1. Enrichment of Chemolithotrophic Iron Oxidizer's from Mine Sites Sample and its Adaptation in Pulverized E Waste Sample of PCBs

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Abstract

The present investigation mentions enrichment of chemolithotrophic iron oxidizing bacteria from chalcopyrite and pyrite mines of the Indian peninsula. Samples from mines numbered as X and Y used for enrichment, these obtained samples were characterized for structural identifications using X-ray diffraction pattern (XRD) techniques. The results obtained show peaks confirming CuFeS2, Fe2O3 and Fe3(SO4) along with presence of CuO2, Fe2O3 minerals respectively. These samples are further crushed into fine powder and used for testing the biological oxidation of iron. Such powdery samples were inoculated in 9K medium with pH maintained at 2 in 250ml Erlenmeyer flask placed in a shaker for shaking rotations of 120 rpm at room temperature for 384hr. This enriched culture shows ferrous iron oxidation 93% and 92% respectively, further activation of enriched cultures carried out by transferring 10% v/v inoculation in fresh 9K medium. It shows a significant decrease in the amount of ferrous iron for X enriched culture and Y enriched culture as 0.3gm/l and 0.2 gm/l respectively. Under biological iron oxidation processes convert ferrous into ferric ions, these ions further act as lixiviate for removing metal from printed circuit boards. Hence its need to adapt enriched chemolithotrophic, to minimize the inhibitory effect of printed circuit boards (PCB).

PCB takes nearly 20 days for the adaptation of culture at 8gm/l. The concentration of ferrous iron in a 9K medium contains 8gm/l for PCB it is found to be 0.5gm/l and 0.4 gm/l. This finding shows that the enriched culture isolates ferrous iron oxidize successfully.

Key words: Bioleaching, Oxidation, 9k Medium, Chemolithotrophic.

Introduction

Globalization has lead to concentrating the world in small electronic devices, containing printed circuit boards of different designs and varying composition of copper and other elements including Ferrous. Hence such generated e-waste needs to be controlled or managed in very proper ways. The bacteria that catalyzed the oxidation of ferrous iron in to ferric iron known as iron bacteria were first observed by Ehrenberg and Winogradsky (Sebrina Hedrich et al 2011) The iron oxidizing acidophilus and extreme acidophiles (less than pH 3) have been isolated from various natural sites like geothermal, costal area and mainly they are found mining of coals, metals ores, pyrite ore, were they get energy from oxidation of iron (D. B. Johnson 1995). Iron being the large volume element on the earth used in almost every appliance for different purposes. Bacterial oxidation of iron i.e. ferrous converted into ferric form. The soluble ferric iron has significant oxidation capacity for extraction of metals and recovery of metals (Mahbubur et al 2010 and E. K. Demir et al 2020). This process follows the principle of microbial leaching, which is carried out in an acidic environment at pH 1.5 and 3, at this pH level most metals ions remain soluble (Punar et al 2014). Continuous demand for household and other modified electronic and electrical equipment leads to a huge volume of electronic waste (Subrada Hait et al 2018 and Y. Hong 2014). The main components of electrical and electronic equipment are printed circuit boards, which regulate functions of devices. These PCBs contain valuable and precious metals like Cu, Fe, Al, Ag, Ni, Pb, Zn, Al Ag, Fe, Mn, Sn, Sd etc. The metal content varies from device to device i.e depending on its function (Joshi et al., 2017). These e-wastes dumped into the environment cause ecological as well as environmental adverse effects and loss of metals. Chemolithotrophic bacteria, iron oxidizing bacteria like acidithiobacillus ferrooxidans and acidithiobacillus thiooxidans, used bioleaching of metals from printed wire boards (Jingwei wang et al 2009). Hence it is needed to isolate a potent iron oxidizing bacteria for recovery of metals from such e-waste.

