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Bioleaching of nickel by *Aspergillus humicola* SKP102 isolated from Indian lateritic overburden

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ABSTRACT

The lateritic deposits spread over the Eastern Ghats of Sukinda Valley, Odisha, India, produce a huge amount of overburden annually as a byproduct of chromite mining. This chromite mining overburden contains nickel, the only source of the metal in the country. During this study *Aspergillus humicola* SKP102, an indigenous fungus isolated from the mining overburden was employed for the leaching of nickel. About 53.89% of the nickel could be leached by the fungus when grown in batch mode using a Czapek dox medium containing 2% (w/v) of the mining overburden. The parameters affecting bioleaching were optimized in order to grow the fungus and leach the metal. Of the different options of cheap carbon sources, straw infusion and molasses emerged as viable options for the growth of the fungus and the leaching of nickel. Two-step and indirect techniques were also used for this purpose, and they resulted in 53.09% and 65.04% Ni leaching respectively. Adding diluted sulfuric acid to the leaching medium resulted in 97.05% nickel recovery from the overburden pulp. *A. humicola* SKP102 could be a potential tool for leaching nickel from the mining overburden.

1. Introduction

Nickel is a very important metal from an industrial point of view as it is used in the making of many industrial consumer products such as magnets, stainless steel plating and alloys of different types. It is also used in the minting of coins and adds a green tint to glass. India's requirement of nickel is mainly met through imports as there is no exclusive nickel deposit in the country (Mohapatra, Bohidar, Pradhan, Kar, & Sukla, 2007). However, around 0.99% of nickel is entrapped in the goethite matrix of the lateritic iron rich ore found in the Sukinda and the Baula-Nuasahi chromite mining belt of Odisha, India. Both open-cast and underground mining techniques are carried out in these areas by the Government and private agencies, these in turn lead to the generation of a large amount of overburden amounting to nearly 8 to 10 times more than the amount of chromite ore (Acharya, Kar, & Sukla, 1998). In these overburden deposits nickel is present in a lateritic form (Behra, Panda, & Sukla, 2011) along with some other important metals like chromium, iron and cobalt. The availability of all these strategic metals in the mining overburden makes it a potential metal pool. However, the conventional hydrometallurgical and pyrometallurgical techniques used for the extraction of these metals from low-grade ores and overburdens have certain disadvantages, such as poor product recovery, high costs of operation, high energy consumption and environmental incompatibility due to high pollution rates (Rohwerder, Gehrke, Kinzler, & Sand, 2003). These economical as well as ecological limitations of conventional metal extraction methods have promoted the idea of using the inherent microflora of the mining environment in the bioprospecting of metal values in an eco-compatible way (Castro et al., 2000). The leaching of metals from so called 'low-grade' ores and the mining overburden has been practiced all around the globe with the intention of sustainable usage of the resources in an ecologically compatible way (Biswas, Samanta, Dey, Mukherjee, & Bannerjee, 2013; Ke & Li, 2006; Pradhan, Pal, Sukla, Roy Chaudhury, & Das, 2008; Rao, Channappa, & Gaddad, 2002; Zilouei Shojaosadati, Kalilzadeh, & Nasernejad, 2003).

During the course of our studies on the heterotrophic leaching of metal values by acidogenic microbiota isolated from the chromite mining environment of Odisha, India, a potent leaching agent identified as *Aspergillus humicola* SKP102 was reported (Ghosh & Paul, 2015). The present communication is an attempt to use this fungal strain for leaching nickel from chromite mining overburden

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and to optimize the cultural conditions for the extraction of nickel in an economically and ecologically compatible manner.

2. Materials and methods

2.1. Chromite mining overburden

The chromite mining overburden sample was collected from the mining sites of the South Kaliapani open cast mines of Sukinda Valley, Jajpur district of Odisha, India. The highly weathered sample was transferred to the laboratory in sterile containers and stored at 4 °C for further use. The sample was sieved through a 0.1 mm sized sieve and was used for the purpose of bioleaching.

2.2. Characterization of the overburden

The moisture content and pH of the overburden sample were determined following Jackson's standard methods (1973). Metal content was determined following the digestion of the overburden sample in a HNO_3:$HClO_3$ (1:3) mixture, filtration through Whatman No. 42 filter paper and quantification in an Atomic Absorption Spectrometer (Varian Spectra AA 20 Plus) using an air-acetylene flame (APHA, AWWA, & WEF, 1998). X-ray diffraction (XRD) analysis was made to determine the mineralogical composition of the overburden sample. The dried and sieved overburden sample was scanned in a Rigaku miniflex diffactometer (Kβ filter) following the method described by Saeed, Bhatti, and Bhatti (2002).

