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Microbial Extraction of Cobalt and Nickel from Lateritic Chromite Overburden using *Aspergillus wentii*

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ABSTRACT

Low-grade nickeliferous lateritic ore from Sukinda region of Orissa, India, was subjected to biohydrometallurgical treatment for the extraction of nickel and cobalt. The mineralogical studies reveal that nickel is entrapped in goethite matrix while cobalt is associated with the manganese phase. *Aspergillus wentii* NCIM 667, a citric acid producing fungal strain, was used for direct (one step and two step) and indirect (using culture filtrate) leaching of the metals under different conditions. The effect of varying pulp density (2%, 5%, 8%) and culture medium composition (*viz.* molasses and sucrose media) was investigated and the leaching conditions optimized. It was found that a maximum of 49.29% Ni and 35.18% Co could be recovered from the heat-treated lateritic chromite overburden by the culture filtrate bioleaching at 80°C with 2% pulp density.

Keywords: *Aspergillus wentii,* Molasses medium, Citric acid, Nickeliferous Laterite, Chromite overburden, Bioleaching.

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INTRODUCTION

Decrease in the dependence on nonrenewable raw materials to meet the demand of primary resources for sustainable development is a crucial factor in the current era. Since the primary natural sources of many strategic metals are limited and dwindling rapidly, application of novel technologies in the area of metal recovery from alternative resources has to be explored and developed. In addition, improvement of existing mining techniques may result in the recovery of metals from sources that have not been of economical interest until today. Metal-winning processes based on the activity of microorganisms offer a possibility to obtain metals from mineral resources not accessible so far by the conventional metallurgical operations [1-2]. Acting as biocatalysts, numerous bacteria and fungi can convert various types of insoluble metal compounds into their water-soluble forms. In addition, it is possible to recover metal values from industrial wastes as secondary sources of raw materials applying biological methods [3].

In the present study chromite overburden of Sukinda mines in the Indian state of Orissa was subjected to bioleaching for the extraction of nickel and cobalt. Sukinda mines contain nearly 98% of the total proved chromium ore reserves of the country [4]. After the chromite mining is done, the left over lateritic overburden contains minor amounts of nickel and other valuable metals like cobalt. Since there is no primary source of nickel in India, ultramafic rocks which are weathered and lateritized to various degrees are the main source of nickel in this country; and the most important nickeliferous laterite deposit of significance in India is the Sukinda ultramafic belt. The complex mineralogy, heterogeneous nature and low nickel content of the nickeliferous laterite make physical beneficiation of the overburden quite difficult, and thus the extraction of nickel is almost impossible from this source [5-7]. Therefore, utilization of the overburden as a source of nickel (as well as cobalt) needs development of alternative technology, and biological processes may provide such a route. This idea led to the initiation of this feasibility study of a fungal strain producing organic acids leach out metal values from ore bodies. In this study, effect of different leaching techniques and pulp density on the extraction of nickel and cobalt using a citric acid producing fungal strain, *Aspergillus wentii* NCIM 667 had been investigated.

It is now well-known that microorganisms take part either directly or indirectly in the leaching of metals from ores and minerals, which is a natural phenomenon occurring through thousands of years. Fungi usually take part in the process via acidolysis, complexation and chelate formation. Nevertheless, the mechanism of bioleaching by the heterotrophs is still not well-understood [8-12]. Previously we reported the efficiency of bioleaching process for the extraction of Ni and Co from the same overburden deposit whereby an oxalic acid producing *Aspergillus niger* strain was used; Considerably high and rapid extraction of the metal values (Ni and Co) from the overburden was observed [13]. Since the efficiency of leaching by heterotrophs depends much on the microbial strain used, more and precisely on the type and quantity of organic acids and other metabolites produced by it (or by the microbial consortium), the present work was undertaken to assess the efficiency of a citric acid producing heterotroph, *Aspergillus wentii* NCIM 667, under various conditions.

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MATERIALS AND METHODS

Materials

The chromite overburden sample was collected from the mining site of Kaliapani open cast mines of Orissa Mining Corporation at Sukinda Valley, Jajpur district, in the Indian state of Orissa. The inorganic salts were of analytical grade; sucrose and molasses were procured from a local grocery shop. Yeast extract was a product of Himedia.

Partial Chemical Analysis and Mineralogy of Chromite Overburden

The overburden sample was first crushed manually and thereafter subjected to jaw crushing followed by roll crushing and finally sieved to obtain a particle size of -75 +53 µm. Metal composition of the sample was determined by an atomic absorption spectrophotometer (Aanalyst 200 Perkin Elmer) after its chemical digestion by 3N HCl. Mineralogical analysis of the raw and pretreated samples were carried out using Rigaku Ultima-III X-ray diffractometer with a Bragg-Brentano geometry and a Cu-kα radiation (λ=0.154nm) to identify the major and minor minerals. Pre-treatment of the raw chromite overburden sample was done in an electric arc furnace operated at 600° C for 5 hours under normal atmospheric condition.