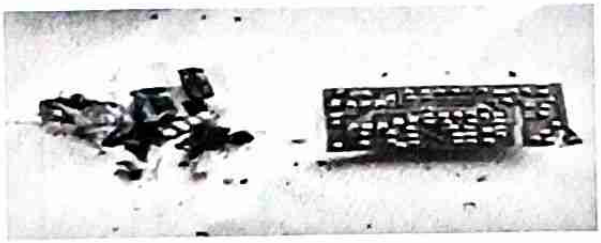
The present investigation mainly emphasizes on enrichment of iron oxidizing chemolithotrophic from two mine samples and the enriched culture were activated and adapted for growth in presence of printed circuit boards.

Material and Method

Two mine samples numbered as X and Y were used for enrichment of chemolithotrophic. The mineral composition was determined using X-ray diffraction (XRD) techniques, measured over Bruker AXS, (D8 Advanced) German model in scanning range of 20-80o (2θ) using CuKα radiations with wavelength of 1.5405Å.

Printed circuit boards (PCB) collected from the electronic and electrical equipment and maintenance centers. Mostly the preference was given to PCB's of Mobile phones, television sets etc.

These collected PCBs were used for further process. Printed circuit boards (PCBs) were neutralized by removal of plastic parts viz. RAM, PCI slot, chip slots etc PCBs pretreatment was conducted by dipping in 10M NaOH solution for 24 hrs, then after washing under running tap water. The washed water was replaced by fresh distilled water until the adhered NaOH was removed. This pre-treatment removes solder mask and chemical coating on PCBs. This may facilitate the ferric iron to efficiently remove the metals from printed circuit boards (Adhapure et al., 2014). Such PCBs samples were grinded using a hammer to convert it into fine partials.



Iron Oxidation

The crushed sample, one gram of two samples X and Y, was transferred to 100 ml complete 9K medium in two different Erlenmeyer flasks for the growth of iron oxidizing bacteria. The flasks were placed on an incubator shaker rotating at 120 rpm at 30o C for 168 hr. The medium was prepared with part A 3.0g (NH4)2SO4, 0.5gm MgSO4.7H2O, 0.5g K2HPO4.3H2O, 0.1g Ca(NO3)2.2H2O, and part B as FeSO47H2O 44.22g the energy source for bacterial growth per liter of distilled water adjusted to pH 2 with 1(N) H2SO4. Part A medium was separately sterilized and part B was sterilized using filter, after sterilization of part A add the part B (silverman & lundgren, 1959) and (Mousavi et al 2015). The growth activity of enriched bacteria was measured using titrimetric methods for determining the remaining ferrous iron in 9 K medium. (Vogel 1969).

Activation of Enriched Cultures

After enrichment of iron oxidizing bacteria, step wise procedure was used for activation of enriched culture by transfer of 10 % v/v of inoculum in a 9 k medium having pH 2. Similar procedure was used for three transfers and measured the remaining ferrous iron concentration in a 9K medium.

Adaptation of Activated Culture with Printed Circuit Boards

To adapt the activated enriched iron oxidizing chemolithotrophic in presence of printed circuit board. The crush sample of PCBs 2 gm/l, was inoculated in 250 ml Erlenmeyer flask

having100 ml of 9 K medium with actively growing 10% v/v inoculum, flask were incubated room temperature at 120 rpm on rotary shaker for 120 hrs. and iron oxidation rate was estimated. In this way, further three transfers were made and the amount of printed circuit board increased by 2 gm/l each transfer, up to 8 gm/l, for adaptation to 2-8g/l bacteria required 20 days

Results and Discussion

Fig 1 and 2 represents the XRD pattern for determination of mineral content into samples site X and Y respectively. The major elements found in the samples are CuFeS2, Fe2O3, Fe3(SO4)4 and CuO2 , Fe2O3.

