

## Efficiency assessment of pure Fe oxidizing microorganisms in iron supplemented and non-supplemented medium and pure S oxidizing microorganisms for bioleaching of mobile phone printed circuit boards

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### ABSTRACT

The microbial culture as well as the components of the bioleaching medium affects the efficiency of microbial leaching. The present study on mobile phone printed circuit boards (MPPCBs) was conducted to know that whether the addition of energy source is required during the microbial leaching or microorganisms can utilize the iron (Fe) content of the MPPCB. The study was conducted with pure Fe oxidizers in Fe supplemented (9g/L, pure Fe 9K), non-supplemented (0g/L, pure Fe 0K) medium and pure sulfur (S) oxidizers supplemented with 3g/L of elemental sulfur (Pure S 3g/L S<sup>0</sup>). The copper (Cu) content of the feed material was 26.3% (w/w) by X-Ray Fluorescence (XRF) spectroscopy. The Cu recovery in pure Fe 0K and pure Fe 9K was 100% while with pure S oxidizers it was 39.41%. The acid consumption in pure Fe 0K and pure Fe 9K was 579.65kg/ton and 559.05kg/ton respectively. The bioleaching rate of Cu was 0.128g/L/h, 0.075g/L/h and 0.023g/l/h in order of pure Fe 9K> pure Fe 0K>pure S. Bioleaching with pure Fe oxidizers in 0K medium was found to be efficient and economical in terms of metal recovery and acid consumption, whereas, bioleaching with pure Fe 9K showed the maximum Cu leaching rate. The Cu recovery was same in both Fe supplemented and non-supplemented medium and microorganisms can utilize the Fe present in feed (PCB) itself.

**KEY WORDS:** BIOLEACHING, COPPER, 9K MEDIUM, 0K MEDIUM

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## INTRODUCTION

A significant amount of valuable metals resides in a printed circuit board (PCB) of an electronic device. There are various established technologies for the extraction of metals from PCBs such as mechanical separation, pyrometallurgy, hydrometallurgy, etc. (Shah, Tipre and Dave, 2014). The application of these techniques for treatment of the waste costs more in comparison to the final product. In that respect, bioleaching has been a promising alternative for metal recovery from low-grade ores and the waste (Cui and Zhang, 2008; Mishra and Rhee, 2010; Erüst *et al.*, 2013; Johnson, 2014). The microorganisms which are primarily used for bioleaching belong to genus *Acidithiobacillus* due to their robust nature and ability to oxidize the inorganic ferrous ( $\text{Fe}^{2+}$ ) and elemental sulfur ( $\text{S}^0$ ) (Rawlings, 2005; Arshadi and Mousavi, 2014; L. Wang *et al.*, 2018; Quatrini and Johnson, 2019).

The 100% Cu recovery by cell-free extract of *Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans* from 5g/L of PCB indicates the indirect bioleaching (Wu *et al.*, 2018). However, the regeneration of the reagent ( $\text{Fe}^{3+}$ ) is necessary for the continuation of the bioleaching process at higher pulp densities. Initial pH and  $\text{Fe}^{2+}$  ion concentration influence the rate of Cu recovery in the bioleaching medium (Xiang *et al.*, 2010). Few studies suggest that Fe content in PCBs can be utilized by bacteria and addition of extra Fe source leads to jarosite precipitation hence, loss of Fe from the bioleaching system (Wang *et al.*, 2018). The bioleaching of PCBs is an indirect non-contact mechanism where the significant role of the bacteria is oxidation of ferrous ( $\text{Fe}^{2+}$ ) into ferric ( $\text{Fe}^{3+}$ ), (Silva *et al.*, 2015; Mostafavi *et al.*, 2018). Low pH environment can prevent the jarosite formation; it favors the microbial growth and enhances the Cu recovery (Wang *et al.*, 2018). The pH values also influence the bioleaching kinetics of *Acidithiobacillus ferrooxidans* to mediate Cu recovery (Yang *et al.*, 2014). Previous studies on bioleaching emphasize that the recovery and rate of Cu bioleaching from PCBs mainly depends on  $\text{Fe}^{2+}$  ion concentration and pH. The parameters such as temperature, pulp density, etc. influence these two factors and consequently affects the bioleaching (Wang *et al.*, 2018; Arinanda *et al.*, 2019).

The present study aimed to investigate bioleaching efficiency of pure Fe oxidizing microorganisms in two different bioleaching mediums, i.e., with and without Fe supplements. The hypothesis for using medium without external energy source is 1. The feed material itself has Fe which can be utilized by the Fe oxidizing microorganisms for bio-oxidation 2. The external Fe added to the medium might be an additional amount and not be used completely by the microorganism 3. Fe

precipitation and jarosite formation is a common factor which influences the bioleaching yield 4. The extra Fe in bioleaching medium tends to enhance the possibility of jarosite formation which consequently increases the process cost due to the input of additional energy source, post residue treatment as well as the negative impact on Cu bioleaching efficiency. Most of the research suggests that addition of Fe might not be required during bioleaching of PCBs as the Fe which dissolves into the bioleaching medium from PCB can be utilized by the microorganisms. (Bryan *et al.*, 2015; Wang *et al.*, 2018).