Fungal Strain and Growth Condition

Aspergillus wentii NCIM 667, a citric acid producing fungal strain was procured from National Chemical Laboratory, Pune, India. It was maintained on potato dextrose agar medium. Two media were used for citric acid production by the strain. The first medium contained in (g/L): $MgSO_4$ $7H_2O$ - 0.5, KH_2PO_4 - 1, NH_4NO_3 - 3, yeast extract- 1 and cane molasses-140 (wet weight), pH was adjusted to 6. Before use molasses (140 g) was treated with 35 ml/L of 1N H_2SO_4 to reduce its sugar content to about 25% sugar level. In practice, the molasses suspension was kept in a boiling water bath for an hour, cooled and neutralized with lime water. It was kept at room temperature over-night and the clear supernatant was decanted. Requisite amounts of other ingredients were then added, pH was adjusted and volume was made up; total sugar content of the medium was estimated to be 15 % (w/v) [14]. The second medium contained sucrose as the c-source and had the following composition in g/L: NH_4NO_3 - 3, KH_2PO_4 - 1, $MgSO_4.7H_2O$ - 0.5, sucrose - 100, yeast extract - 1 initial pH of the medium was adjusted to 6.8 with 0.5 N NaOH. Spores from 7-days old potato dextrose agar slants at 30° C were suspended in 10 mL 0.1% (v/v) tween 20 solution and were counted under a light microscope in a Neubauer chamber. Suitably diluted 1 mL of suspension containing $2x10^7$ spores was added to 100 mL liquid medium in a 500-mL conical flask. The flasks were kept on a rotary incubator shaker at 30°C for scheduled period to allow growth; thereafter the mycelia were separated from the culture filtrate which was preserved at 4°C until used in leaching experiments.

Synthetic Citric Acid Leaching

Leaching of the metal values with citric acid solution was conducted to evaluate the effect of other metabolites in the culture filtrate. The synthetic citric acid solution contained the same concentrations of citric acid as in the culture filtrate of different days.

Methods of Bioleaching

Bioleaching was performed in 500-mL Erlenmeyer flasks containing 100 mL of sucrose or molasses medium and pretreated chromite overburden at three different pulp densities (2, 5, 8% (w/v)). All glass apparatus and media were sterilized at 121°C for 15 min prior to inoculation with fungal spores. All direct bioleaching experiments were conducted in an orbital shaking incubator at 30±1 °C and 150 rpm whereas the culture filtrate leaching experiments were carried out in a shaking water bath at 80±0.4°C. Three different methods of bioleaching were investigated. The first method was one-step bioleaching; in this method the fungus was grown in presence of chromite overburden. The second method was two-step bioleaching, in which the fungus was first allowed to grow in molasses or sucrose medium for 2 days and then the chromite overburden sample was added; during this period, pH of the culture started to decrease indicating organic acid production. The third method was culture filtrate leaching. In this method, the fungus was grown in molasses or sucrose medium for a period not exceeding 21days; the cultures were harvested at an interval of 7 days and the cell-free culture filtrate were collected. The filtrates containing fungal metabolites were used for the leaching of chromite overburden samples that were added to the filtrates. Control experiments were conducted using fresh sucrose medium.

Analytical Methods

After every pre-determined interval, the fungal biomass from each flask was separated by filtration, and the filtrate was analyzed for citric acid content. The citric acid was detected and its concentration was measured by HPLC using a Model Agilent 1200 series HPLC Analyzer having Zorbax eclipse XDB-C18 column (250 x 4.6 mm, i.d.). Two mobile phases [first phase methanol:water (30:70 v/v); second phase- 0.05M KH_2PO_4 of pH 2.5] at a flow rate of 1 mL/min at 25˚C were used. As standard, 20 µL of 100 mM solution of HPLC-grade citric acid was injected. The concentration of organic acid was measured at 214 nm. All elemental analysis was done using an atomic absorption spectrophotometer (Aanalyst 200, Perkin Elmer). The pH of culture filtrate, leached liquors and growth media was measured using a digital pH meter. The fungal biomass obtained after filtration was washed with water, carefully transferred to a pre-weighed Petri dish and dried at 80 °C for 24 hs to determine the dry weight of the biomass.

