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Copper and Nickel Extracting Fungi from Low and Lean Grade Ores From Mine Areas of Chhattisgarh

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Abstract : Bioleaching is most effectual and low cost method of recovery of metals from low and lean grade ores against conventional chemical techniques as it requires less energy inputs for the recovery of small quantities of metal left after physical processing of high grade ores. Four potent fungi belonging to genera *Aspergillus* and *Penicillium* were explored for copper and nickel extraction from low and lean grade ores. *Aspergillus niger* NFCCI-3303 extracted higher amount of copper and nickel as compared to other three isolated fungi.

Keywords: Copper, Nickel, Low grade ores, Acid production, *Aspergillus*, *Penicillium*

Introduction

Chhattisgarh is a newly carved state from Madhya Pradesh in the year 2000 and is located in central India. It is the 10th largest state in India, with an area of 135,194 km^2. It is one of the fastest-developing states of the country. Chhattisgarh is one of the foremost mineral rich states of India. Eight mines of Chhattisgarh region *viz*., Lakshman coalfield mine, Kusmunda mine, Dalli mine, Rajhara mine, BIOP mine, Hirri mine, Rawan mine, Nandini mine and one ore-dumping site of Bhilai Steel Plant were explored for the study. Twenty nine acidophilic fungi were isolated from these study sites. After quantitative acid production, four isolates were selected for screening purpose using chalcopyrite and nickel laterite as leaching material.

Microbes are principally exploited for commercial application in industries. Their ability to solubilize metals from solid material has been explored in the recent past which has opened completely new prospects for their application in mineral biotechnology (Sabat & Gupta, 2009). Microbes of mining environments have become very important for current investigations. Several species of fungi have been used for the bioleaching process. Enormous literature has been published concerning the ability of fungi to extract metals from different resources (Ghobani *et al*., 2008; Anjum *et al*., 2009; Nouren *et al.*, 2011; Behera & Shukla, 2012 & Choudhary *et al*., 2014). Different heterotrophic microorganisms are widely distributed all over the mine areas. In past many researchers have emphasized on isolation of fungi for metal dissolution which can be useful towards biohydrometallurgy (Mishra *et al*., 2008; Bohinder *et al*., 2009; Behera *et al*., 2010; Gharich *et al*., 2013; Amin *et al*., 2014 & Kumari *et al*., 2015) . In the present study acidophilic fungi was isolated from different mine areas of Chhattisgarh region and best acid producing fungi was used for efficient copper and nickel solubilization from respective ores.

Materials and Methods

1.1 Isolation of Fungi

The fungus was isolated from eight mine areas of Chhattisgarh region *viz*., Laxman coalfield mine (22.35°N & 82.50° E), Kusmunda mine (22.35° N & 82.75° E), Dalli mine - Rajhara mine (20.56° N & 81.05° E), BIOP mine (18.60° N & 81.25° E), Hirri mine (21.98° N & 82.31° E), Rawan mine (21.24° N & 81.64° E), Nandini mine (21.37° N & 81.42° E) and one ore-dumping site of Bhilai Steel Plant (21.18° N & 81.39° E). Based on quantitative acid production four potent fungal isolates were selected for screening purpose *viz*., *Aspergillus niger* gr. NFCCI-3303 (LAK-2), *Aspergillus niger* gr. NFCCI-3307 (DM-1), *Penicillium* sp. NFCCI-3305 (HM-6) and *Penicillium* sp. NFCCI-3306 (KUS-2).

1.2 Ore Material

The ore material for leaching studies was acquired from various mine sites. The low grade chalcopyrite ore was collected with the assistance of faculty of School of Studies in Geology and WRM, Pt. Ravishankar Shukla University, Raipur and the nickel laterite was collected from Sukinda mine (21.03° N & 85.77° E), Orissa, India.