The research on bioleaching of PCBs are on laboratory scale due few limitations of its largescale operations such as toxicity, time requirement as well as the cost. The present paper focuses on the current needs for the commercialization of the bioleaching system by comparing the bioleaching yield of Cu from waste MPPCBs in Fe free and supplemented growth medium for Fe oxidizing microorganisms. The paper also studied the bioleaching efficiency of only S oxidizing microorganisms. The bioleaching experiments were compared in respect of acid consumption, Cu recovery and time required for complete bio-oxidation of Cu.

## MATERIAL AND METHODS

### Mobile phone printed circuit boards (MPPCBs):

Printed circuit boards were separated by manual sorting of the waste mobile phones collected from the mobile phone repair shops in Alwar, Rajasthan, India. The crushing of MPPCBs was carried out at the Mineral Processing department of CSIR-Institute of Minerals and Materials Technology, Bhubaneswar, India. The PCBs were ground to a particle size below 250  $\mu\text{m}$  in an impact crusher followed by sieving. The elemental composition of this grounded material (feed) was analyzed by X-Ray Fluorescence analyzer (Bruker) (Table 1).

The XRF analysis revealed that Cu was 26.3% of the total metal constituents in the feed material. The recovery of Cu was then focused along with Ni (1.08%) and Zn (1.08%). The X-ray diffraction (XRD) and Scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) (Nova Nano FE-SEM 450 (FEI) analysis was done to determine the mineralogical phases and elemental weight percentage (%) of the feed material (Figure 1 and 2).

The small dotted arrangement patterns The same pattern of arrangement found in the Sulphur (S), Copper (Cu) and Iron (Fe) indicates the presence of the copper mineral complex with iron and sulfur, which was also observed in the mineralogy obtained from the XRD studies (Figure 1 and 2)

Table 1. Metal content (%) in mobile phone printed circuit boards (MPPCBs).

MPPCB Metal content (%)	Cu	Si	Ca	Br	Al	Fe	Sn	Ni	P	Zn	S	Ti	Pb	
	26.3	21.7	14.7	9.6	6.1	5.3	3.1	1.1	1.2	1.1	0.8	0.8	0.7	
	Ag	Mg	Sb	Cr	Ta	Sr	Cl	Nd	Mn	K	Au	Ga	Co	Nb
	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.1	0.1	0.1	0.1	0.01	0.01	0.01

### Bioleaching experiments

The bioleaching of crushed MPPCBs was done in three sets of experiments. Among three two bioleaching experiments namely pure Fe 0K and pure Fe 9K were done with pure culture of *Leptospirillum* dominated Fe

oxidizing bacteria in bioleaching medium without Fe supplement (0g/L, Fe) and with Fe supplement (9g/L, Fe) respectively. Another bioleaching experiment Pure S (3g/L) was done with *At. thiooxidans* dominated pure S oxidizing bacterial culture supplemented with 3g/L of