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RESULTS AND DISCUSSION

Characterization of Chromite Overburden

The chromite overburden sample was collected from the Kaliapani open cast mines of Orissa Mining Corporation is highly weathered. It is rich in oxides of iron and contains minor amounts of nickel and cobalt. Nickel in the ore is reported to be entrapped in the goethite matrix and cobalt is primarily associated with the manganese mineral phase [5]. The partial chemical analysis of the Chromite Overburden shows that, the raw ore contains 0.87% nickel, 0.03% cobalt, 48.88% iron, 1.88% chromium and 0.37% manganese, whereas the heat-treated ore contained 0.97% nickel, 0.04% cobalt, 51.79% iron, 1.9% chromium and 0.61% manganese were detected. Pre-treatment of the raw ore at 600° C results in complete conversion of goethite into hematite as is evident from Figure 1 showing the XRD profile of both raw and the heat-treated ore samples. It is apparent that heat-treatment caused homogeneous distribution of nickel particles throughout the matrix making the sample more amenable to leaching. Chemical analysis revealed an increase in nickel and cobalt content after the heat-treatment, because of the removal of moisture and other volatile fractions associated with the ore. The treatment also increased the porosity of the ore particle surface resulting in more penetration of the leach solution facilitating better dissolution and mobilization of metal values from the ore matrix.

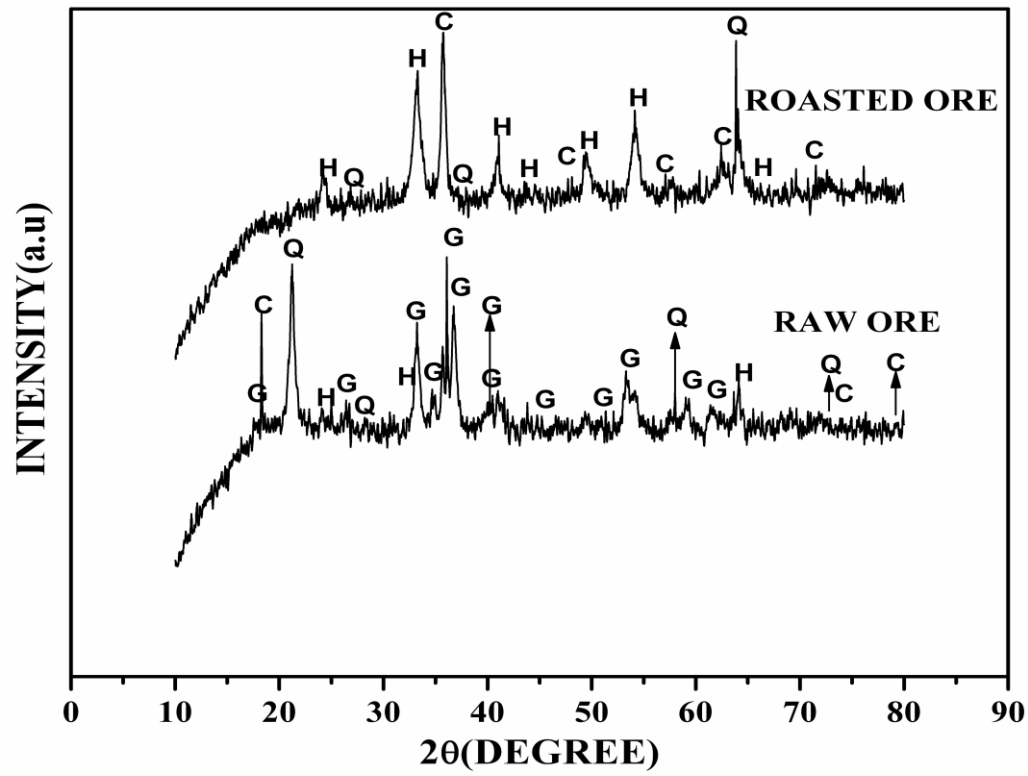

Figure 1: XRD analysis of raw and roasted chromite overburden.

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Production of Citric Acid

Citric acid, an intermediate of primary metabolism, is not usually secreted by a microbe under natural conditions in noticeable amounts. Hence any appreciable secretion must be the consequence of some severe metabolic irregularity caused by drastic metabolic imbalances or genetic deficiencies in the strain. Hence only a few species of microorganism have been reported to excrete substantial amounts of citric acid into the culture medium only under certain conditions [15]. To start with, we tried to get a citric acid producing microbe from other laboratories in India, and obtained an *Aspergillus wentii* strain (NCIM 667) from National Chemical Laboratory (CSIR), Pune. The medium suggested for maintenance of the strain was potato-dextrose agar/broth which did not support sufficient production of citric acid. It is well-known that starchy materials do not support citric acid production and high concentration of sugars (like sucrose or molasses) supports its production. Other medium components (N-source, phosphate concentration, trace elements) also influence citric acid excretion profoundly [15]. Hence we used a medium suggested by Sikander et al [14] using both sucrose and molasses as C-source. Figure 2 shows that the microbe excreted 33.1 gm/L and 21.5 gm/L citric acid after 21 days from molasses and sucrose medium, respectively.