1.3 Characterization of ore sample

The elemental composition of the ores was determined by Scanning Electron Microscope (Zeiss EVO18 special Edition equipped with EDX 'Oxford Instruments' X-Max^N) using Aztec software. The mineral composition was determined by XRD (X-Ray Diffraction) using X'pert powder 'PANalytical'. The results obtained were analyzed with the help of available XRD data for minerals using the High Score plus software version 3.0e.

1.4 Bioleaching efficiency:

The fungal isolates were screened for solubilization of metals from collected ores. All bioleaching experiments were conducted using modified Czapek-Dox minimal medium supplemented with 1% of ground chalcopyrite or 1% nickel laterite ores (100 mesh). 100 ml of the medium in 250 ml Erlenmeyer flask was inoculated with 4 mm fungal disc. Control flask was separately maintained without fungal inoculums. All the flasks were incubated at room temperature (28±2°C) on a rotary shaker (100 rpm) for 10 days. After 10 days, mycelia were removed from the medium using Whatman No. 1 filter paper; 5 ml culture filtrate was taken and diluted with 5% HCl to 100 ml in volumetric flask. The concentration of soluble copper and nickel were determined by atomic absorption spectrophotometer (LABINDIA, AA8000) with an air-acetylene flame and expressed as g/ L. Metal recovery or leaching efficiency was calculated as follows:

Metal recovery % = Metal content in leachate / Initial metal content in ore × 100

1.5 Preparation of standard copper and nickel solution

Standard copper and nickel metal solution (1g/ L; Merck), were used for the preparation of respective standard copper and nickel solution by diluting with double distilled water for Atomic absorption spectrophotometer (LABINDIA, AA8000).

Results

The X-Ray diffraction pattern of the chalcopyrite revealed that the main mineralogical phase identified was bornite, quartz, chalcopyrite, covellite and pyrite (Figure 1) whereas nickel lateritic ore was mainly associated with chromite, maghemite, magnetite, goethite, ferrihydrite and quartz (Figure 2). The EDX analysis reveals that the percentage of copper and nickel was 2.76% and 0.42% along with other trace metals. The leaching efficiency of copper by all the four fungal isolates is shown in (Figure 3). The solubilization of copper was found to be between 3.64±1.27 to 7.71±0.52 g/ L by the fungal isolates. *Aspergillus niger* gr. NFCCI-3303 (LAK-2) exhibited maximum solubilization of copper from the respective ore (7.71±0.52 g/ L), followed by *Aspergillus niger* gr. NFCCI-3307 (DM-1) with a value of 5.23±0.51 g/ L and *Penicillium* sp. NFCCI-3305 (HM-6) with a value of 3.64±1.27 g/ L. The isolate *Penicillium* sp. NFCCI-3306 (KUS-2) recorded minimum solubilization (3.64±1.27 g/ L). The percentage of extraction of the ore by four fungal isolates ranged from 13.18% to 27.92%. The leaching efficiency of nickel by the same fungal isolates is presented in (Figure 4). The range of solubilization of nickel was recorded to be within 0.04±0.01 to 0.37±0.03 g/ L. The maximum solubilization of nickel was recorded for *Aspergillus niger* gr. NFCCI-3303 (LAK-2) to a tune of 0.37±0.03 g/ L. The percentage of extraction was 8.81%. The minimum solubilization was exhibited by *Penicillium* sp. NFCCI-3306 (KUS-2). The percent extraction of nickel was high with *Aspergillus niger* gr. NFCCI-3303.