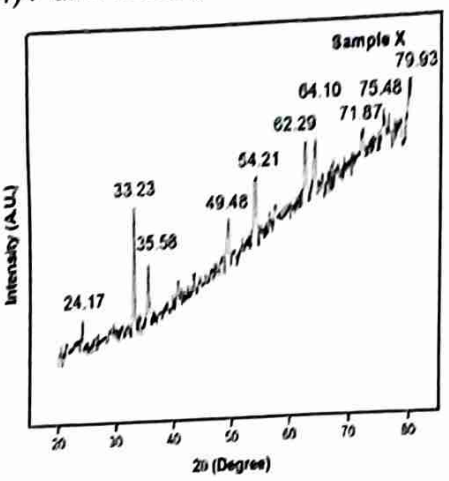


Figure 1. X-ray diffraction pattern obtained for iron samples X

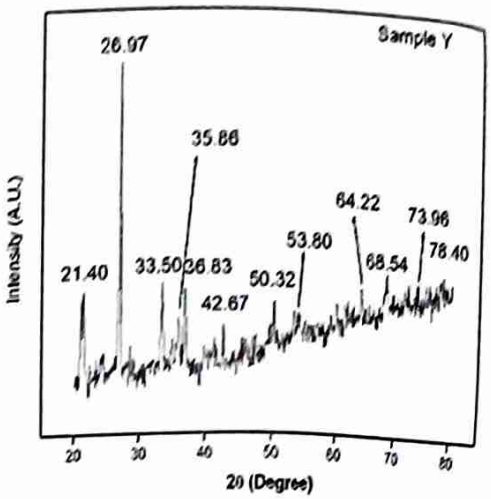


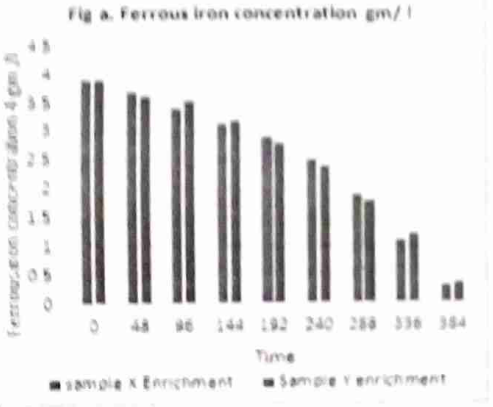
Figure 2. X-ray diffraction pattern obtained for iron samples Y

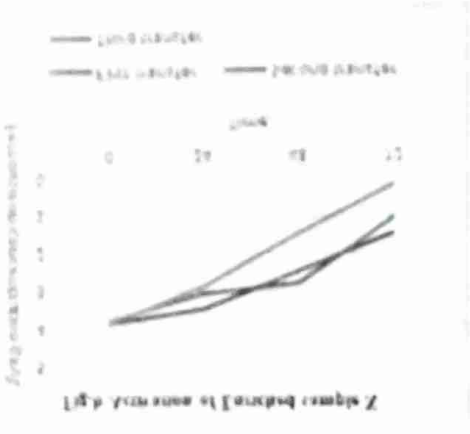
Figure1. X-ray diffraction pattern obtained for iron samples X-Figure 2. X-ray diffraction pattern obtained for iron samples Y

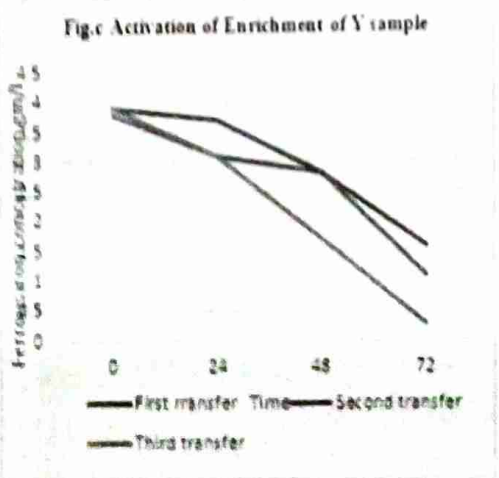
The growth activities of enriched cultures of both samples were analyzed by measuring remaining ferrous iron concentration in 9K medium. The results of iron oxidation test shown in Fig. 2(a) which shows Ferrous iron oxidation rate carried out by estimating the remaining soluble ferrous iron using the standard potassium dichromate titrimetric method. Initially the 9K medium contains 4gm/l concentration of ferrous ions, the sample X and Y took 16 days for oxidation of 93% and 92 % respectively.