2.3. Fungal culture and maintenance

Aspergillus humicola SKP102, used throughout this study, was isolated from the mining overburden sample collected from the chromite mining sites of the South Kaliapani region of Odisha, India (Ghosh & Paul, 2015). The strain was maintained on slopes of Czapek Dox medium by regular sub-culturing at an interval of 30 days. As and when required the fungal strain was preserved at 4 °C.

2.4. Metal tolerance of the isolate

Metal tolerance of the isolate was determined using the broth dilution method of Calomiris, Armstrong, and Seidler (1984). Separately sterilized metal solutions were added aseptically to the liquid medium (20 ml medium/100 ml flask) inoculated with homogenized fungal spore suspension (10^6 spores/ml) and incubated at 30 °C under continuous shaking (120 rpm) using a rotary shaker for 8 days. Growth of the isolate was recorded by calculating the dry weight of the mycelial biomass. The minimum concentration of the metal in the medium completely inhibiting the growth of fungi was considered as the minimum inhibitory concentration (MIC).

2.5. Identification of acid(s) produced by the fungus

The mycelium free culture medium after 8 days of growth was lyophilized at −56 °C using an LSL Secfroid Lyophilizer and used for the determination of extracellular organic acids produced using the High Performance Liquid Chromatographic technique. The experiments were performed in Water's 510 HPLC, using the E18 column and a flow rate of 1 ml/min. The solvent used was 6 mM H_3PO_4 and the peaks were detected at 210 nm of UV light following the methods of Bosshard, Bachofen, and Brandl (1996).

2.6. Biological leaching of metals

Biological leaching experiments were performed in batch mode following direct and indirect approaches.

2.6.1. Direct one-step leaching

Homogenized spore suspension of *A. humicola* SKP102 was prepared in 1% Tween 80 and used as the source of inoculum in the direct one-step leaching experiments. Pulp (2%) was added to 50 ml of Czapek dox medium in 250 ml Erlenmeyer flasks along with fungal spore suspension with a concentration of 10^6 fungal spores/ml medium. The set up was incubated under continuous shaking at 120 rpm on a rotary shaker at 30 °C for 30 days. Supernatant from the growing culture was collected at regular intervals and the amount of nickel released in the medium was measured using an Atomic Absorption Spectrophotometer (Varian AA 20 Plus) with air-acetylene flame at 232.0 nm. To optimize the conditions for direct one-step leaching parameters, such as effect of aeration, pulp density, particle size, inoculum density, non-conventional carbon sources and sulfuric acid supplementation, were evaluated.

2.6.2. Direct two-step leaching

In the case of direct two-step bioleaching, the Czapek-dox medium (50 ml/250 ml flask) was inoculated with 10^6 spores/ml and incubated under continuous shaking (120 rpm) for 8 days at 30 °C. After the desired period of growth, overburden pulp (0.1 mm sized) was added aseptically at a level of 2% and incubated for an additional 30 days at 30 °C keeping all other conditions unchanged. Metal values released in the medium were estimated at 10 day intervals after the addition of pulp as in direct one-step leaching.

2.6.3. Indirect leaching

Indirect bioleaching of metals was tested using the mycelia-free culture filtrate of the fungal isolate. The fungal isolate was grown in 50 ml of leaching medium (Czapek-dox medium) contained in 250 ml Erlenmeyer flask for 8 days at 30 °C under continuous shaking at 120 rpm. After 8 days of incubation, the fungal biomass was separated by centrifugation (Remi R24 centrifuge, 10,000 × g for 10 min). 2% overburden pulp (0.1 mm sized) was added to the supernatant and this was incubated under the same conditions for 30 days. The metal leached in the medium was estimated as in direct one-step bioleaching.

All the experiments were performed in triplicate and the results represent mean ± standard deviation.

3. Results

3.1. Characteristics of the mining overburden

The overburden sample collected from the mining environment of South Kaliapani, Sukinda valley [21°0′N-21°5′N:85°43′E-86°0′E] belonging to the Keonjhar district of Odisha, India was used in the present study. Results of the physico-chemical and mineralogical studies of the overburden, as shown in Table 1 and Fig. 1, reveal that the sample was low in moisture content and exhibited a pH of 7.57. The metal content of the overburden was high, and contained 7.2 mg/g of Ni(II) along with iron, chromium, magnesium and manganese and was used as the pulp for Ni(II) leaching studies.

An X-ray diffraction study of the overburden sample reflected the general mineralogical pattern of the area. The lateritic minerals, namely, Goethite, Limonite, Haematite, Magnetite, Chromite, Spinel and Ulvospinel, were present in the sample and this along with the presence of clay mineral Illite is indicative of the mixing of mining overburden with the top soil as a result of weathering activities.

3.2. Metal tolerance and acid production by A. humicola SKP102

The metal tolerance profile of the *A. humicola* strain SKP102 was determined during the growth in the metal supplemented Czapek dox medium and expressed as minimum inhibitory concentration

Table 1
Physico-chemical characteristics of the overburden sample collected from South Kaliapani region, Sukinda valley, Odisha, India.