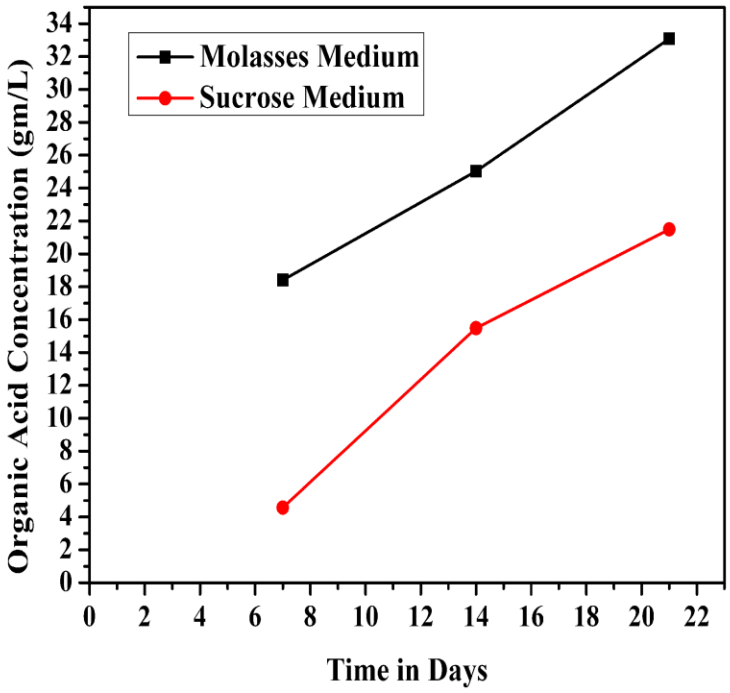

Figure 2: Production of organic acid using different culture media.

Recovery of Metal Values Using *Aspergillus wentii* Culture Filtrate

Leaching of the two strategic metals Ni and Co from chromite overburden was always much higher from the roasted ore compared to that from the raw ore [4-5, 13]. Maximum amounts of the metals were leached from the roasted ore, 21 days-grown culture filtrate and at

80°C (Fig 3a and 3b). The experiments were carried out for 3 hs because no significant increase in metal recovery was achieved beyond this period. Culture filtrate collected after fermentation beyond 21 days did not show any improvement in leaching capacity (data not shown); hence, fermentation was carried out up to 21 days.

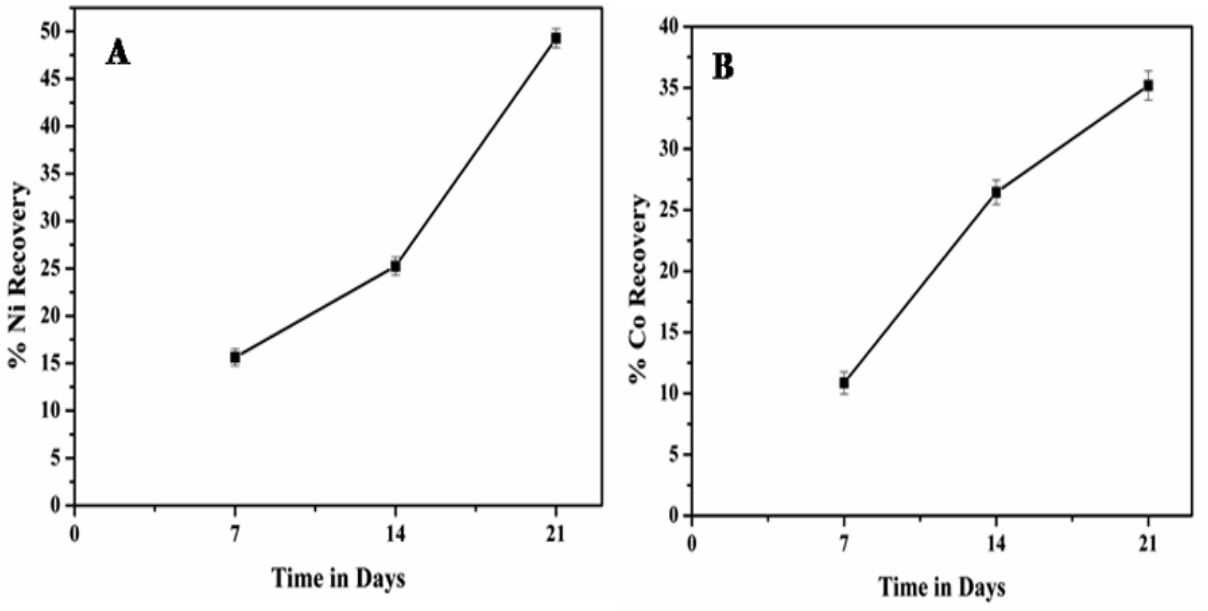

Figure 3: Percentage metal recovery using culture filtrate of *Aspergillus wentii*: (A) Nickel; (B) Cobalt.

