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Full Length Research Paper

Bio-extraction of metal ions from laterite ore by *Penicillium chrysogenum*

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The main objective of this study was to find a more feasible and economical method to extract metal ions from laterite ore by *Penicillium chrysogenum*. The effect of different substrates on microbial recovery of metal ions from laterite ore using indigenous strain of *P. chrysogenum* was observed. Maximum recovery of aluminum (86.78%), iron (97.78%), manganese (77.61%), nickel (57.31%) and chromium (34.32%) was recorded in case of shaking flasks experiments up to 24 days of incubation. Metal ions solubilization was also compared with the samples, which were not shaken and maximum recovery of Al (83.54 %), Fe (96.12 %), Mn (88.56 %), Ni (46.53 %) and Cr (37.82 %), were attained up to 24 days of incubation period. Enhanced recovery of Fe and Al may be due to the result of the acidic effect of the environment and the chelating capacity of organic acids.

Key words: Bioleaching, *Penicillium chrysogenum*, agriculture wastes, laterite ore.

INTRODUCTION

High-grade ores reserves are withdrawing all over the world at an upsetting rate as a result of speedy increase in demands for metals. The recovery of mineral value from the low-grade ores using present technology is prohibitively expensive due to high energy and capital costs. Presently, available physico-chemical methods are not environmental friendly and safer. Bio-beneficiation is taking into consideration as eco-friendly, promising and revolutionary solutions to these problems and is gaining more importance due to depletion of high-grade ores and enforcement of strict anti-pollution laws (Pradhan et al., 2006).

Due to rapid technological and industrial developments, many industrial sites are contaminated with heavy metals and organic compounds, which are toxic to any kind of living organisms, particularly human beings. Therefore, industries and public offices are obliged to implement the concepts of structured environmental management system more strictly. Moreover, reliable remediation techniques are required to recycle these industrial wastes like electronic scraps, used catalysts and clean up the site (Bayraktar, 2005). As a result, metal production has to be met more often from lower grade or complex ores, from mines and industrial wastes (Rawlings, 2004).

Usually in tropical climates and by intense and prolonged weathering, laterite soil is produced. Plentiful oxygen, water, and warmth can leach most water-soluble minerals from particles of parent rock and leave a non soluble residue enriched in hydroxides of aluminum, iron, magnesium, nickel, and titanium. Laterites high in specific metals are often strip mined as ores. Laterite which is rich in aluminum is phrased as aluminous laterite or bauxite. Aluminous laterite is formed from clay minerals such as kaolinite (Al_4 (Si_4O_{10}) $(OH)_8$) by the leaching of silica (SiO_2). The residue left by the leaching of silica, aluminum hydroxide $Al(OH)_3$, is termed, gibbsite. Gibbsite's dehydrated forms, diaspore and bohemite (both $HAlO_2$), are also common components of aluminous laterite. Aluminous laterite is the world's primary source of aluminum. Laterite is rich in iron and nickel and is termed ferruginous laterite. Laterite formed from rocks particularly rich in nickel may contain a high percentage of the mineral garnierite $(NiMg)_6Si_4O_{10}$ $(OH)_8$. A range of mixed laterites exists between the aluminous and ferruginous extremes. Nickeliferous laterites are an important commercial source of nickel (Le et al., 2006).

Microbial metal-extraction processes are usually more

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economical and eco-friendly than physicochemical processes (Rawlings, 2004; Akcil, 2004). They do not use large amounts of energy as compared to roasting and smelting and do not produce sulphur dioxide, another harmful gas (Mishra et al., 2004). Microbial technology offers an economic alternative for the mining industry, at a time when high grade mineral resources are being depleted (Rawlings et al., 2003). Generally, bioleaching refers to the conversion of metals into their water soluble forms by microorganisms (Olson et al., 2003; Ndlovu, 2008). Microorganisms like heterotrophs require carbon as an energy source, and this requirement can be fulfilled by using organic wastes. Acidolysis is the principal mechanism in bioleaching of metal ions by fungus. The fungus produces organic acids such as citric, oxalic, malic and gluconic acids during bioleaching (Mulligan et al., 2004; Johnson, 2006; Anjum et al., 2010).

The main objective of this study was to investigate bioleaching as an economical, environment friendly process and to determine the ability of microbes like *Pencillium chrysogenum* to extract Al, Fe, Mn, Ni and Cr from laterite ore using different organic wastes as substrates.

