyanide generation was highest (69 mg/L in 5 days) when 1.0×10^{-2} mol/L Na_2HPO_4 was added into the medium; further increase in Na_2HPO_4 level decreased the cyanide concentration in the solution as observed previously also (Lawson *et al.*, 1999). The amount of cyanide generation

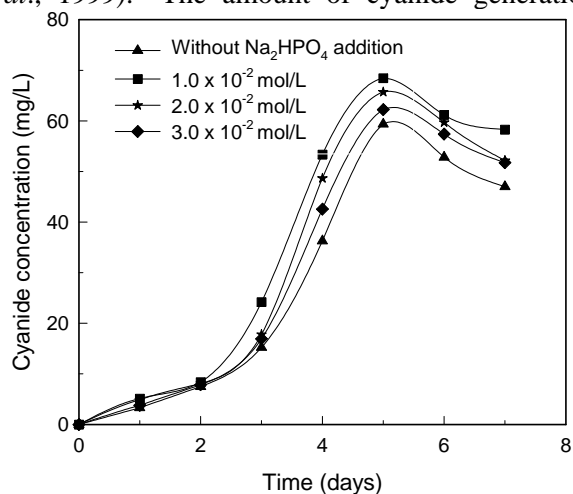
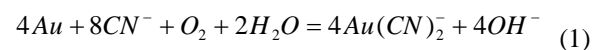


Figure 4. Cyanide generated by *C. violaceum* at varying phosphate concentration in the YP medium (4×10^{-3} mol/L MgSO_4 , temperature 30°C , initial pH 7.4).

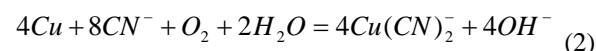
(data not given in Fig. 4)) was found to increase to 73 mg/L at pH 9.0 in 5 days time and then decreased to 63 and 58 mg/L at pH 10.0 and 11.0, respectively during this period.

3.2. Bioleaching of gold and copper from waste mobile phone PCBs by *C. violaceum*

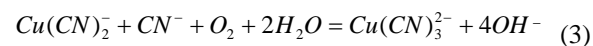
Before examining the potential leaching of gold from waste mobile phone PCBs by *C. violaceum* in YP medium containing 4.0×10^{-3} mol/L MgSO_4 , the effect of pH on cell population and cyanide generation was investigated. Results presented in Figure 5(a) show that cell population decreased with increase in pH of the media. At pH 7.4 cell population was maximum (1.18×10^9 cells/mL) in 5 days time while at pH 11.0 the cell population decreased to a lower value of 8.7×10^8 cells/mL. On the other hand, the cyanide concentration, presented in Figure 5(b), increased from 59 mg/L to a level of 68 mg/L in 5 days when pH was raised from 7.4 to 9.0; the cyanide concentration dropped to 56 mg/L at pH 9.0 in 7 days time under this condition. At pH 11.0, the cyanide concentration was low and was found to be 50 mg/L in 7 days time. In presence of cyanide generated in the YP medium, both gold and copper can dissolve, in which copper cyanide is present in the form of various complexes depending on the pH and other conditions whereas gold mostly forms dicyanide anion $[\text{Au}(\text{CN})_2]^-$. Since leaching of gold in the cyanidation process critically depends on the solubilization of copper in the sample, it is therefore, desired to dwell upon the dissolution behavior of the latter also along with gold as a part of the present research. As can be seen below (reactions 1–4), dissolution of gold and copper as their cyanide complexes is thermodynamically favourable at 298K in presence of oxygen (Alonso-Gonzales *et al.*, 2010; Rees *et al.*, 1999):



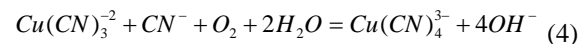
$$\Delta G_{298}^0 = -392.2 \text{ kJ/mol}$$



$$\Delta G_{298}^0 = -322 \text{ kJ/mol}$$



$$\Delta G_{298}^0 = -228.6 \text{ kJ/mol}$$



$$\Delta G_{298}^0 = -163.3 \text{ kJ/mol}$$

Copper cyanide complexes, particularly tetracyanate is more stable at above 9.0 pH (Marsden *et al.*, 1960) and -tricyanate (stable in the pH range 7.0 - 12.0) can dissolve gold when cyanide concentration is high (Vukcevic *et al.*,

1996; Vukcevic *et al.*, 1997; Rees *et al.*, 1999). With little or low free cyanide, $\text{Cu}(\text{CN})_2^-$ complex will mostly prevail which may not oxidize gold.