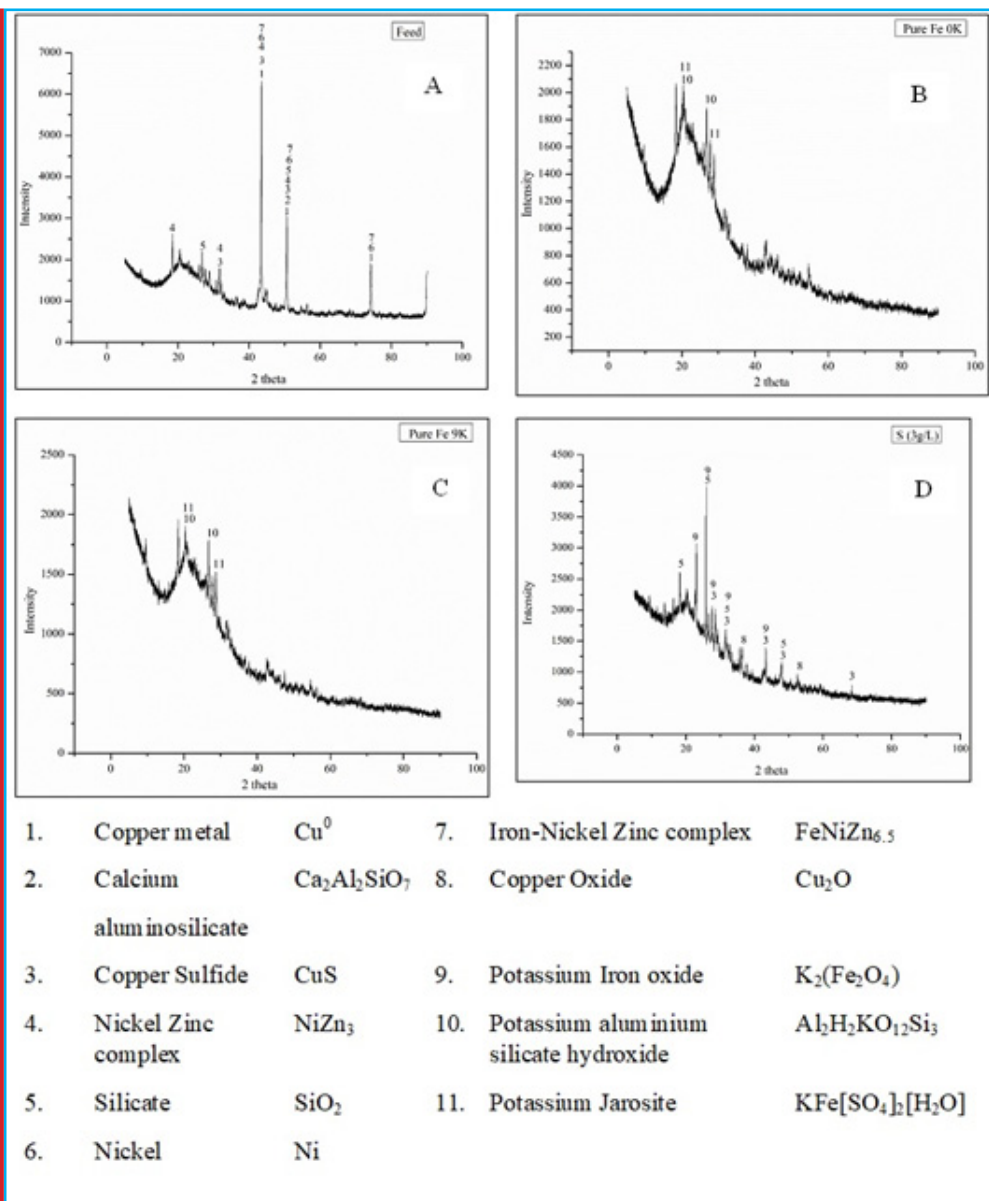


FIGURE 1. XRD Diffractogram of feed and bioleached residues of pure Fe 0K, pure Fe 9K and pure S(3g/L).