Effect of Pulp Density on Metal Recovery

Pulp density is an important parameter that influences metal recovery from ores. In this study, we found that the same is equally applicable for the chromite overburden using any bioleaching process. Figure 4 & 5 shows that the optimum pulp density for the maximum recovery of metal values from the overburden was 2% for all the processes and two culture media used. A direct relationship exists between the percentage of metal recovery and fungal growth. Higher fungal growth led to higher citric acid and other secondary metabolite production resulting in higher percentage of metal recovery. At pulp densities higher than the optimum, leaching of Ni and Co decreased in all the processes. In case of one step and two step bioleaching this decrease was due to the high toxic metal concentration in the culture medium which may inhibit fungal growth and organic acid production while in case of culture filtrate bioleaching the decrease in metal recovery might be due to the high level of iron in the overburden trapping more citric acid and other metabolites for its chelation and complexation at higher pulp densities. Gang materials present in the overburden may react or absorb acid, lowering its effective concentration at higher pulp densities. At an optimum pulp density of 2% 49.3±3% Ni and 35.2±2% Co was obtained while 16.8±1.9 and 12.5±2% of Ni and 18.2±1.8 and 14.4±2% of Co was obtained in case of 5% and 8% pulp density respectively. Table 1 and table 2 show a comprehensive summarization of results leading to optimization of pulp density for molasses and sucrose medium respectively.

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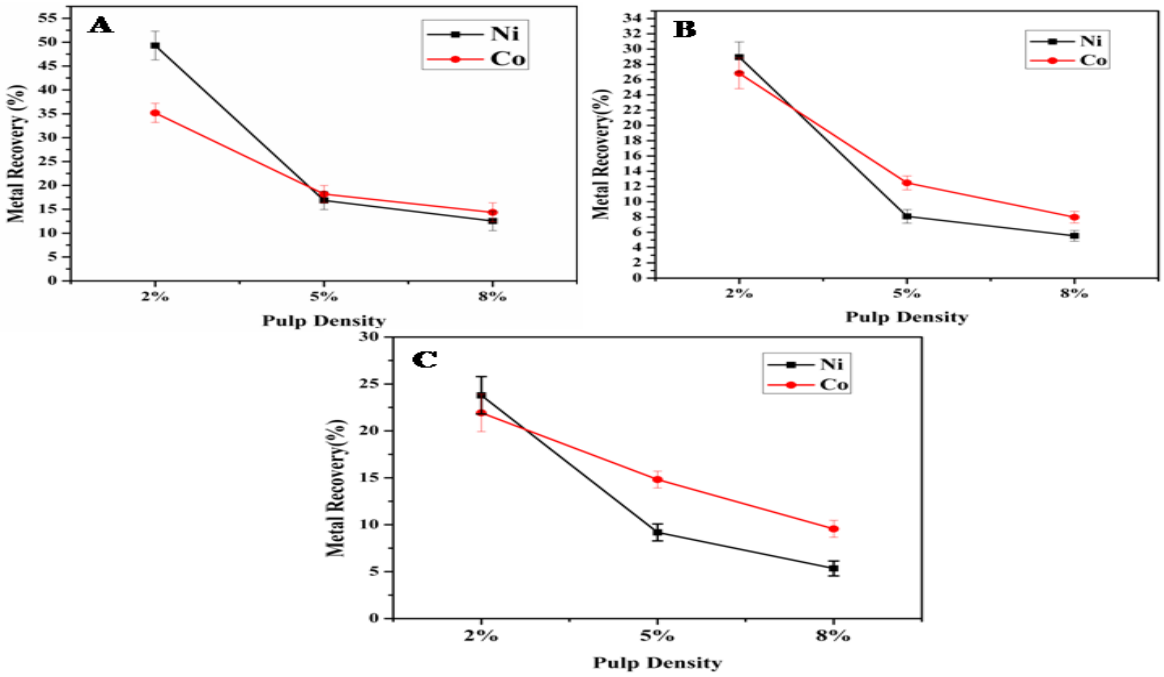

Figure 4: Effect of pulp densities on recovery of metals using different bioleaching methods and molasses medium: (A) Culture filtrate; (B) Two-step process; (C) One-step process.