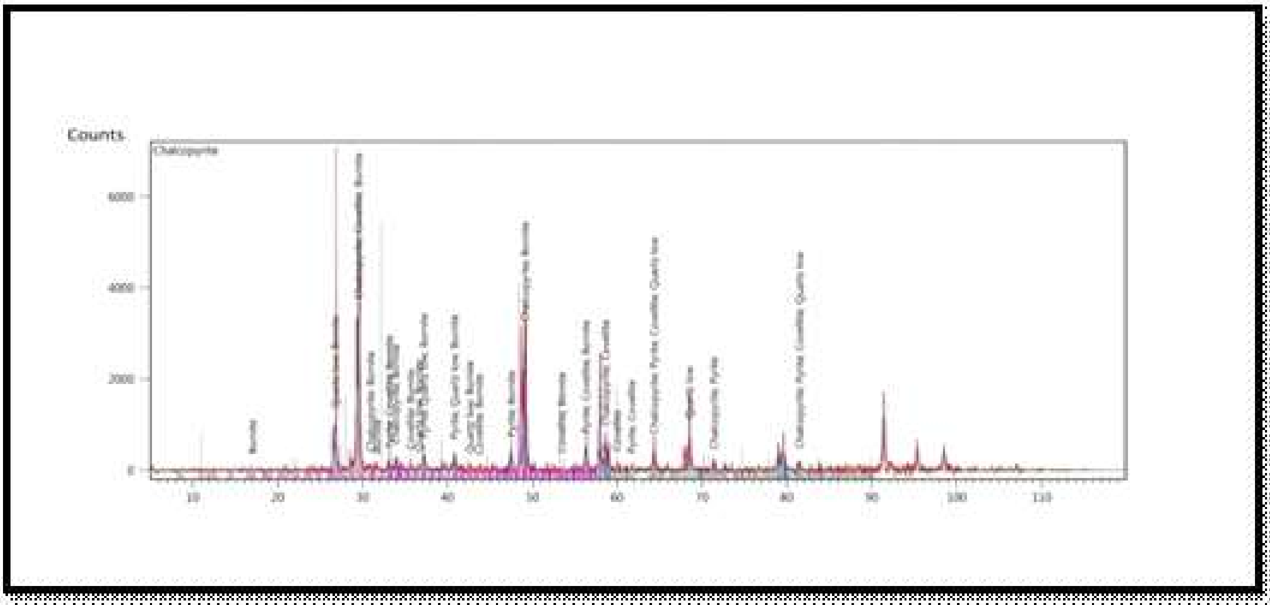

Figure 1: X-ray diffraction analysis of chalcopyrite ore showing peak of bornite, quartz, chalcopyrite, quartz, covillite and pyrite. The x-axis is 2ϴ (angle of diffraction) and the y-axis is the intensity.