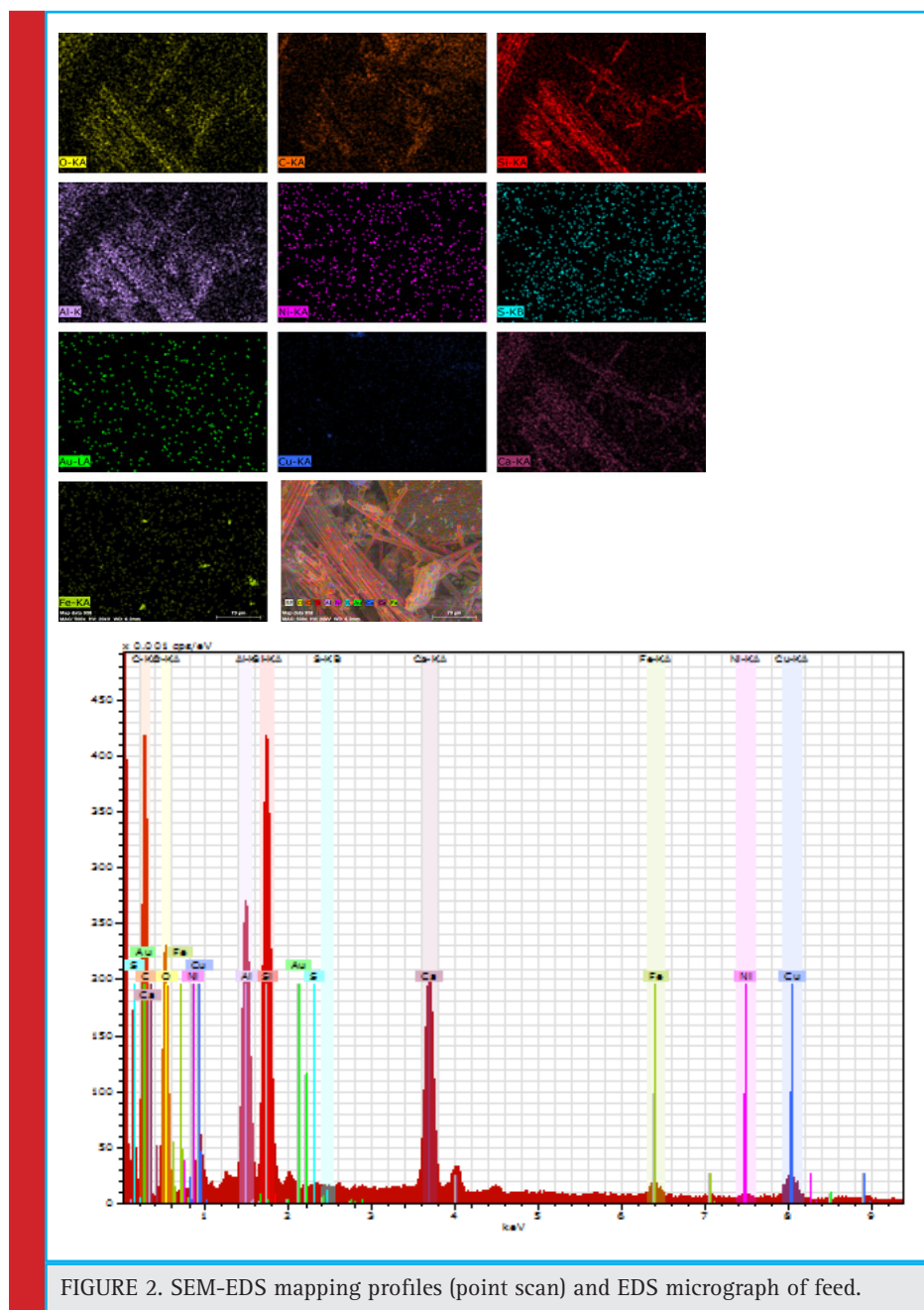


FIGURE 2. SEM-EDS mapping profiles (point scan) and EDS micrograph of feed.

elemental sulfur ( $S^0$ ). The inoculums used for bioleaching experiments were the activated microbial cultures grown in their respective mediums. The Fe oxidizing microbial culture was grown in a 0K medium [( $NH_4$ ) $_2$  $SO_4$ , 3.0 g/L; KCl, 0.1 g/L;  $K_2HPO_4$ , 0.5 g/L;  $MgSO_4 \cdot 7H_2O$ , 0.5 g/L;  $Ca(NO_3)_2 \cdot 4H_2O$ , 0.01 g/L] (Silverman and Lundgren, 1959), supplemented with 9g/L of ferrous ( $Fe^{2+}$ ) in the form of ferrous sulfate ( $FeSO_4 \cdot 7H_2O$ ) as an energy source. The pH was controlled at 1.5 and the temperature at 30°C. On the other hand, *At. thiooxidans* was grown in a X-Umea basal salt medium ( $Na_2SO_4 \cdot 10H_2O$ ,

3.2g/L; ( $NH_4$ ) $_2$  $SO_4$ , 3.0 g/L; KCl, 0.1 g/L;  $K_2HPO_4$ , 0.5 g/L;  $MgSO_4 \cdot 7H_2O$ , 0.5 g/L;  $Ca(NO_3)_2 \cdot 4H_2O$ , 0.01 g/L) supplemented with 3g/L of elemental sulfur ( $S^0$ ) as the energy source. The growth of S oxidizing microorganisms was started at 3 pH and 30°C. The growth of Fe oxidizing microorganisms was analyzed by the regular measurement of pH, redox potential, ferrous ( $Fe^{2+}$ ) and total iron. The maximum value of oxidation-reduction potential (ORP) and zero residual  $Fe^{2+}$  ion was indicated activated Fe oxidizers. The increasing sulfate ion ( $SO_4^{2-}$ ) concentration and decreasing trend in pH val-

ues were marked in the activated S oxidizing microbial culture.

The bioleaching experiments were done with a working volume of 1L (v/v), 90% (v/v) of growth medium and 10% (v/v) of the inoculums. The pH of the bioleaching medium was maintained at pH 1.5 by addition of 5M  $H_2SO_4$  for the activity of Fe oxidizing microorganisms. The bioleaching medium composition was the same as the growth medium for pure S oxidizing microorganisms. No pH adjustments were made during the bioleaching of S-oxidizing microorganisms. Temperature for both the bioleaching experiments was maintained at 35°C. 50g/L of the PCB powder was added in the bioleaching medium (growth medium + activated culture) in the starting of all the three experiments. The daily addition of  $H_2O$  compensated the evaporation loss of water ( $H_2O$ ). The changes in pH during bioleaching experiments were regularly monitored by a pH meter (Eutech). The changes in  $Fe^{2+}$  concentration and ferrous/ferric ( $Fe^{2+}/Fe^{3+}$ ) couple during bioleaching with pure Fe oxidizing microorganisms was monitored by a titrimetric method using cerium sulfate with 1,10-Phenanthroline as an indicator and an ORP meter having platinum electrode with Ag, AgCl reference electrode. The concentration of  $SO_4^{2-}$  was analyzed by using a turbidimetric method by visible spectrophotometric method (420 nm) described in the American Public Health Association, 1975 (APHA) (Kolmert, Wikström and Hallberg, 2000).

The metal ion concentration in all the three experiments was determined by atomic absorption spectrophotometer (AAS) (Thermo Scientific iCE 3000 SERIES). A bright field microscope did the planktonic viable

microbial cell count at 100X magnification on a Neubauer hemocytometer. After constant values of metal ion concentration and other parameters, the bioleaching experiments were harvested by solid-liquid separation. Bioleached residue was washed thoroughly using 1.5 pH water and air dried. The mineralogical analysis of both feed and the bioleached residue was determined by "PANanalytical Powder XRD." The samples for XRD were pulverized first to ensure the homogeneity then the diffraction patterns were measured at angles between  $10^\circ$  to  $90^\circ$ , the step size was 0.02 angle/sec. The crystalline phases were identified by using the joint committee for powder diffraction standards (JCPDS) file.