Sample no.	Moisture content, %	pH	Metal content (mg/g)						
			Fe	Ni	Cr	Co	Mg	Mn	Zn
02	52.23 ± 0.5	7.57 ± 0.0	368.5 ± 8.5	7.2 ± 1.1	8.67 ± 1.5	0.88 ± 0.2	8.15 ± 0.9	5.13 ± 0.5	1.0 ± 0.0

Values represent the average of triplicate readings ± SD.

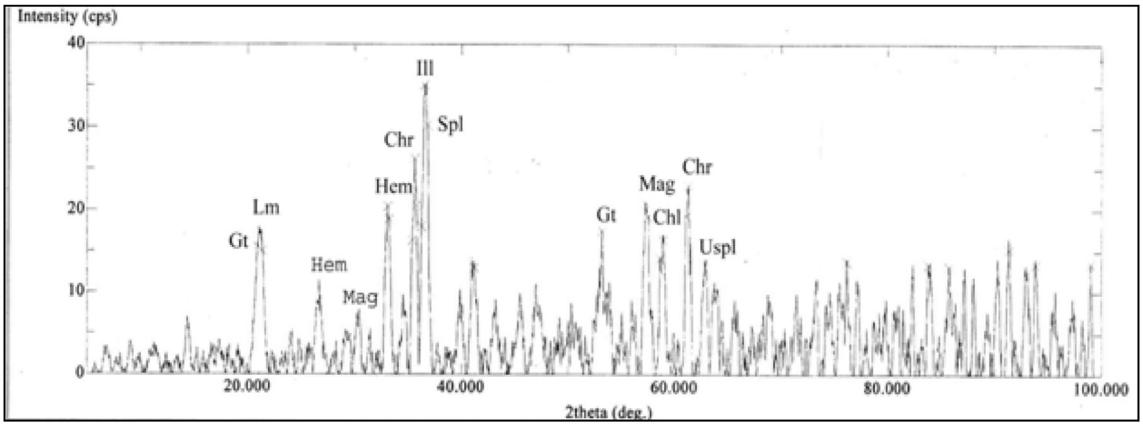

Fig. 1. X-ray diffraction pattern of the overburden sample collected from South Kaliapani (Sample no. 02) showing the presence of Gt-Goethite, Lm-Limonite, Hem-Haematite, Mag-Magnetite, Chr-Chromite, Ill-Illite, Spl-Spinel and Uspl-Ulvospinel (Mineral abbreviations following Kretz, 1983).

of the metal. Results, as summarized in Table 2, reveal that *A. humicola* SKP102 was tolerant to as high as almost 9.0 mM of Ni(II), and also showed considerable resistance towards Fe(II), Zn(II) and Cu(II). However, the fungus was relatively sensitive to Cr(VI), Co(II) and Hg(II), as exhibited by the low MIC values.

Growth associated production of organic acids by *A. humicola* SKP102 was also studied, and the results show that the fungus after 8 days of growth in Czapek dox broth under continuous shaking produced solely oxalic acid (8.47 g/l).

3.3. Time course of direct one-step metal leaching

Time course for metal leaching was performed by *A. humicola* SKP102 to understand the pattern of leaching over time and to standardize the incubation period required for optimum bio-leaching. Experiments were conducted in batch mode with the fungus (10^6 spores/ml) inoculated in Czapek dox broth containing 2% overburden material of 0.1 mm particle size in triplicate sets. The flasks were incubated at 30 °C under continuous shaking for 30 days and samples were withdrawn at an interval of every 5 days. The amount of Ni released into the medium was quantified using an Atomic Absorption Spectrophotometer (AA 20 Plus).

Results (Fig. 2) show that, nickel leaching by *A. humicola* SKP102 increased with the time of incubation and reached its maximum on the 30th day of incubation. The amount of nickel leached increased rapidly during the first 20 days of incubation, thereafter, the amount of nickel leached declined as revealed by the flattening of the curve and ultimately after 30 days of incubation, about 53.89% of Ni was released from the overburden. Change in the pH of the leaching medium was also studied as a function against time and it was observed that there was a sharp decline of pH (3.0) until the 20th day of incubation, and the pH values increased.

Table 2
Metal tolerance profile and growth associated production of organic acid by *A. humicola* SKP102.