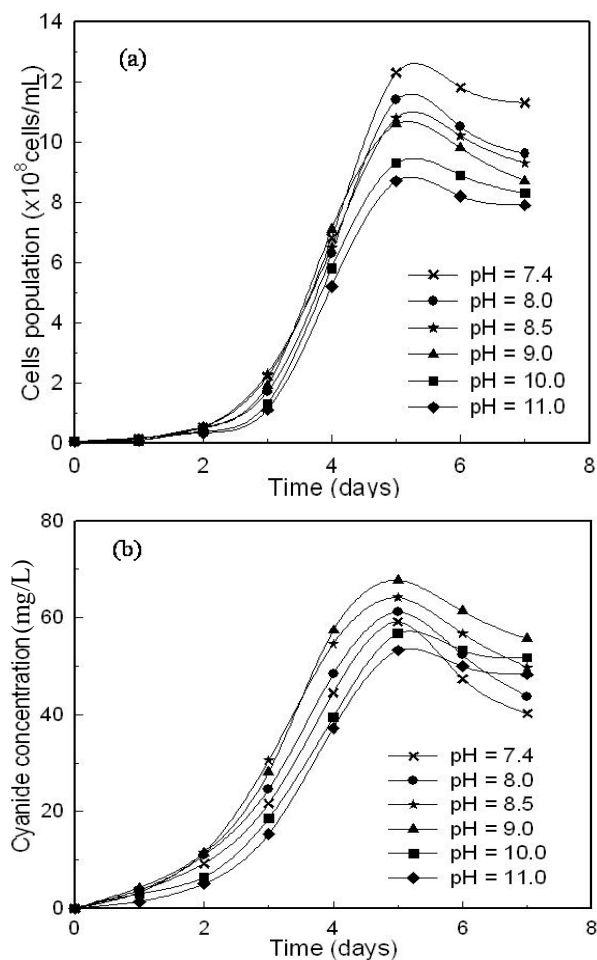


Figure 5. Effect of pH on cell population of *C. violaceum* (a) and cyanide generation (b) in the YP medium (4.0×10^{-3} mol/L MgSO_4 , temperature 30°C).

a. Effect of pH in cyanidation/ leaching of metals in presence of magnesium sulphate

Effect of pH on the leaching of gold and copper from waste mobile phone PCBs was studied at 30°C and 15 g/L pulp density in presence of 4.0×10^{-3} mol/L MgSO_4 in YP medium, and results are shown in Figure 6.

It is apparent that the gold dissolution from the waste PCBs increased from 7.8% (0.35 mg/L) to the highest value of 11% (0.46 mg/L) when the pH was increased from 8.0 to 11.0 (Figure 6 (a)). The trend of copper leaching was observed to be similar to that of gold. Copper leaching efficiency was found to increase with increasing pH, but was high of 11.4% (878 mg/L) at pH 10.0 and then decreased marginally to 10.2% (815 v) when pH was further raised to 11.0 in 8 days of leaching (Figure 6(b)). Though, the cyanide generation was higher (68 mg/L in 5 days and 56 mg/L in 7 days) at pH 9.0 (Figure 5(b)), but high gold and copper

leaching at the pH 11.0 and 10.0, respectively may be attributed to the requirement of the higher pH to form stable metal cyanide complexes under the conditions of the present work (Marsden and House., 1960). At pH 11.0 the cyanide concentration was analysed to be 54 mg/L in 5 days and 50 mg/L in 7/ 8 days time when gold leaching was maximum (11%).