## RESULTS AND DISCUSSION

The pH of without Fe supplement (OK) bioleaching medium was high during the initial three days of the experiment due to the alkaline nature of the PCBs and bio-oxidation of  $Fe^{2+}$  into  $Fe^{3+}$  (Figure 3).

After that, no significant increase in pH was observed. The highest concentration of  $Fe^{3+}$  was marked on the 4<sup>th</sup> day of the experiment with a notable rise in ORP values after that the ORP was in an increasing trend along with microbial cell count. The bioleaching experiment in OK medium was initiated with a total iron concentration of 0.92g/L and reached 3.51g/L on the 3<sup>rd</sup> day (Figure 4) which indicates complete dissolution of Fe presented in the feed (2.66g/L).

The decreasing trend in total Fe concentration with a notable low pH value after the 3<sup>rd</sup> day of the experiment

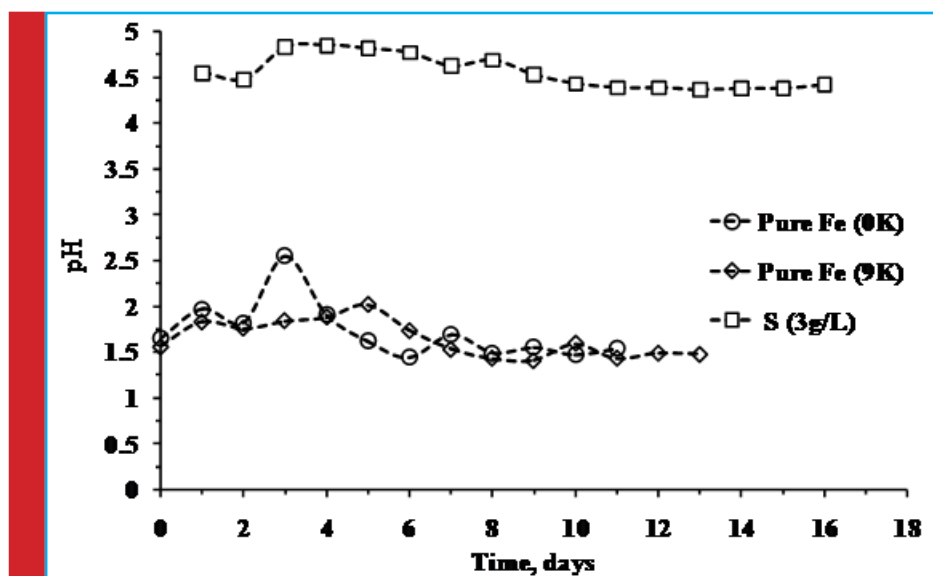
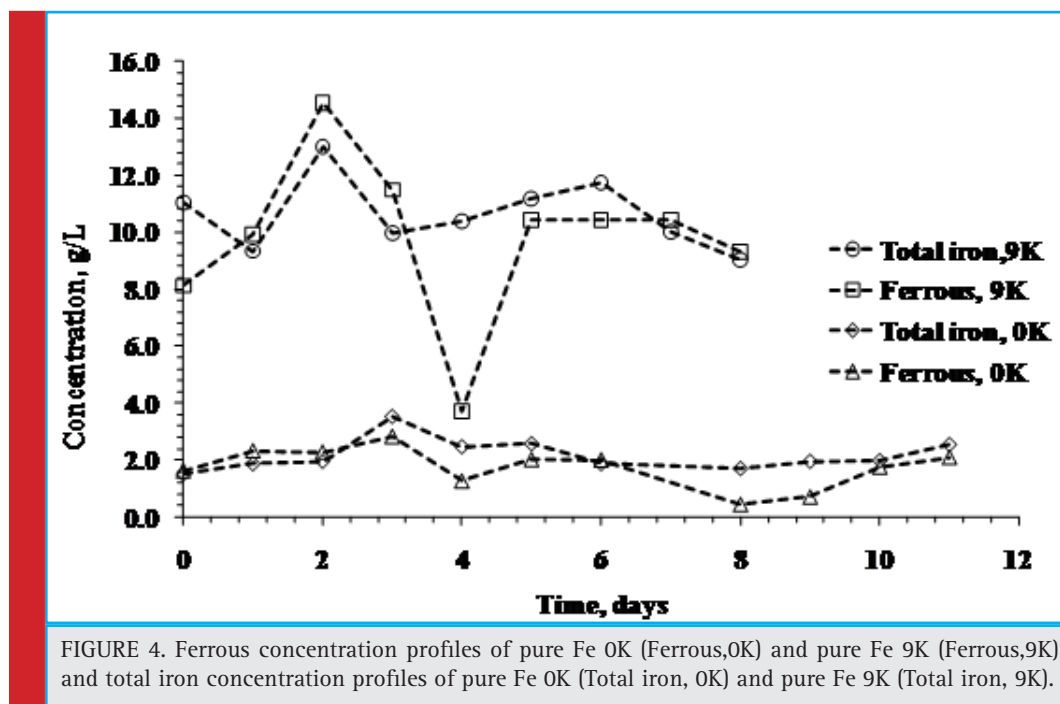


FIGURE 3. Change in pH profile with time during the bioleaching experiments.