Growth (mg/g)	10.9
Oxalic acid produced (g/l)	8.47
Minimum inhibitory concentration of metals (mM)	
Ni	9.0
Cr	3.0
Co	2.5
Cu	5.0
Zn	7.0
Hg	0.01
Fe	>12

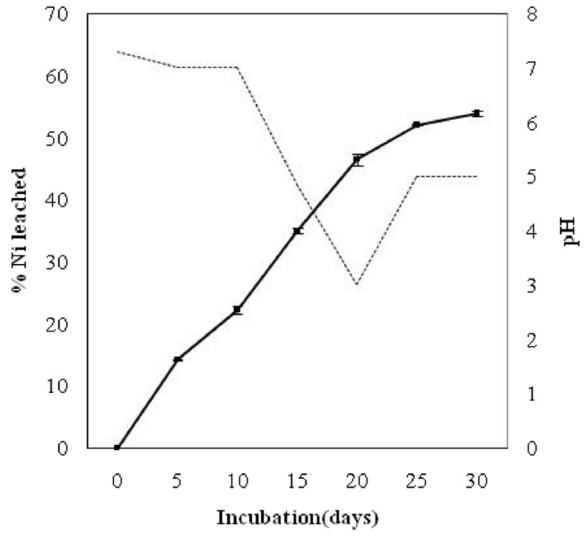

Fig. 2. Time course of Ni bioleaching by *A. humicola* SKP102 under shake condition (-■-Ni and —pH). (Incubation 30 days, pulp 2% w/v and 0.1 mm particle size, inoculum 10^6 spores/ml, shaking 120 rpm, Ni leached was measured using an Atomic Absorption Spectroscopy. Results represent mean ± S.D. of triplicate sets).

3.4. Effect of aeration

The bioleaching of Ni was conducted with *A. humicola* SKP102 under stationary and shake culture conditions to observe the

importance, if any, of aeration on the leaching of metal values from the overburden. The results when tabulated (Table 3) in a comparative manner revealed that aeration of the culture medium due to continuous shaking (120 rpm) has enhanced the rate of bioleaching to greater than that of the incubation under stationary condition. After 30 days of incubation under shaking condition, *A. humicola* SKP102 leached almost three times more Ni (53.8%) from the overburden as compared to a stationary condition (17.78%).

3.5. Effect of pulp density

The concentration of pulp in the leaching medium has an impact on the rate of bioleaching, hence, various concentrations of pulp (1–4% w/v) were used for the standardization of the optimum pulp concentration. Results (Fig. 3) reveal that, after 30 days of incubation, metal leaching was best with 2% (w/v) pulp density when compared to the other concentrations used. Lower as well as higher pulp concentration had an inverse effect on the rate of bioleaching. *A. humicola* SKP102 leached 56.01% Ni from the overburden sample after 30 days of incubation at 2% pulp density, while after 30 days of incubation 52.1, 54.1, and 50.26% nickel was leached with 1, 3 and 4% pulp densities respectively.

3.6. Effect of particle size

The particle size of the overburden pulp has been seen to affect the rate of bioleaching, hence, this parameter was also studied by using the overburden sample sieved through meshes of various sizes (0.1–0.8 mm). It can be concluded from the results (Fig. 4) that *A. humicola* SKP102 showed better bioleaching with the minimum particle size (0.1 mm) after 30 days of incubation. There was a sharp decline in the leaching of nickel as the particle size of the overburden was increased, indicating the fact that increase in the size of the pulp has an adverse effect on bioleaching.

A. humicola SKP102 was capable of leaching 60.4% nickel from the overburden with particle size with a maximum diameter of 0.1 mm, the amount of metal leached decreased gradually as the particle size increased from 0.2 mm (54.2%) to 0.5 mm (50.1%). The lowest amount of nickel leaching was recorded with 0.8 mm particle size, where, after 30 days only 47.1% Ni could be solubilized.

3.7. Effect of the initial fungal inoculum dose

The amount of fungal inoculum can affect the rate of the bioleaching of metal values from the overburden. Different doses of fungal inocula (10^5–10^8 spores/ml) were added to the leaching medium in order to assess the effect of inoculum doses on metal leaching whilst keeping all the other parameters unchanged.

From the results (Fig. 5) of leaching metals by varying the initial fungal inoculum doses, it can be concluded that the rate of bioleaching rapidly increases when the initial inoculum dose is increased up to a level of 10^8 spores/ml in the bioleaching medium. Nickel leaching by *A. humicola* SKP102 increased from 45.37 to 64.62% as the initial inoculum dose was increased from 10^5 to 10^8 spores/ml of the leaching medium.

Table 3
Comparative account of Ni leaching by *A. humicola* SKP102 under stationary and shake culture conditions.

Incubation, days	% Ni leached	
	Stationary culture	Shake culture
10	1.68 ± 0.0	19.6 ± 0.1
20	14.5 ± 2.4	44.6 ± 0.2
30	17.78 ± 1.6	53.8 ± 0.2

Incubation 30 days, pulp 2% w/v, 0.1 mm particle size, inoculum 10^6 spores/ml, nickel leached was measured using an Atomic Absorption Spectroscopy. Results represent Mean ± S.D. of triplicate sets.