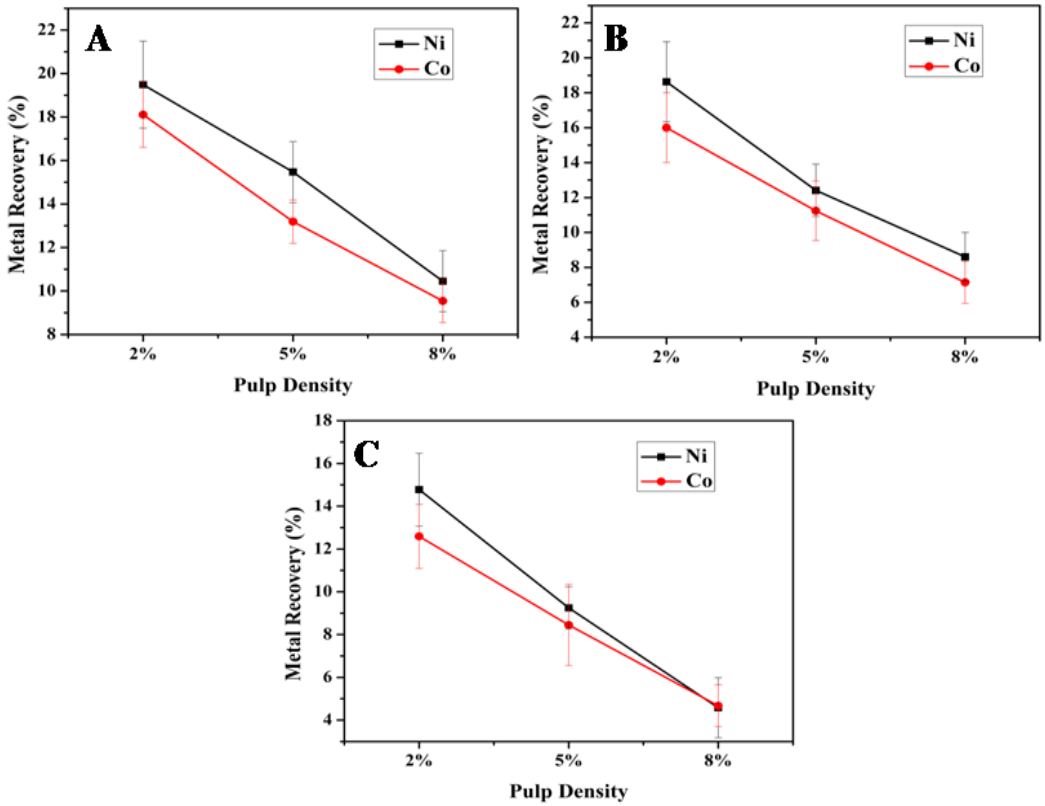

Figure 5: Effect of pulp densities on recovery of metals using different bioleaching methods and sucrose medium: (A) Culture filtrate; (B) Two-step process; (C) One-step process.

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Elements	Pulp Density (%)	Percentage Recovery								
		One Step			Two Step			Culture Filtrate		
		Days			Days			Days		
		7	14	21	7	14	21	7	14	21
Ni	2	9.77	15.18	23.77	16.47	22.39	28.95	19.54	29.48	49.29
	5	2.88	4.54	9.18	8.09	9.08	8.09	7.14	13.08	16.85
	8	1.54	3.57	5.34	1.47	4.52	5.54	5.49	8.51	12.54
Co	2	14.9	18.00	21.92	10.98	10.98	26.83	10.86	26.45	35.18
	5	6.35	10.81	14.81	7.18	7.18	12.48	7.93	11.43	18.19
	8	2.78	5.87	9.56	2.35	4.68	7.98	6.57	9.87	14.35

Table 1: Summarization of results for the optimization of pulp density using molasses medium.

Elements	Pulp Density (%)	Percentage Recovery								
		One Step			Two Step			Culture Filtrate		
		Days			Days			Days		
		7	14	21	7	14	21	7	14	21
Ni	2	4.21	10.47	14.78	13.03	14.3	18.63	10.63	15.08	19.49
	5	1.45	5.87	9.25	5.84	8.54	12.41	4.49	11.75	15.47
	8	0.87	2.41	4.58	1.25	3.54	8.6	2.87	6.47	10.45
Co	2	3.74	8.54	12.59	6.56	14.66	16	9.46	13.95	18.11
	5	2.3	5.78	8.45	3.65	8.12	11.24	3.58	11.43	13.19
	8	0.54	1.24	4.67	2.47	4.52	7.14	1.95	4.65	9.54

Table 2: Summarization of results for the optimization of pulp density using sucrose medium.

Comparison of Bioleaching Methods

As shown in Figure 6, the most favorable results for both the metals were obtained from the culture filtrate bioleaching process at a pulp density of 2% (w/v) where 49.3±3% Ni and 35.2±2% Co were obtained. It is also evident that culture filtrate bioleaching is favourable for the extraction of both the metal values even in case of the experiments conducted with sucrose medium though the percentage recovery of metals was less (19.5±2% Ni and 18.1±2% Co). In control experiments (data not shown) using fresh medium (molasses/sucrose medium), the recovery yield of Ni and Co was negligible at all pulp densities.