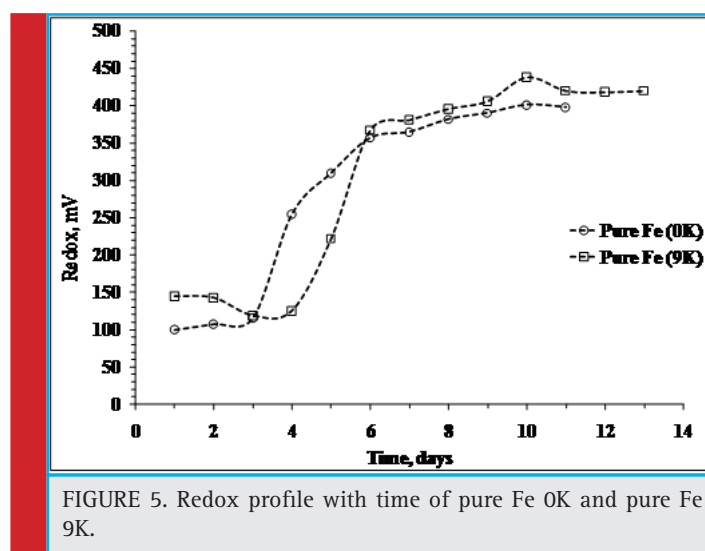


was due to formation jarosite. The presence of potassium jarosite peaks (characteristic in a Fe oxidizing microbial bioleaching system) in XRD data of bioleached residue confirms jarosite formation (Figure 1B). The significant increase in pH values in Fe supplemented medium (9K) was observed from the 4<sup>th</sup> day with increasing values of ORP and microbial cell count. The acid was mainly added during initial days to maintain a pH value of 1.5 which is accounted for the growth of *Leptospirillum ferriphilum* and highest Cu recovery ( Wang *et al.*, 2018).

The pH values in both the experiments were low during the last days, and no more acid was added. The ORP

values in both the systems were unstable after addition of PCBs in the bioleaching medium; hence, hard to evaluate. The stable ORP values from the 1<sup>st</sup> day of the experiments were low and started increasing from 4<sup>th</sup> and 5<sup>th</sup> day respectively. At the end of the operations, the ORP value of 0K bioleaching medium was 406mV while, 438mV in the 9K bioleaching medium (Figure 5).

As soon as the PCB powder was added in bioleaching medium the initial microbial cell count of the 0K and 9K bioleaching medium was decreased from a value of 2.80E+08 to 7.20E+06 and 4.80E+06 respectively (Figure 6).



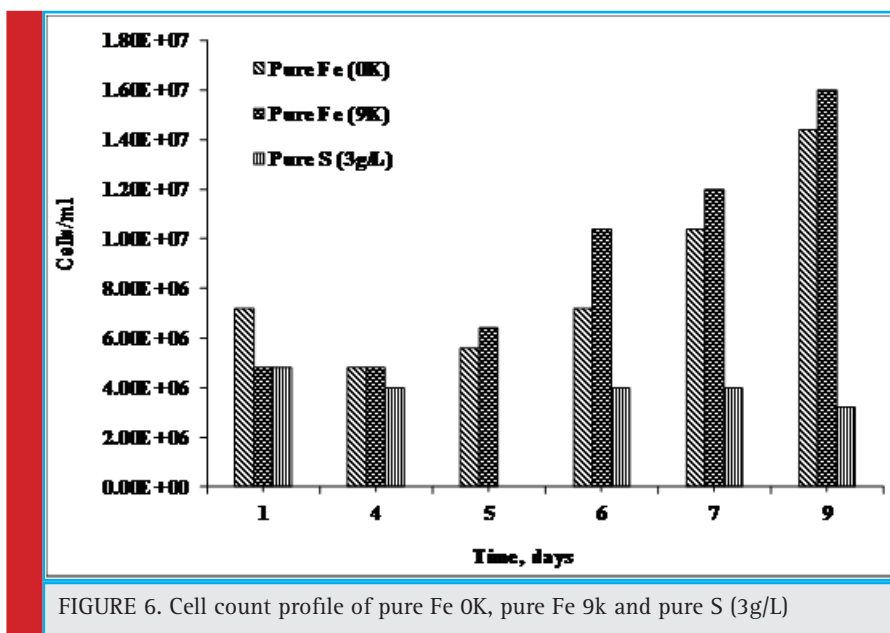


FIGURE 6. Cell count profile of pure Fe 0K, pure Fe 9k and pure S (3g/L)

The microbial cell count was recovered after the 5<sup>th</sup> day in the amount of 1.60E+07 cells/mL of bioleaching medium. The total amount of acid consumption was 559.05 kg/ton and 579.65kg/ton in 0K and 9K bioleaching medium respectively. Complete recovery of Cu (100% recovery) took place in 8 days and six days of Pure Fe 0K medium and pure Fe 9K medium respectively. The recovery of Zn and Ni in pure Fe 0K and 9K systems was 75.57%, 98.14%, 75.12%, and 22.10% respectively (Figure 7).