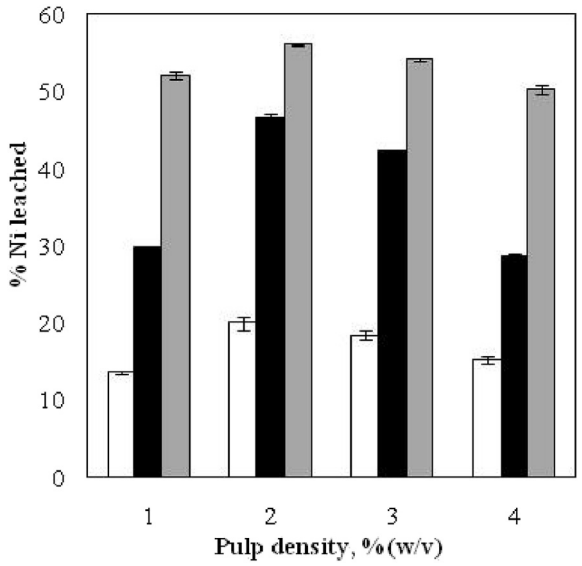

Fig. 3. Effect of pulp density of the overburden on the bioleaching of Ni by *A. humicola* SKP102. (Medium containing 1–4% pulp (0.1 mm particle size) was inoculated with 10^6 spores/ml medium and incubated for 10 (-□-), 20 (-■-) and 30 (-▪-) days under continuous shaking at 120 rpm. Nickel leached was measured using an Atomic Absorption Spectroscopy. Results represent mean ± S.D. of triplicate sets).

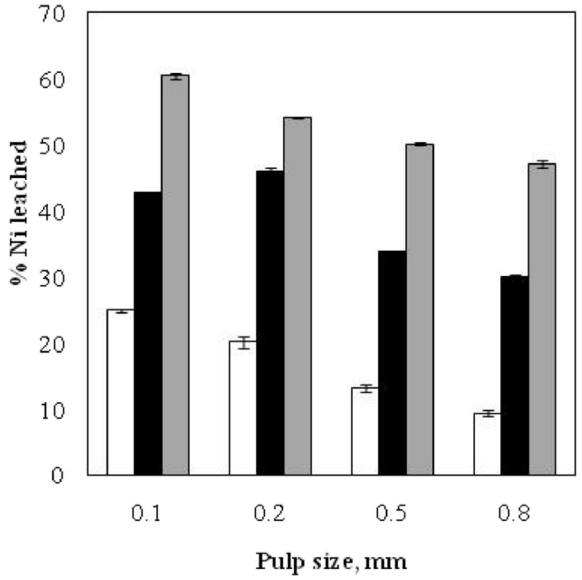

Fig. 4. Effect of particle size of the overburden on bioleaching of Ni by *A. humicola* SKP102. (Medium containing 2% pulp (0.1–0.4 mm particle size) was inoculated with 10^6 spores/ml medium and incubated for 10 (-□-), 20 (-■-) and 30 (-▪-) days under continuous shaking at 120 rpm. The nickel leached was measured using an Atomic Absorption Spectroscopy. Results represent mean ± S.D. of triplicate sets).

3.8. Use of non-conventional substrates

Various cheap and readily available carbon sources, i.e. molasses, cane sugar, straw infusion and potato peel, were used for the purpose of minimizing the cost of leaching. Decoction of straw

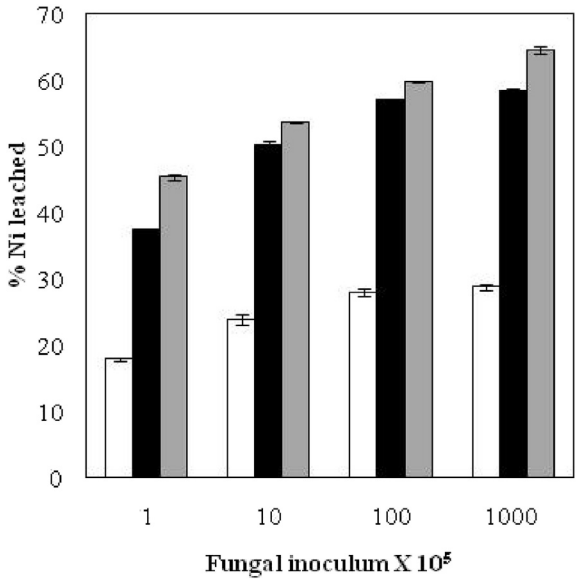

Fig. 5. Effect of initial dose of fungal inoculum on bioleaching of Ni by *A. humicola* SKP102. (Medium containing 2% pulp (0.1 mm particle size) was inoculated with 10^5-10^8 spores/ml medium and incubated for 10 (-□-), 20 (-■-) and 30 (-▪-) days under continuous shaking at 120 rpm. Nickel leached was measured using an Atomic Absorption Spectroscopy. Results represent mean ± S.D. of triplicate sets).

and potato peel was prepared by boiling them in water, whereas, molasses and cane sugar were used as the source of carbon in place of sucrose in the Czapek-dox medium. The leaching media prepared containing 2% overburden pulp was inoculated with 10^6 fungal spores/ml for the leaching of nickel.