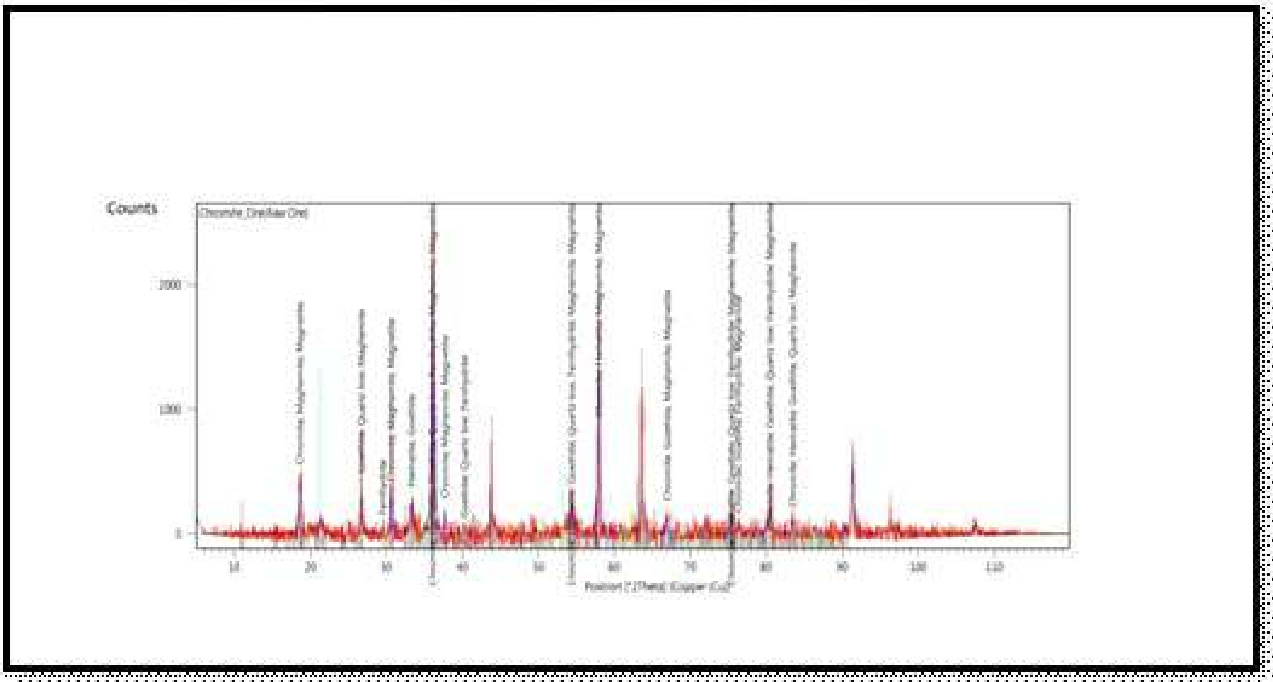

Figure 2: X-ray diffraction analysis of nickel lateritic ore showing the peak of chromite, maghemite, magnetite, goethite, ferrihydrite and quartz. The x-axis is 2ϴ (angle of diffraction) and the y-axis is the intensity.

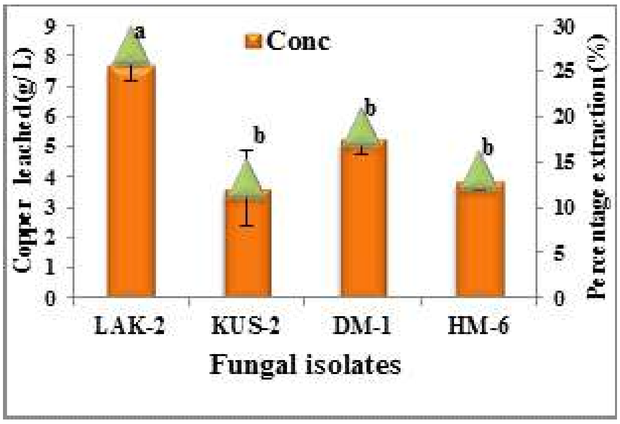

Figure 3: Screening of four selected fungal isolates for bioleaching efficiency of copper from chalcopyrite.

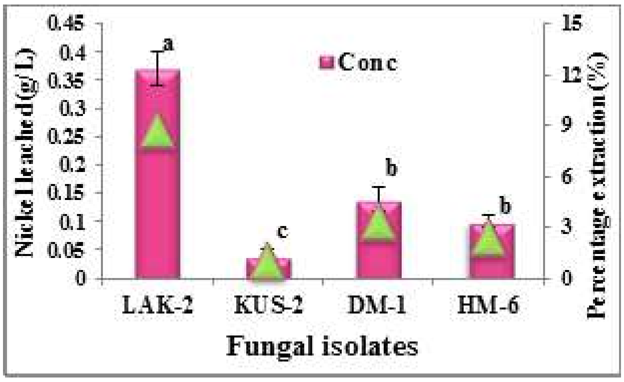

Figure 4: Screening of four selected fungal isolates for bioleaching efficiency of nickel lateritic ore.

Discussions

The chalcopyrite and lateritic nickel ore samples were evaluated to establish their suitability to serve as a raw material for copper and nickel leaching. The mineralogical characterization of the chalcopyrite ore by XRD-analysis revealed the presence of chalcopyrite and covellite as major phase along with other associated iron-bearing phases like magnetite (Fe_3O_4) and pyrite (FeS_2). The elemental composition of the chalcopyrite EDX analysis indicated that it was a low grade ore with 2.76% of copper along with other trace elements, which is uneconomical for commercial exploitation through conventional methods. The categorization of an ore to fit in low and lean category varies not only with the element but also with geographical region. Baba *et al*. (2011) exploited low grade chalcopyrite ore having 7.16 % copper present in it for the biooxidation by a mixed bacteria consortium predominantly having *Acidithiobacillus ferrooxidans* (Nakade, 2012) used low grade chalcopyrite (having 32.8% copper) for bioleaching of copper by *Thiobacillus thiooxidans* N-9.