The Cu dissolution rate was 0.128g/L/h in pure Fe 9K system while it was 0.075g/L/h in pure Fe 0K system (Figure 8).

The 50g/L PCB was added in X-Umea salt medium at pH 1 and initial sulfate ( $\text{SO}_4^{2-}$ ) concentration of 6.14g/L

(inoculum+ media). The initial cell count of the bioleaching media was decreased from 6.64E+08 to 4.80E+06 as soon as PCB was added in the medium (Figure 6). The microbial cell count, pH as well as the sulfate concentration was not recovered with time. The acid was also added in initial few days to maintain the pH at three optimized for the growth of S oxidizing microorganisms. At pH 3, Fe does not remain in the soluble form which ensures only biogenic proton leaching of PCB. The  $\text{SO}_4^{2-}$  contributed from 18.19M of  $\text{H}_2\text{SO}_4$  with a purity of 96% and a density of 1.84g/mL was 6.93g/L. The total sulfate detected at the end of the experiment was 11.20g/L which was lesser than the total added  $\text{SO}_4^{2-}$  concentration. The Cu recovery in pure S oxidizing system was only 39.41% which quite lower in comparison to the

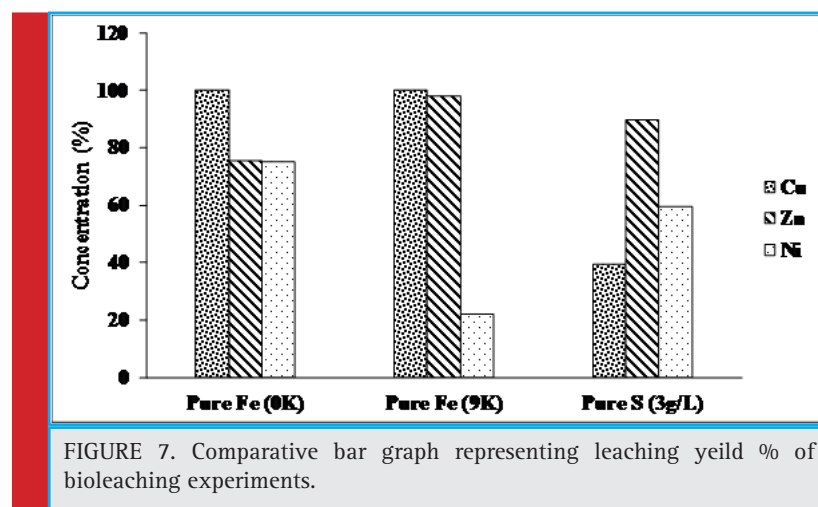


FIGURE 7. Comparative bar graph representing leaching yield % of bioleaching experiments.

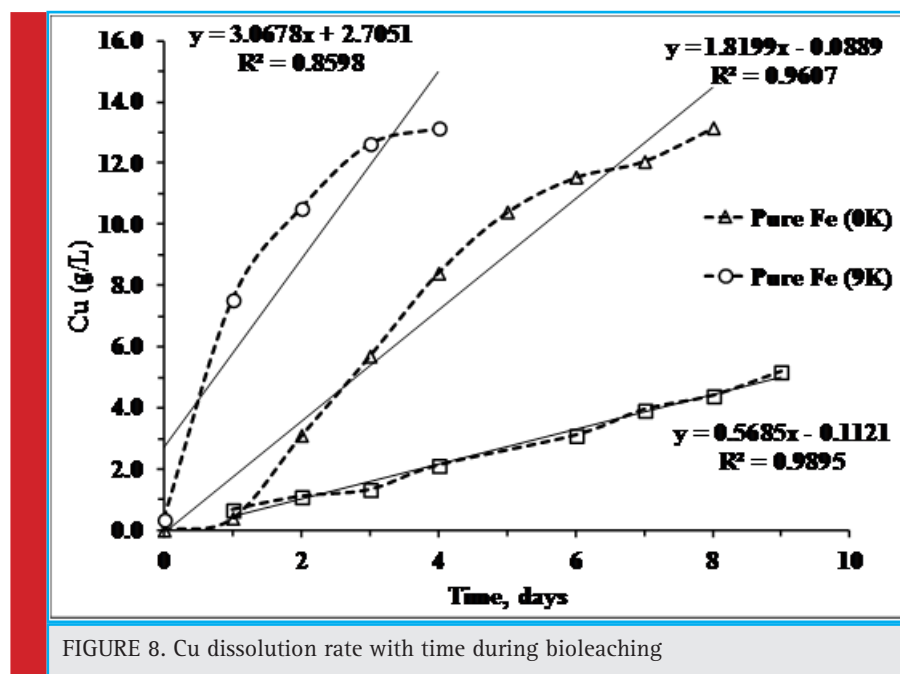


FIGURE 8. Cu dissolution rate with time during bioleaching

other two experiments with a slower dissolution rate of 0.023g/L/h. Recovery of the other two metals *viz* Ni and Zn was 59.38% and 89.68% respectively (Figure 7 and 8).