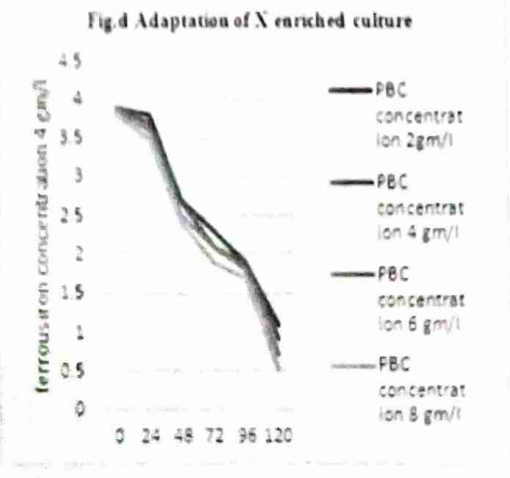
The activation of enriched culture for maximum concentration of ferrous iron oxidized in less time was carried out, the iron oxidation test shows significant result to reduced ferrous iron concentration in third transfer both cultures utilized maximum concentration of ferrous irons (Fig b and c). X-enriched culture reduced ferrous iron concentration to 0.3 gm/l and Y enriched sample 0.2gm/l, these enriched culture were adapted for different concentration (2gm/l, 4 gm. /l, 6 gm./l, 8gm/l) of pulverized samples of PCBs. This activated culture was adapted to a gradually

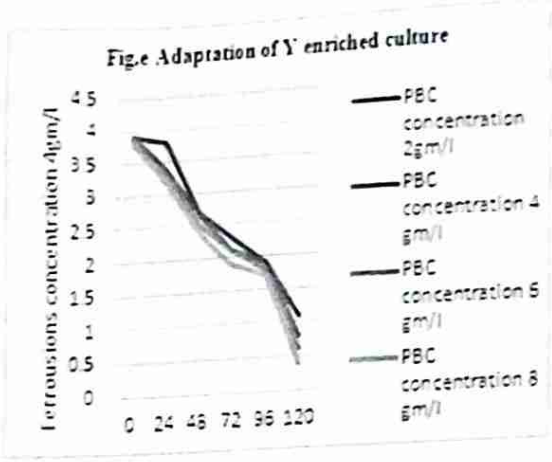
increased dose of PCBs because toxicity of metal ions in PCBs inhibit the growth of bacteria, hence it is needed to adapt the activated culture (Y. Xiang et al 2010). During the adaptation of activated cultures, growth of chemolithotrophic bacteria measuring the concentration of ferrous iron carried for 5 days. The adapted culture tolerated at 8gm/l PCBs (Fig. d & e) required twenty days. The concentration of ferrous iron in a 9K medium contained 8gm/l PCBs was found to be 0.5gm/l and 0.4 gm/l. The literature review, authors reported, on A. thiooxidans and A. ferrooxidans when maximum amount of bioleaching achieved from PCBs, it is to maximize its addition amount steps wise while avoiding inhibition of bacterial growth at the same (G. lang et 2010). Unlike other acidophilus, this enriched culture shows iron oxidation at pH 2, which means they are included in acidophiles isolated from pyrite mine samples. Hubau et al used acidophilic consortia for generation of biogenic iron lixiviate for bioleaching of printed circuit boards (A. Hubau et al 2018). The oxidation of ferrous to ferric irons conversion plays a very important role in bioleaching of copper from printed circuit boards (A. Isuldar et al 2015). The enriched culture shows iron oxidation in an acidic environment, further optimization of growth parameters for achieving maximum amount of bioleaching from printed circuit boards.











Conclusion

From the above studies it can be inferred that, the enrichment of chemolithotrophic bacteria from two mine sample, initial stage shows low rate of iron oxidation but after activation an improvement in iron oxidation rate was observed. This enriched culture is adapted for growth in presence of 8 mg/l PCBs and shows iron oxidations which means it converts ferrous ions into ferric ions. These adapted enriched cultures were used for bioprocessing of metals from printed circuit boards.

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