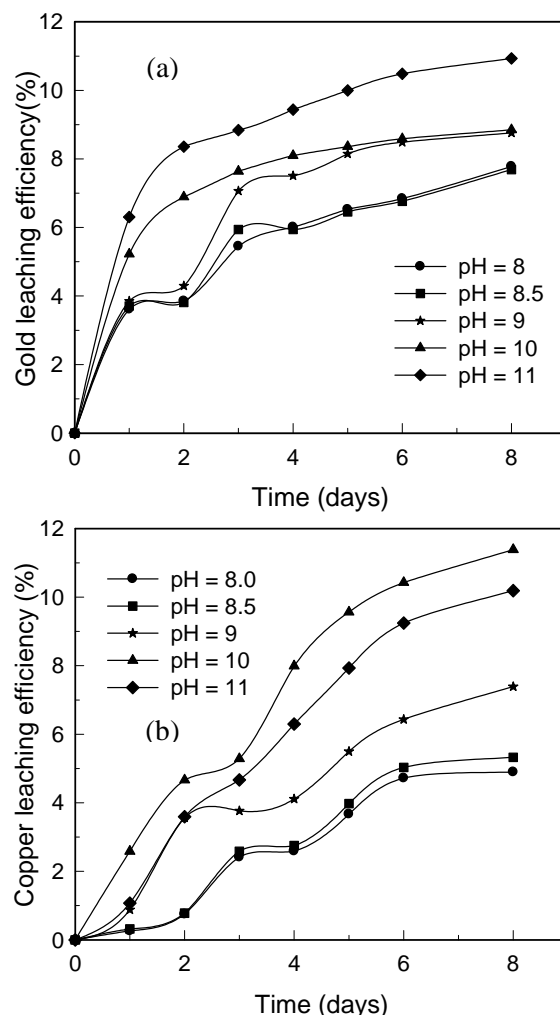


Figure 6. Effect of pH on gold (a) and copper (b) leaching in presence of magnesium sulphate in the YP medium (4.0×10^{-3} mol/L MgSO_4 , pulp density 15g/L, temperature 30°C).

b. Effect of pH in cyanidation/ leaching of metals in presence of Na_2HPO_4

Effect of addition of 1.0×10^{-2} mol/L Na_2HPO_4 into the YP medium on metal dissolution was investigated at varying pH in the range 8.0 – 11.0 and results are presented in Figure 7.

In the presence of Na_2HPO_4 the trend of gold and copper dissolution was similar to that observed in presence of Mg^{2+} till a pH of 9.0. Gold leaching was not very different in the pH range 8.0 – 11.0 with maximum dissolution of 9.2% Au at pH 11.0 (Figure 7(a)). Similarly, copper recovery was low within the pH range 8.0 – 9.0. However, copper