The low pH and redox values of 9K bioleaching medium during initial days indicate that as soon as PCB was added in the bioleaching medium the Fe<sup>3+</sup> ion attacked on it and converted into Fe<sup>2+</sup>. The total Fe profile of 9K bioleaching medium shows that high Fe<sup>3+</sup> ion concentration on the 0<sup>th</sup> hour was immediately reduced into Fe<sup>2+</sup>. The Fe<sup>2+</sup> to Fe<sup>3+</sup> bio-oxidation was then marked with an increasing ORP after a microbial growth lag phase of 4 days. The Fe<sup>2+</sup> to Fe<sup>3+</sup> bio-oxidation thus started in later days of the experiment. The results are consistent with a previous study stated that the Fe<sup>2+</sup> to Fe<sup>3+</sup> bio-oxidation is the rate-limiting step during bioleaching (Wu *et al.*, 2018). The significant portion of the acid was added during the initial four days of the bioleaching without Fe supplements while in 9K medium bioleaching the acid addition was done during the experiment. This acid was required for the bio-oxidation of Fe<sup>2+</sup> into Fe<sup>3+</sup> which was high in the 9K medium. The more acid consumption by Fe oxidizing microorganisms in 9K medium shows that addition of

Fe puts an extra burden on microbes as the Cu recovery in both the bioleaching mediums was similar with less consumption of acid in OK (Table 2).

A study on PCBs found that 0.25g/L of Fe<sub>2</sub>SO<sub>4</sub> is sufficient to mobilize Cu from 50g/L of PCBs in a multistage system, (S. Wang *et al.*, 2018). The Fe<sup>2+</sup> to Fe<sup>3+</sup> bio-oxidation and jarosite formation are the two main reasons behind low pH values during the last days in OK medium which can be confirmed by an increasing trend of redox potential, viable cell count, decrease in Fe<sup>2+</sup> ion concentration. Hence, the Fe content in PCBs itself was sufficient to be utilized by the microorganisms for bioleaching.

The changes in pH, SO<sub>4</sub><sup>2-</sup> as well as the cell count profile of bioleaching in the presence of pure S oxidizing bacteria, was not significant. The bioleaching experiment was initiated with an SO<sub>4</sub><sup>2-</sup> concentration of 6.14g/L. The increased SO<sub>4</sub><sup>2-</sup> in the bioleaching system was contributed by the acid addition. The acid production by the S oxidizing microorganisms was insignificant. According to the previous study, the S oxidizers are more sensitive for the higher metal concentration as well as the toxicity related to PCBs (Wang *et al.*, 2009). A previous study on bioleaching of PCBs also concluded the Cu recovery

Table 2. Summary of bioleaching experiments.

	Pure Fe OK	Pure Fe 9K	Pure S (3g/L)
Feed weight, g	50	50	50
Bioleach residue, g	35.44	35.06	48.9
Acid Consumption, kg Conc. H <sub>2</sub> SO <sub>4</sub> /ton PCBs	579.65	559.05	-



in presence to *At. thiooxidans* was less in comparison to the abiotic chemical leaching (Hong and Valix, 2014). The results were persistent with our study also where Ni and Zn dissolution were high in comparison to Cu. The XRD data of pure S oxidizers bioleached residue reveals the presence of CuS and Cu<sub>2</sub>O which according to a previous study tends to precipitate Cu, forms a passivation layer and results in an incomplete Cu dissolution (Hong and Valix, 2014). The faster Cu recovery rate in 9K medium shows that the presence of Fe<sup>3+</sup> during first few hours helped in Cu dissolution. However, the bio-oxidation of Fe<sup>2+</sup> into Fe<sup>3+</sup> took a time of three days, and an increased amount of Fe<sup>3+</sup> was again noted on the 4<sup>th</sup> day of the experiment. The metal dissolution in pure S oxidizers was due to proton leaching by addition of H<sub>2</sub>SO<sub>4</sub> and not by the biogenic acid.

## CONCLUSION

The present study was done to check the feasibility of MPPCBs bioleaching by using pure Fe oxidizing microorganisms in with and without Fe supplemented medium and by pure S oxidizing microorganisms. The Cu recovery was 100% in both the experiments with a faster dissolution rate of Cu in the 9K medium. The Cu dissolution rate was 0.1278g/L/h and 0.075g/L/h in pure Fe 9K and 0K medium whereas 0.023g/L/h in S oxidizers. The complete recovery of Cu took six days and 8days in pure Fe 9K and 0K medium respectively. The acid consumption in 0K medium was less in comparisons to 9K medium. The recovery of Ni and Zn was also satisfactory in bioleaching pure Fe 0K medium. The bioleaching with pure Fe oxidizers in 0K medium was found to be more feasible in terms of metal recovery, acid consumption, leaching time and total cost of the process. Whereas, 9g/L Fe supplemented medium was efficient to enhance the Cu recovery rate. The pure S oxidizing microorganisms supplemented with 3g/L of S<sup>0</sup> were found inefficient for the bioleaching of 50g/L of MPPCBs.

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