MATERIALS AND METHODS

Fungal strain and growth conditions

P. chrysogenum was isolated from the laterite ore and then cultivated for purification on slants of potato dextrose agar (PDA) medium (3.9% m/v) as described by Bousshard et al. (1996). Slants were incubated (Incubator, Sanyo, Germany) for 74 h at 28°C to produce an adequate number of spores. Afterward, the spores were counted using a Petroff-Hausser counting chamber. For growth in the liquid medium, the culture medium was composed of (g/L): KH_2PO_4, 5.0; NH_4NO_3, 2.0; $(NH_4)_2SO_4$, 4.0; $MgSO_4.7H_2O$, 0.2; peptone, 2.0; trisodium citrate, 2.5; yeast extract, 1.0 and the volume made up to 1000 ml with distilled water (Bhatti et al., 2007). Six sets of 250 ml flasks containing 100 ml of liquid medium were prepared each in triplicate samples. Medium in each flask was autoclaved. After sterilization, 5% (m/v) of the given substrate was added in each flask except for the control; the flasks were then inoculated with 1 ml of *P. chrysogenum* spore suspension as inoculum (approximately 1.8×10^7 spores ml^{-1}). All the flasks were sealed with removable cotton and incubated in an orbital shaker (Gellen Kamp, England) at 28°C and 120 rpm for 15 days of growth period.

Source and analysis of ore sample

Lateritic ore used in this study was collected from the Mineralogical Center, Chakwal, Pakistan. Sample was crushed and prepared by milling to produce a mean particle size of 200 mesh fractions by ASTM sieving machine and was used for its chemical as well as mineralogical analysis and for shake flask bioleaching experiments. For the digestion of laterite ore sample, 1 g of ore sample was added in a digestion flask. Then HNO_3 and $HClO_4$ in ratio (3: 1) were added in the same flask. Flask was put on the hot plate for 5 to 6 h at 80 to 90°C until the ore sample was completely digested and white fumes were given off. At the end 4 to 5 ml of HCl was added. The digested sample was cooled, filtered and was diluted to 50 ml with distilled water (Carolina et al., 2007). Filtrate was then analyzed for the metal concentration by atomic absorption spectroscopy (AAS) (Hitachi Z-8200 Japan) as described by Castro et al. (2000).

Pretreatment of substrates

Before the addition of substrates in the media, substrates were subjected to pretreatment process. Glucose was filtered- sterilized and then added in medium 1. Potato peel , black chick peas bran, grape fruit peel, musambi peel, were immersed in sulphuric acid of pH 2 for 24 h and molasses was diluted with the ratio of 1:3 and autoclaved for 5 min then all of these were added in medium nos. 2, 3, 4, 5 and 6. The control medium contained no substrate (Tables 1 and 2).

Chemical leaching of metal ions

Chemical leaching experiments were carried out with citric, oxalic, malic and tartaric acids to determine the effects of different organic acids on the extraction of metal ions from ore. Four different concentrations of organic acid 0. 5% (w/v), 1% (w/v) and 1.5% (w/v) with 1% (w/v) of ore residue (pulp density) in triplicate, were subjected to shake flasks treatment for the period of 24 days. The pH was noted during the leaching period after every 2 day. Supernatants were withdrawn at the end, filtered and analyzed for metals dissolved in each sample by atomic absorption spectrophotometry (Perkin Elmer, Analyst 300).

Bioleaching of the ore by *P. chrysogenum*

After one week of microbial growth, all the culture media in the flasks were autoclaved, centrifuged (8000×g for 10 min at 15°C) and filtered before high performance liquid chromatography (HPLC) analysis for organic acid metabolites. Culture supernatants containing organic acid metabolites were used to leach the metal ions from ore residue. Varied concentration of sample (2, 3, 4, 5, 6 g) was added in each medium of flasks containing culture supernatants and incubated on an orbital shaker to keep everything in a homogeneous slurry form at 28°C and 120 rpm for leaching period of 24 days. While in the 2nd set of experiments, substrates concentration was varied (2, 4, 6, 8, 10 g). Samples were collected after every 3 day and analyzed for metal ions. Same experiment was repeated with non- shaking conditions as shown in Tables 1 and 2. Then analyses of metabolites (example citric, oxalic, tartaric and malic acids) of all media were performed by following the modified HPLC method as described by Escobal et al. (1996). After centrifugation and filtration, samples were vortexed before HPLC analysis. The mobile phase consisted of 0.25% of acetic acid, was filtered and sonicated to remove any of the suspended particles. An HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with S-1121 dual piston solvent delivery system and S-3210 UV/VIS diode array detector and software package for data acquisition was used. A 20 µl of filtered sample was injected in to an analytical Hypersil (Thermo Hypersil, GmbH, Germany) ODS reverse phase (C18) column (250×4.6 mm; 5 µm particle size) fitted with a C_{18} guard column. The chromatographic separation was performed by isocratic elution of the mobile phase at a flow rate of 1.0 $mlmin^{-1}$ at 30°C. Detection was performed at wavelength of 254 nm. Organic acids were identified by comparing the retention times and quantified on the basis of peak area percent of the unknowns with those of pure standards of oxalic, citric, tartaric and malic acids. The peak areas were recorded and calculated by a computer with, chromatography data acquisition and integration software (SRI Instrument, Torrance, California, USA).