Results as reflected in Fig. 6 indicate that of the four cheap carbon sources used, straw infusion and molasses increased the rate of bioleaching when compared to the Czapek-dox broth. In the case of *A. humicola* SKP102, nickel leaching increased from 50.61 with Czapek-dox to 66.09 with molasses and 74.24 with straw infusion. However, use of cane sugar and potato peel as a sugar supplement was not effective and the rate of nickel leaching decreased in both cases. Only, 31.75 and 17.3 Ni was leached using cane sugar and potato peel respectively after 30 days of incubation.

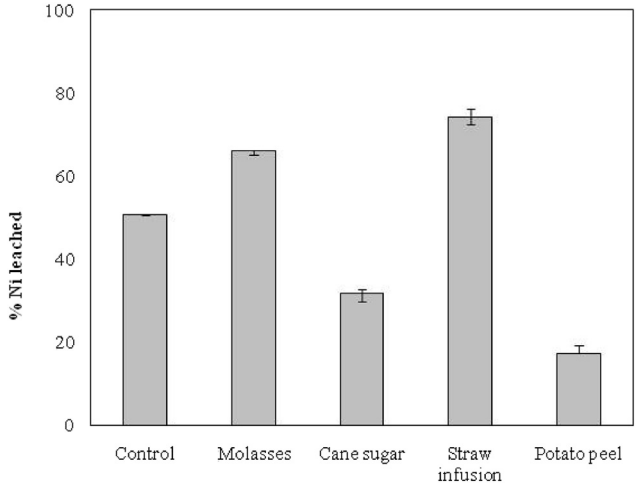

Fig. 6. Effect of non-conventional substrates on bioleaching of Ni by *A. humicola* SKP102. (Medium containing 2% pulp (0.1 mm particle size) was inoculated with 10^6 spores/ml medium and incubated for 30 days under continuous shaking at 120 rpm. Nickel leached was measured using an Atomic Absorption Spectroscopy. Results represent mean ± S.D. of triplicate sets).

3.9. Effect of sulfuric acid supplementation

Additional use of dilute sulfuric acid (0.1N) in a tolerable dose along with fungal spores was attempted in order to improve the rate of bioleaching and the results were remarkable.

Highly efficient metal leaching was exhibited by *A. humicola* SKP102 when the leaching medium was supplemented with dilute sulfuric acid (0.1 N). Over 30 days of incubation, the fungus with sulfuric acid amended medium showed better leaching with respect to just the sulfuric acid as well as the fungus alone in the Czapek dox medium. About 97.05% Ni, was leached in the acid supplemented medium which was 40% more than the leaching achieved by only 0.1 N sulfuric acid and 47% more than that using *A. humicola* SKP102.

3.10. Comparison of bioleaching by direct one-step, two-step and indirect leaching techniques

While direct one-step leaching was performed as the methods described earlier, for the two-step process, *A. humicola* SKP102 was inoculated in a Czapek-dox medium (10^6 spores/ml) and incubated for 8 days under continuous shaking at 30 °C. 2% pulp was added on the 8th day to the fermented medium. Subsequently, leaching experiments were conducted for 30 days, keeping all other parameters unchanged.

In the case of indirect leaching, *A. humicola* SKP102 was grown in a Czapek-dox medium for 8 days under continuous shaking at 30 °C for 8 days. The fermented medium was centrifuged aseptically and the separately sterilized pulp (0.1 mm) was added to mycelia-free culture filtrate at a level of 2% and incubated at 30 °C for 30 days.

Both direct and indirect techniques for bioleaching showed promising results (Fig. 8) and were indicative of improvement in metal leaching over the direct bioleaching processes employed. Leaching increased consistently for the 30 days of incubation, within 10 days 18% Ni was leached in the one-step technique, 20.6% in the two-step technique and 27.36% in the indirect method. Metal leaching continued until 30 days of incubation when, there was 50.61% of leaching in the one-step technique, up to 59.63% in the two-step technique and finally 65.4% in the case of indirect bioleaching.

4. Discussion

The physico-chemical (Table 1) analysis of the overburden sample collected from Sukinda valley Odisha, India showed near neutral pH and high metal content that corroborated the earlier findings of Acharya et al. (1998). Dwivedi, (1970), in a similar context however, reported that in mining areas low pH may be due to the presence of a significant quantity of pyrite or other acid forming minerals in the soil. Mineralogical analysis of the overburden sample, as revealed by the XRD technique, reflects the presence of lateritic minerals like haematite, goethite, chromite, limonite etc. along with clay minerals like ulvospinel and spinel (Fig. 1).