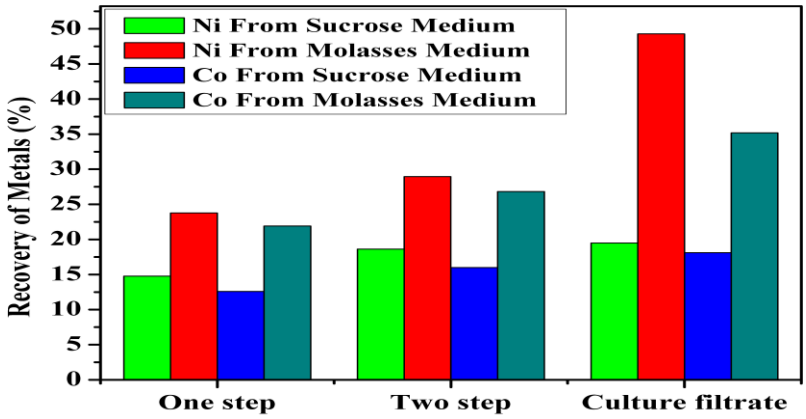

Figure 6: Metal recovery at optimum pulp density in different bioleaching and using different growth media.

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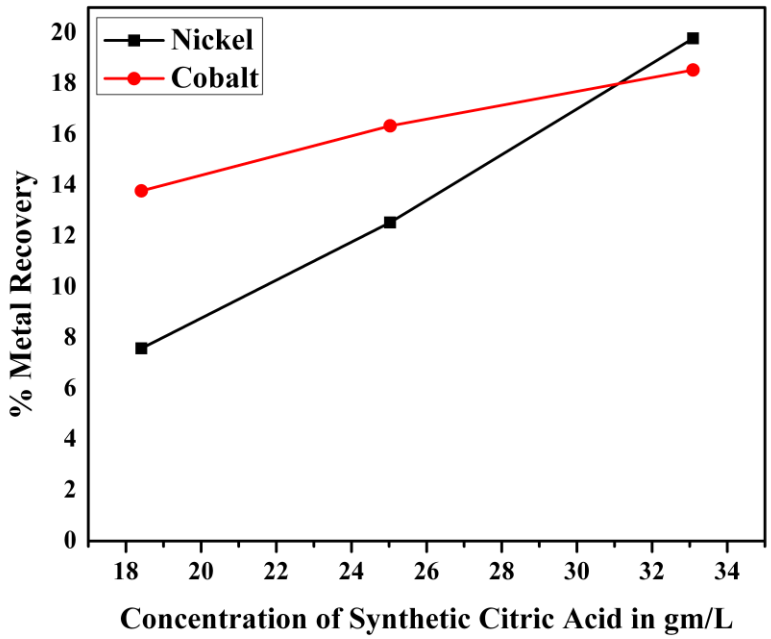

Figure 7: Recovery of metals using synthetic citric acid.

Figure 7 shows the results of synthetic citric acid leaching of chromite overburden using the same citric acid concentrations secreted by *A. wentii* NCIM 667. The figure 7 shows that 19.8% Ni and 18.5% Co were recovered, which were much less than the highest recovery percentage obtained in case of fungal culture filtrate leaching. The difference suggests that metabolites other than the organic acids were involved in metal leaching.

Effect of Culture Medium on Recovery of Metals

Figure 8 shows recovery of metal values from the chromite overburden using two different culture media. Comparison of the results shows that 49.3±3% Ni and 35.2±2% Co were leached using molasses medium whereas 19.5±2% Ni and 18.1±2% Co were obtained using sucrose medium. This difference in percentage recovery is attributed to the decreased production of citric acid as primary metabolite in case of sucrose medium shown in figure 7. Thus citric acid plays the key role in the recovery of metal values because its concentration in the lixiviant is directly proportional to the percentage recovery of metals. In addition, other secreted metabolites participate in the leaching process enhancing the metal recovery (already mentioned in the section 3.5). Figure 9 shows the drop in pH of the culture broth and increase in fungal biomass during the course of fungal growth using different culture media. The medium pH decreased to 3.6 from an initial pH of 6 in 7 days and 2.8 at the end of 21 days in case of molasses medium whereas in case of sucrose medium from an initial of 6.8 the pH decreased to 6 in 7 days and 4 at the end of 21 days. On the other hand, the fungal dry weight increased from 4.59 gm/L after 7 days to 5.71 gm/L at the end of 21 days in case of molasses medium whereas in case of sucrose medium the dry weight increased from 1.17 gm/L after 7

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days to 4.48 gm/L at the end of 21 days. The results clearly indicate the better efficiency and favourability of molasses over sucrose medium.