recovery was found to increase substantially to

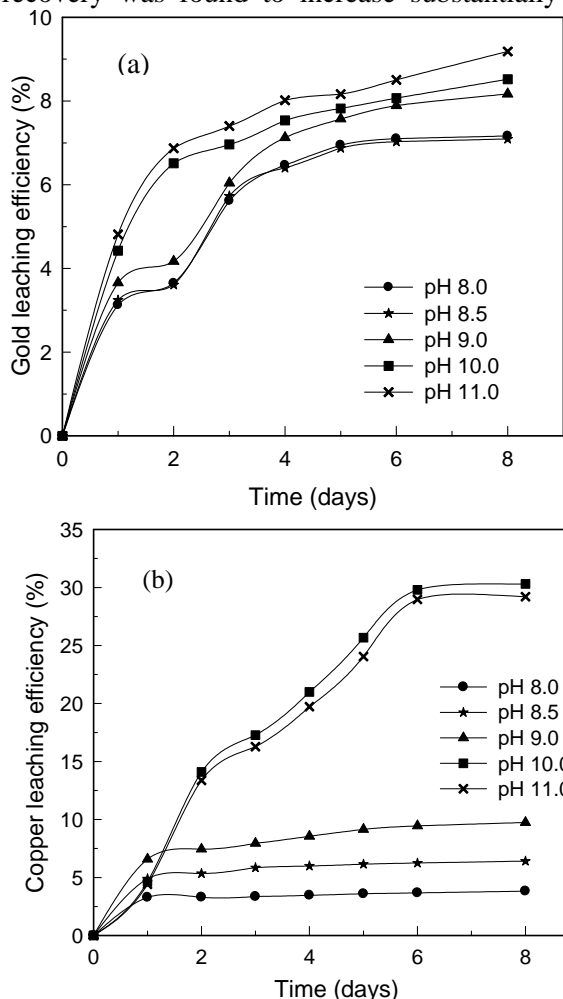


Figure 7. Effect of pH on gold (a) and copper (b) leaching in presence of Na_2HPO_4 in YP medium (1.0×10^{-2} mol/L Na_2HPO_4 , pulp density 15 g/L, temperature 30°C).

30.3% and 29.2% at the higher pH of 10.0 and 11.0 (Figure 7(b)), respectively. It is thus apparent that the high amount of cyanide generated (Fig. 4) in presence of Na_2HPO_4 in comparison to MgSO_4 (Fig. 5b) has resulted in improved copper leaching than dissolving gold from the PCBs sample under the above conditions. The pH is thus critical along with the cyanide concentration.

c. Effect of lead nitrate in cyanidation/ leaching of metals

Results on addition of 3.0×10^{-6} mol/L $\text{Pb}(\text{NO}_3)_2$ into the YP medium in absence of Na_2HPO_4 and MgSO_4 are presented in Figure 8 for gold and copper bioleaching at pH 11.0 and 10.0, respectively. To compare the effect of other additives, the dissolution patterns of gold and copper in presence of Na_2HPO_4 and MgSO_4 are also shown under the same conditions in Fig. 8. Based on the optimum pH reflected from Fig. 6 and 7, the leach recovery of gold and copper are also briefly mentioned (without giving the plots)

at pH 10.0 and 11.0, respectively in presence of small amount of lead nitrate. Copper bioleaching at pH 10.0 was found to be higher (38.1%) than that of pH 11.0 (29.3%, data not shown) in presence of small amount of lead nitrate. However, higher gold bioleaching (10.1%) was observed at pH 11.0 (Fig. 8 a) than that of pH 10.0 (9.4%, data not shown). Figure 8(b) also shows that copper bioleaching can be increased by 3 to 4 times when magnesium sulphate was substituted by Na_2HPO_4 and $\text{Pb}(\text{NO}_3)_2$ in the YP media. As can be seen, copper bioleaching was found to be 11.4% (878 mg/L) with MgSO_4 which increased to 30.3% (2,156 mg/L) and 38.1% (2,668 mg/L) in presence of small amounts of Na_2HPO_4 and $\text{Pb}(\text{NO}_3)_2$, respectively. In contrast to copper, gold leaching however, was only marginally higher (11%) in presence of MgSO_4 at pH 11.0 than the recovery of 9.2% and 10.1% Au in presence of Na_2HPO_4 and $\text{Pb}(\text{NO}_3)_2$, respectively (Figure 8 (a)).

From these results, it can be said that addition of a low concentration of Na_2HPO_4 and $\text{Pb}(\text{NO}_3)_2$ has enhanced copper leaching because of generation of higher amount of cyanide, typically 69 and 66 mg/L, respectively at pH 7.4, as compared to that of MgSO_4 (59 mg/L cyanide at pH 7.4). Similarly, at higher pH of 10.0 and 11.0 the concentration of cyanide generated in presence of Na_2HPO_4 was clearly higher (63 and 58 mg/L in 5 days) as compared to that of MgSO_4 (57 and 54 mg/L cyanide) favouring copper bio-dissolution.

Regarding the higher concentration of copper in presence of $\text{Pb}(\text{II})$ in comparison to Na_2HPO_4 , it may be attributed to the activation of the copper surface by the former leading to higher reactivity with cyanide. Higher solubility of copper over gold may also be related to the electrochemical behavior of the two metals as gold is nobler ($E_{\text{Au}^{3+}/\text{Au}}^0 : 1.52\text{V}$) than copper ($E_{\text{Cu}^{2+}/\text{Cu}}^0 : 0.34\text{V}$), and therefore, copper may dissolve preferentially while consuming bio-genic cyanide. Secondly, gold leaching will be facilitated by high concentration of cyanide (Marsden *et al.*, 1960)

and the low level of bio-generated CN^- in the present case has clearly favoured the copper leaching at the expense of gold. As mentioned above, the $\text{Cu}(\text{CN})_2^-$ complex available due to the low cyanide and DO concentration in this study will have a little ability to dissolve gold.