Reports reveal that, metal tolerance and adaptation to multi-metal environment are essential pre-requisites for microorganisms involved in heavy metal bioleaching. There are reports of better metal leaching by metal tolerant strains of *Aspergillus niger* and *A. foetidus* (Ke & Li, 2006; Le, Tang, Ryan, & Valix, 2006; Valix, Usai, & Malik, 2001). During this piece of work, the high metal tolerance of *A. humicola* SKP102 (Table 2) and its subsequent use as an effective bioleaching agent (Fig. 2) supports the earlier observations and predictions.

Citric acid is reported to be the best leaching agent for nickel by Burgstaller, Strasser, Wobking, and Schinner (1992) and Coto, Bruguera, Abin, Gamboa, and Gomez (2001) which is

contradictory to the present findings (Table 2). However, it has been indicated that oxalic acid facilitates the solubilization of manganese from the ferro-manganese nodule, and that the solbilization of manganese releases nickel concomitantly. Since mineralogical studies revealed the presence of nickel, cobalt and manganese in the same mineral phase of the chromite mine overburden, the fact that oxalic acid is the best leaching agent for all four metals studied can be validated.

In this study, the pH of the growth medium declined initially and then increased gradually with an increase in the dissolution of metals (Fig. 2). The production of acid helps in partially dissolving ore material and thereby releasing the metal value into the solution. The acid produced appears to have a dual effect; it increases metal dissolution by lowering the pH and increases the load of soluble metals in the solution by complexing/chelating into a soluble organo-metallic coordination complex (Burgstaller & Schinner, 1993). The observation that once the pH of the leaching medium started to increase there was no further improvement in metal dissolution corroborates the findings of Behra et al. (2011).

Oxygen supply has also been found to be an important parameter in controlling bioleaching (Jain & Sharma, 2004). Supply of oxygen is seen to have positive effects on bioleaching rates (Table 3), similar reports have been made on the positive effects of oxygen on the production of citric acid during the leaching of zinc from filter dust by *Penicillium simplicissimum* Burgstaller et al., 1992). Franz, Burgstaller, and Schinner (1991) have also observed increased solubilization of zinc from filter dust with increased shaker speed.

The concentration of pulp or the metal containing part also exerts an effect on bioleaching. Very low concentration of the pulp is undesirable as bioleaching of the metal will decrease in this case due to the unavailability of metals for bioleaching. On the other hand, very high concentration of the pulp will exert toxic effects on fungal growth leading to poor bioleaching. This study reveals that 2% pulp density is the optimum level for the bioleaching of Ni by *A. humicola* SKP102, lower (1%) as well as higher (3 and 4%) concentrations of pulp result in lower bioleaching rates (Fig. 3). Burgstaller and Schinner (1993) reported that an increase in the concentration of the leaching substrate resulted in total inhibition of the growth of *P. simplicisissimum*. Vasan, Modak, and Natarajan (2001) observed an increment in the rate of Ca leaching from bauxite at 5% pulp density over 10%.

It has been reported several times, in agreement with the results of this study (Fig. 4), that the finer the particle size, the better the rate of bioleaching. It may be that the finer the particle, the more readily available the metal of choice is with the interacting acid.

The density of the fungal inoculum has an effect on the rate of the bioleaching of metals from their ores, therefore, optimization of the inoculum density is necessary to achieve maximum bioleaching (Fig. 5). Burgstaller et al. (1992) reported a 50% enhancement in the rate of Zn extraction from filter dust upon doubling the size of the inoculum of *P. simplicissimum*. Acharya, Kar, and Sukla (2002) reported that 10% (v/v) inoculum size of *P. citrinum* actually leads to the better leaching of Mn than 5% or 2% (v/v) inoculum.

The use of different cheap waste products for fungal growth can be a way to reduce the effective cost of the bioleaching process. Results of this study (Fig. 6) show that straw infusion and molasses improved the bioleaching of nickel with *A. humicola* SKP102. Mulligan and Galvez-Cloutier (2000) reported that Cu leaching from low grade ore was best when *A. niger* was grown in sucrose, with the two next best options being molasses and corn cobs. However, it was also indicated that pre-treatment of the fungal biomass will improve the process even further. Mulligan, Kamali, and Gibbs (2004) analyzed the use of various cheap carbon sources like potato-peel, sawdust etc. for the effective bioleaching of Cu from low grade mining ore using *A. niger*. Rao et al. (2002) used different types of sugars like glucose, maltose, mannitol, sucrose and lactose for bioleaching Cu from chalcopyrite using various fungal strains. Sulfuric acid supplementation in dilute concentration improved the rate of nickel bioleaching by *A. humicola* (Fig. 7) in a manner similar to bacterial leaching of nickel bearing pyrroholite as documented by Ke and Li (2006).