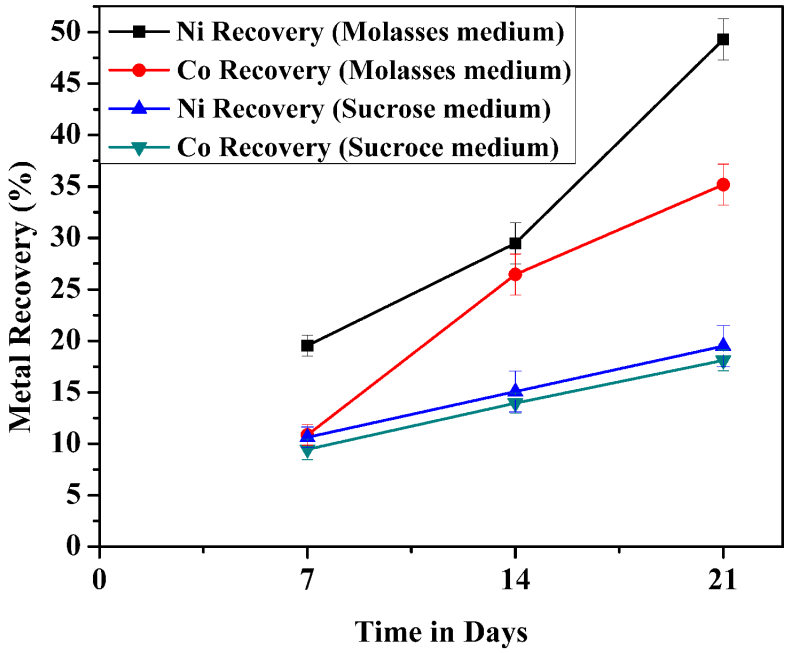

Figure 8: Recovery of metal values using different culture media.

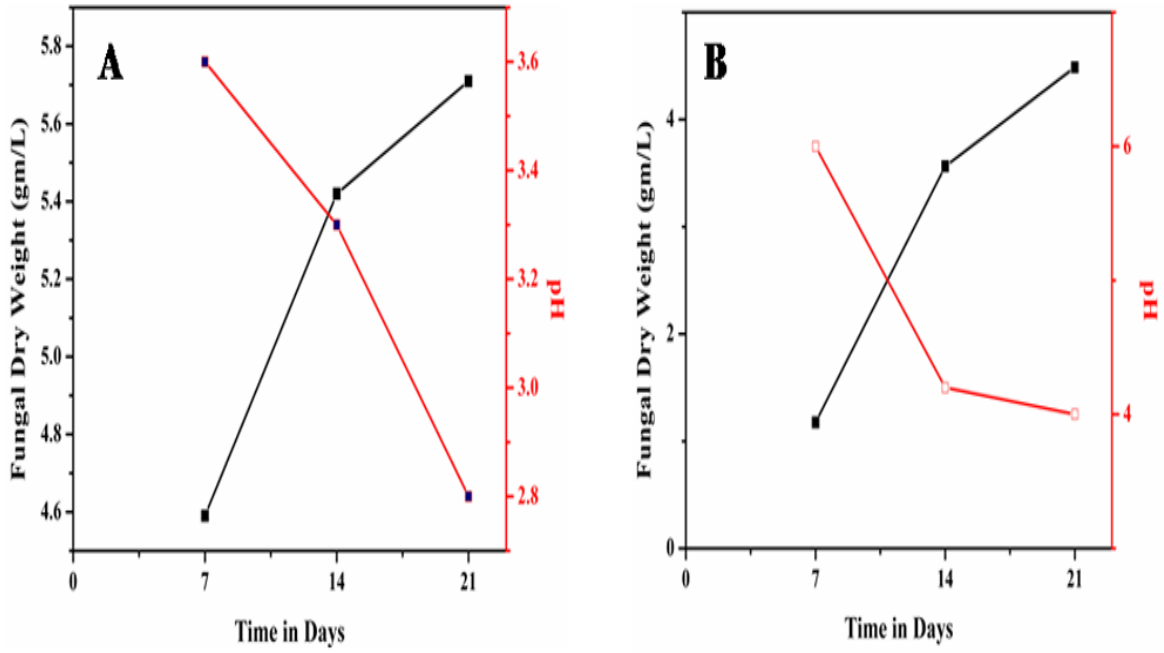

Figure 9: Plot showing pH of fungal culture filtrate and dry weight of fungal biomass using (A) Molasses medium; (B) Sucrose medium.

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CONCLUSION

This work shows that metal values from chromite overburden can be mobilized by different methods of bioleaching using *Aspergillus wentii* NCIM 667. Culture filtrate bioleaching was found to be most effective for recovery of both nickel and cobalt. Optimum recovery of both the metals employing any of the three bioleaching processes at 2% pulp density using molasses medium was achieved. Though bioleaching process offers many advantages over other conventional methods due to its relatively simple methodology, requirement of mild operating conditions, low energy input, reduced skilled labour requirements, and eco-friendliness, but it requires a longer period of operation. These results suggest that optimizing the process of bioleaching using *Aspergillus wentii* NCIM 667 could facilitate the creation of an alternative to conventional methods in treating underutilized natural resources like one used for the present study.

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