A look at Figure 9 on the variation of redox potential during leaching of waste PCBs shows the low value of Eh (about 2 mV in 6 days) at pH 11.0 (Figure 9(b)) favouring gold leaching as compared to the leaching at lower pH of 10.0 and below. The leaching behaviour of gold and copper

at pH 9.0 and below was different than that of pH 10.0 and 11.0. This may be attributed to stability of iron hexacyanide complex $[\text{Fe}(\text{CN})_6]^{4-}$ till pH 9.0 which might decompose above this pH and thus contributing to the dissolution of metals (Rees *et al.*, 1999).

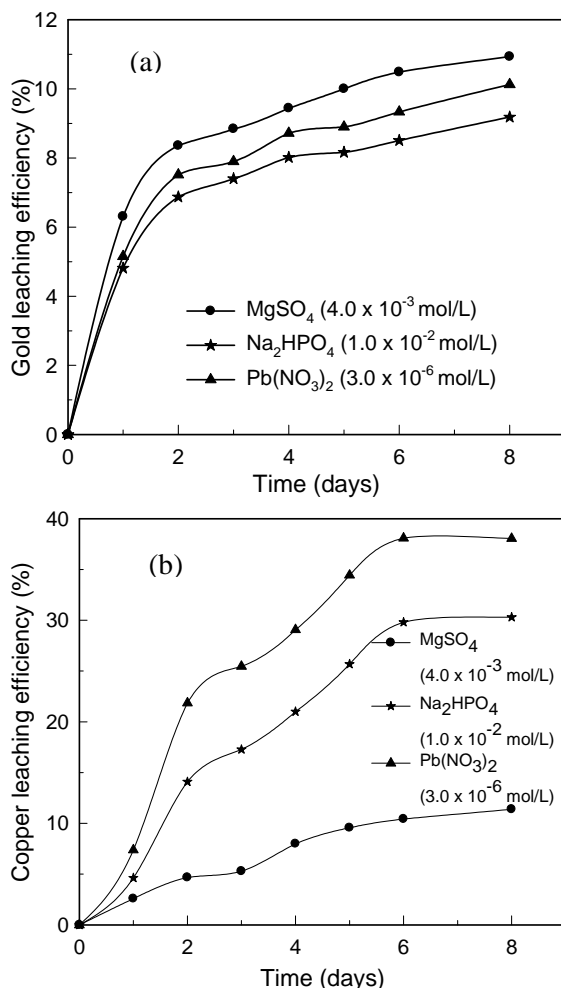


Figure 8. Effect of different metal ions on the bioleaching of gold at pH 11.0 (a) and copper at pH 10.0 (b) in the YP medium (pulp density 15 g/L, temperature 30°C).

Our recent studies (data not included) by adding 0.004% (v/v) hydrogen peroxide to supplement the DO level during the leaching of waste mobile phone PCBs by *C. violaceum* have shown the improvement in copper recovery by 8–10% and gold by 2–3% over above results depending on the metal ions present.

In view of the above results it appears that gold bioleaching in presence of high copper content in the PCBs is adversely affected and thus, calls for reducing the copper content in the sample. The low leaching of gold may affect eventually the exploitation of the results. In order to make the present process/ technology applicable for accomplishing the complete recycling of the

waste mobile phone PCBs, there is a need to improve the gold recovery. The precious metals particularly gold is found to be mostly present as bonding wire and coated thin film in the PCBs. As observed in our earlier research (Lee *et al.*, 2010), by the physical beneficiation of the waste PCBs such as grinding, separation by gravity etc, gold can be enriched (about 80–85wt %) in the separated non-metallic portion containing a little metallic. It may thus, be desirable to reduce the copper content by physical beneficiation and study the bioleaching of gold from the gold enriched non-metallic component with a little metallic by using *C. violaceum*. The major metallic portion enriched with copper and other metals can be treated by a suitable hydrometallurgical method to recycle the waste mobile phone PCBs completely.