The X-ray diffraction pattern of raw nickel lateritic ore mainly associated with chromite, maghemite, magnetite, goethite, ferrihydrite and quartz. Behera *et al.,* (2010) studied the X-ray diffraction of raw overburden lateritic ore and reported the presence of the goethite, a hydrated iron oxide (-FeOOH). The elemental analysis by EDX in the present study on laterite nickel ore revealed that the sample of raw ore was typically a low grade ore with the presence of 0.42% nickel along with other metal. Laterite ore is highly weathered material in which the main nickel bearing mineral is the hydrated iron oxide or goethite (FeOOH). Behera *et al*. (2010) reported a range of 0.4-0.9% nickel in chromite mine of Sukinda Valley, Orissa.

The efficient four acid producing strains *viz*., *Aspergillus niger* gr. NFCCI-3303 (LAK-2), *Penicillium* sp NFCCI-3306 (KUS-2), *Aspergillus niger* gr. NFCCI-3307 (DM-1) and *Penicillium* sp NFCCI-3305 (HM-6) were selected for bioleaching of copper and nickel metals from their respective ores. *Aspergillus* and *Penicillium* has been documented for their better biohydrometallurgy process for the recovery of metals (Castro *et al.*, 2000; Mulligan & Kamali, 2003; Zhou *et al*., 2008 & Siddiqui *et al*., 2009). The bioleaching studies in present context revealed that both the *Aspergillus niger* strains isolated from two different mines showed better potentiality for copper and nickel extraction from their respective ores over *Penicillium* sp. However, among the two *Peniciliium* sp., *Penicilium* sp NFCCI-3305 showed better leaching as compared from *Penicilium* sp NFCCI-3306. *Aspergillus niger* has been reported to exhibit good potential in generating a variety of metal solubilization organic acids during cellular metabolism (Mulligen *et al*., 2004; Behera *et al*., 2011; Marcincakova *et al.,* 2015). Also, *A. niger* has been documented to be more efficient in solublizing nickel as compared from the leaching ability of *A. fumigates* and *Penicillium* sp (Bohindar *et al*., 2009). It has been suggested that several species of fungi can be used for biomining (Siddiqui *et al*., 2009). They reported that *Aspergillus niger* and *Penicillium simplicissimum* were the best strains for the mobilization of metals. Thus in the present study *Aspergillus niger* NFCCI-3303 was found to be more effective for leaching purpose.

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References

Amin M.M.., El-AassyI.E., El-Feky, M.G., Sallam, A.M., El-Sayed, E.M., Nada A.A., and Harpy, N.M., (2014): Fungal leaching of rare earth elements from lower carboniferous carbonaceous shales, southwestern Sinai, Egypt. *Rom. J. Biophys.*, **24,** 25-41.

Anjum F., Bhatt, H.N., Ghauri M.A., Bhatti I.A., Asgher M., and Asi, M.R., (2009)**:** Bioleaching of copper, cobalt and zinc from black shale by *Penicillium notatum*, *Afr. J. Biotechnol.*, **19**, 5038-5045.

Baba A.A., Ezekafor E.E., Adekola, F.A., Ahmed, R.N., and Panda, S., (2011)**:** Bio-oxidation of a low grade chalcopyrite ore by mixed culture of acidophilic bacteria, *J. Ecobiotechnol.*, **3,** 01-06

Behera, S.K. and Sukla, L.B., (2012): Microbial extraction of nickel from chromite overburdens in the presence of surfactant. *Trans. Nonferrous Met. Soc. China*, **22**, 2840-2845.

Behera, S.K., Panda, P.P., and Sukla, L.B., (2011**):** Microbial recovery of nickel and cobalt from pre-treated chromite overburdens of Sukinda mines using *Aspergillus niger. Rec. Res. Sci. Technol.*, **3**, 28-33.