In order to reduce the effect of toxic heavy metals present in the overburden in fungal metabolism, two-step and indirect bioleaching techniques were employed. This led to a sharp increase in the bioleaching potency of the fungi (Fig. 8). This improvement is due to the reduced interaction of the fungal mycelia with toxic metals present in the overburden sample. Several other researchers used the same technique and observed similar results, Ren, Li, Geng, and Li (2009) reported that heavy metal bioleaching by *A. niger* increased from 56% to 97.5% of copper with a two-step process compared to the

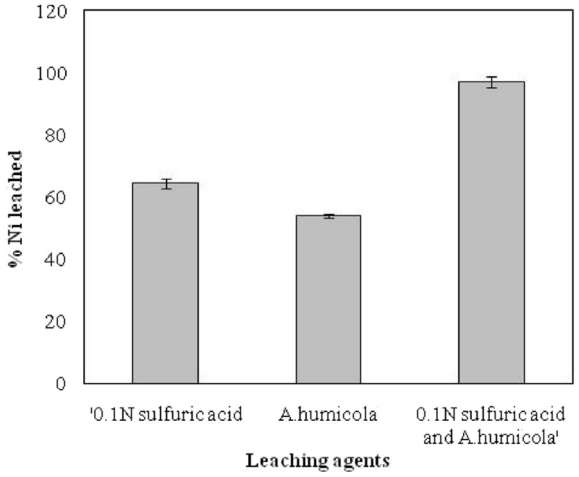

Fig. 7. Effect of dilute sulfuric acid supplementation on bioleaching of Ni by *A. humicola* SKP102. A medium containing 2% pulp (0.1 mm particle size) was inoculated with 10^6 spores/ml and incubated for 30 days under continuous shaking at 120 rpm. Nickel leached was measured using an Atomic Absorption Spectroscopy. Results represent mean ± S.D. of triplicate sets).

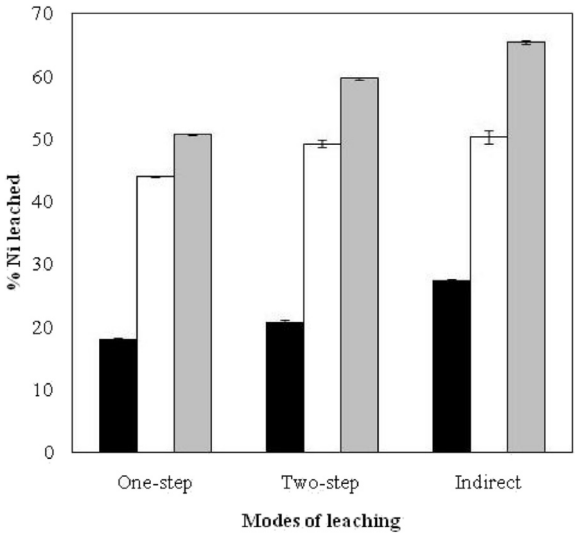

Fig. 8. Comparative account of bioleaching of Ni from overburden by one-step, two-step and indirect methods by *A. humicola* SKP102 for 10 days (-■-), 20 days (-□-) and 30 days (--■--) of incubation. (Nickel leached was measured using an Atomic Absorption Spectroscopy. Results represent mean ± S.D. of triplicate sets).

conventional one-step bioleaching technique. Castro et al. (2000) also indicated better Ni and Zn recovery from silicate using *A. niger*. However, according to Valix et al. (2001) bioleaching with a direct method showed better results in comparison to using an indirect method. The reason for this was said to be that in the direct method there is continuous interaction between the ore and mycelium, thus, the attachment of the metal to the mycellial matter results in the better bioleaching of metals. Several other reports also indicate that direct bioleaching techniques are better than indirect techniques. Behra, Sukla, and Mishra (2010), reported 10% Ni and 18% Co bioleaching in the direct method compared to 5% Ni and 10% Co bioleaching in the indirect method. Bohidar, Mohapatra, and Sukla (2009), reported that the direct bioleaching technique showed better Ni leaching using *A. niger*, *A. fumigatus* and *Penicillium* sp. The present findings corroborate those of Acharya et al. (2002) and Mulligan and Galvez-Cloutier (2003).

5. Conclusion

The lateritic deposit of Odisha, India, generates a huge amount of overburden during the course of chromite mining. These overburdens containing a considerable amount of nickel represent the only geogenous source of the metal in the country. Exploration of the overburden collected from this region has resulted in the isolation of a potent, oxalic acid producing multi-metal tolerant fungus, *Aspergillus humicola* SKP102. The isolate showed efficient nickel leaching abilities when screened for different process parameters and also responded well when grown in different cheap media like straw infusion and molasses. Supplementation of dilute sulfuric acid in the medium resulted in 97% Ni removal from the overburden sample used. Finally, it may be concluded that considering the nickel leaching from the mining overburden, further investigations are required for using the *A. humicola* SKP102 strain on a large scale basis and *in-situ* condition.

Conflict of interest

No potential conflict of interest was reported by the authors.

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