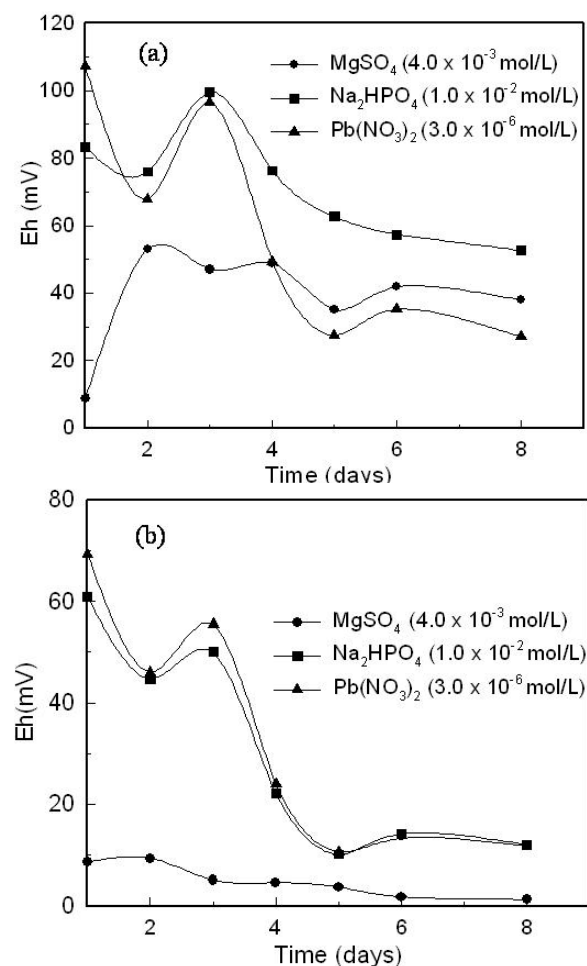


Figure 9. Variation of Eh during bioleaching of copper at pH 10.0 (a) and gold at pH 11.0 (b) from waste mobile phone PCBs with *C.violaceum* in the YP medium (pulp density 15g/L, temperature 30°C).

Comparing the amount of biogenic cyanide by *C. violaceum* in presence of various metal ions in a particular medium (YP medium) and using the cyanide so generated in gold leaching from the

waste mobile phone PCBs in order to recycle it while understanding the effect of high copper present in the sample on the biodissolution of gold, are the main features of this research.

4. Conclusion

Cyanide generation by cyanogenic bacteria – *Chromobacterium violaceum* in YP medium can be enhanced by adding a low amount of metal ions. The addition of magnesium sulphate and ferrous sulphate in the medium was found to be equally effective for cyanide generation by the bacteria, but the presence of Na_2HPO_4 and lead nitrate was still better. Dissolved oxygen concentration did not affect cyanide generation by *C. violaceum*. The results on bioleaching of valuable metals from waste mobile phone PCBs show that maximum gold can be leached out (11% in 8 days) at pH 11.0 in presence of 4.0×10^{-3} mol/L MgSO_4 in YP medium; however, the copper recovery was high (11.4%) at pH 10.0. When 1.0×10^{-2} mol/L Na_2HPO_4 and 3.0×10^{-6} mol/L $\text{Pb}(\text{NO}_3)_2$ was added in the YP medium, copper recovery was found to increase almost 3 times (30.3%) and 4 times (38.1%) at pH 10.0 in 8 days. Presence of phosphate and lead nitrate in the media favoured copper bio-dissolution, but was not effective for gold leaching. The low concentration of cyanide generated by metabolic activity of *C. violaceum* and dissolved oxygen favoured copper bioleaching at the expense of gold from the PCBs. The results show the possibility of recovering gold and copper values by using a cyanogenic bacteria – *C. violaceum*.

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