Behera, S.K., Sukla, L.B., and Mishra, B.K., (2010): Leaching of nickel laterite using fungus mediated organic acid and synthetic organic acid: A comparative study. *Proceedings of the XI International Seminar on Mineral Processing Technology* (Eds. R Singh, A Das, P K Banerjee, KK Bhattacharyya and NG Goswami), (NML Jamshedpur), pp 946-954.

Bohindar, S., Mohapatra, S., and Sukla, L.B, (2009): Nickle recovery from chromite of Sukinda using fungal strains, *Intl. J. Integr. Biol.* **5,** 103-107.

Castro, I.M., Fietto, J.L.R., Vieira, R.X., Tropia, M..J.M., Campos, L.M.M., Paniago, E.B. and Brandao, R.L., (2000)**:** Bioleaching of zinc and nickel from silicate using *Aspergillus niger* culture, *Hydrometallurgy.* **57,** 39-49.

Chaudhary, N., Banerjee, T., Ibrahim, N. and Sibi, G., (2014): Fungal leaching of iron ore: isolation, characterization and bioleaching studies of *Penicillium verruculosum. Am. J. Microbiol.*, **5**, 27-32.

Gharieb, M.M., Abdulkawi, A.A., and Ali, M.I., (2013)**:** Biological solubilization of hematite by some fungi, *Aust. Mycol.* **31**, 45-52.

Ghorbani, Y., Oliazadeh, M., and Shahvedi, A., (2008)**:** Aluminum solubilization from red mud by some indigenous fungi in Iran, *J. Appl. Biosci.*, **7**, 201-213.

Kumari D., Pan X., Achal, V., Zhang, D., Al-Misned & Mortuza, M.G., (2015): Multiple metal-resistant bacteria and fungi form acidic copper mine tailings of Xinjiang, China, *Environ. Earth Sci.,* **74,** 3113-3112.

Marcincakova, R., Kadukoka, J., Mrazikova, A., Velgosora O. and Vojtko, M., (2015): Lithium bioleaching from lepidolite using the yeast *Rhodoturula rubra*, *J. Pol. Mine. Eng. Soc.*, **16,** 1- 6.

Mishra, A., Pradhan, N., Kar, R.N., Shukla, L.B., and Mishra, B.K., (2008): Microbial recovery of uranium using native fungal strains, *Hydrometallurgy*, **95**, 175-177.

Mulligan, C.N, and Kamali, M., (2003): Bioleaching of copper and other metals from low-grade oxidized mining ores by *Aspergillus niger*, *J. Chem. Technol. Biotechnol.,* **78**, 497-503.

Mulligan, C.N., Kamali, M., and Gibbs, B.F., (2004)**:** Bioleaching of heavy metals from a low-grade mining ore using *Aspergillus niger*, *J. Hazard. Mater.*, **110,** 77-84.

Nakade, D.B., (2012), Biomining of copper using halophilic *Thiobacillus ferroxidans* N-9, *Cent. Eur. J. Exp. Biol.,* **1**, 1-6.

Nouren, S., Bhatti, H.N. and Ilyas, S., (2011)**:** Bioleaching of copper, aluminum, magnesium and manganese from brown shale by *Ganoderma lucidum. Afr. J. Biotechnol.,* **10**, 10664-10673.

Sabat, J. and Gupta, N., (2009): Iron ore solubilization by *Penicillium restrictum*: Effect of carbon and incubation days, *Am-Eura J. Agron.,* **2**, 43-44.

Siddiqui, M.H., Kumar, A., Kesari, K., and Arif, J.M., (2009)**:** Biomining - A useful approach toward metal extraction, *Am-Eura J. Agron.*, **2**, 84-88.

Zhou, Y., Huang, X., Huang, G., Bai, X., Tang, X., and Li Y., (2008)**:** Cu and Fe bioleaching in low-grade chalcopyrite and bioleaching mechanisms using *Penicillium janthinellum* strain GXCR, *Chin. J. Biotechnol.*, **24**, 1993-2002.