Table 1. Varied pulp density with constant substrate concentration.

S/N	Substrate	Substrate quantity (g)	Ore sample quantity (g)	Condition taken
1	Glucose	5	2, 3, 4, 5, 6	Shaking as well as non-shaking
2	Potato peel	5	2, 3, 4, 5, 6	Shaking as well as non-shaking
3	Black chick peas bran	5	2, 3, 4, 5, 6	Shaking as well as non-shaking
4	Grapefruit peel	5	2, 3, 4, 5, 6	Shaking as well as non-shaking
5	Musambi peel	5	2, 3, 4, 5, 6	Shaking as well as non-shaking
6	Molasses	5	2, 3, 4, 5, 6	Shaking as well as non-shaking

Table 2. Varied substrate concentration with constant pulp density.

S/N	Substrate	Substrate quantity (g)	Ore sample quantity (g)	Condition taken
1	Glucose	2, 4, 6, 8, 10	1	Shaking as well as non-shaking
2	Potato peel	2, 4, 6 , 8, 10	1	Shaking as well as non-shaking
3	Black chickpeas bran	2, 4, 6, 8, 10	1	Shaking as well as non-shaking
4	Grapefruit peel	2, 4, 6, 8, 10	1	Shaking as well as non-shaking
5	Musambi peel	2, 4, 6, 8, 10	1	Shaking as well as non-shaking
6	Molasses	2, 4, 6, 8, 10	1	Shaking as well as non-shaking

pH determination and metal ions analysis

The pH values of the leached samples collected at regular intervals were documented by using a digital pH meter (TOA, Japan) to pursue bioleaching studies of laterite ore. Samples collected on every second day of leaching period, were subjected to metal ions analysis by atomic absorption spectrophotometry (Perkin Elmer, Analyst 300). At the end of leaching period, residue samples were washed with water thrice and oven dried. Residues were subjected to wet digestion process by using nitric acid and hydrogen peroxide as described by Environment Canada (1990).

Statistical analysis

All experiments of the samples before and after leaching were performed in triplicate and the results were reported as mean ± SD (Steel et al., 1997).

RESULTS AND DISCUSSION

Laterite ore sample

Laterite ore sample was collected from Mineralogical Center Chakwal, Pakistan that was red-brown residual soil, insoluble in water, relatively inert and non reactive. It was slightly acidic in nature and having pH 6.6. The iron oxides goethite and hematite cause the red-brown color of laterites as it is formed by the leaching of silica and by enrichment with aluminum and iron oxides, especially in humid climates. Atomic absorption spectrophotometry showed that it mainly composed of (mg/kg): Fe (2014); Al (25.1); Ni (8.5), Mn (4.5) and Cr (3.2).

Chemical leaching

To evaluate the effectiveness of different organic acids in leaching of metal ions from laterite ore, chemical leaching tests were performed. The effect of these acids for the solubilization of heavy metal ions from the laterite ore was compared and has been shown in Figure 1a to d. In the chemical leaching experiments, the citric acid pH was between 1.9 and 3.1, oxalic acid pH ranges from 1.2 to 2.9, tartaric acid pH was in the range of 1.8 and 2.8 and of malic acid was between 1.9 to 3.1.

Citric acid showed more potential than oxalic and tartaric acid to solubilize nickel (Ni). Up to 94% Ni was solubilized with 1.5% citric acid and minimum Ni concentration (25%) was observed in case of 0.5% tartaric acid as illustrated in Figure 1. Our results are in accordance to Tzeferis et al. (1994) who reported tartaric acid as the least desired acid in nickel solubilization. Valix et al. (2000) also reported the enhancement in nickel solubilization with the concentration of acids.

In case of iron (Fe) solubilization, oxalic acid was considered as more efficient than other acids. Maximum recovery of Fe (95%) was recorded with 1% oxalic acid and least solubilization showed at 0.5% tartaric acid which is 54%. Tzeferis et al. (1994) postulated that oxalic acid was capable of complexing and reducing iron (II) citrate and iron (III) citrate that are stable and dissolve slowly. Citric acid showed maximum solubilization for the Cr (88%) at higher concentration, that is, 1.5%, while the minimum solubilization of Cr (32%) was with tartaric acid concentration (0.5%).

In the case of aluminum (Al), citric acid showed more affinity to solubilize the Al. Maximum solubilization (64%) was observed with 1.5% citric acid while oxalic acid demonstrated insignificant leaching behaviour for Al solubilization (33%). Citric acid forms multidentate complexes with metal ions as it has 3 replaceable H^+ ions.

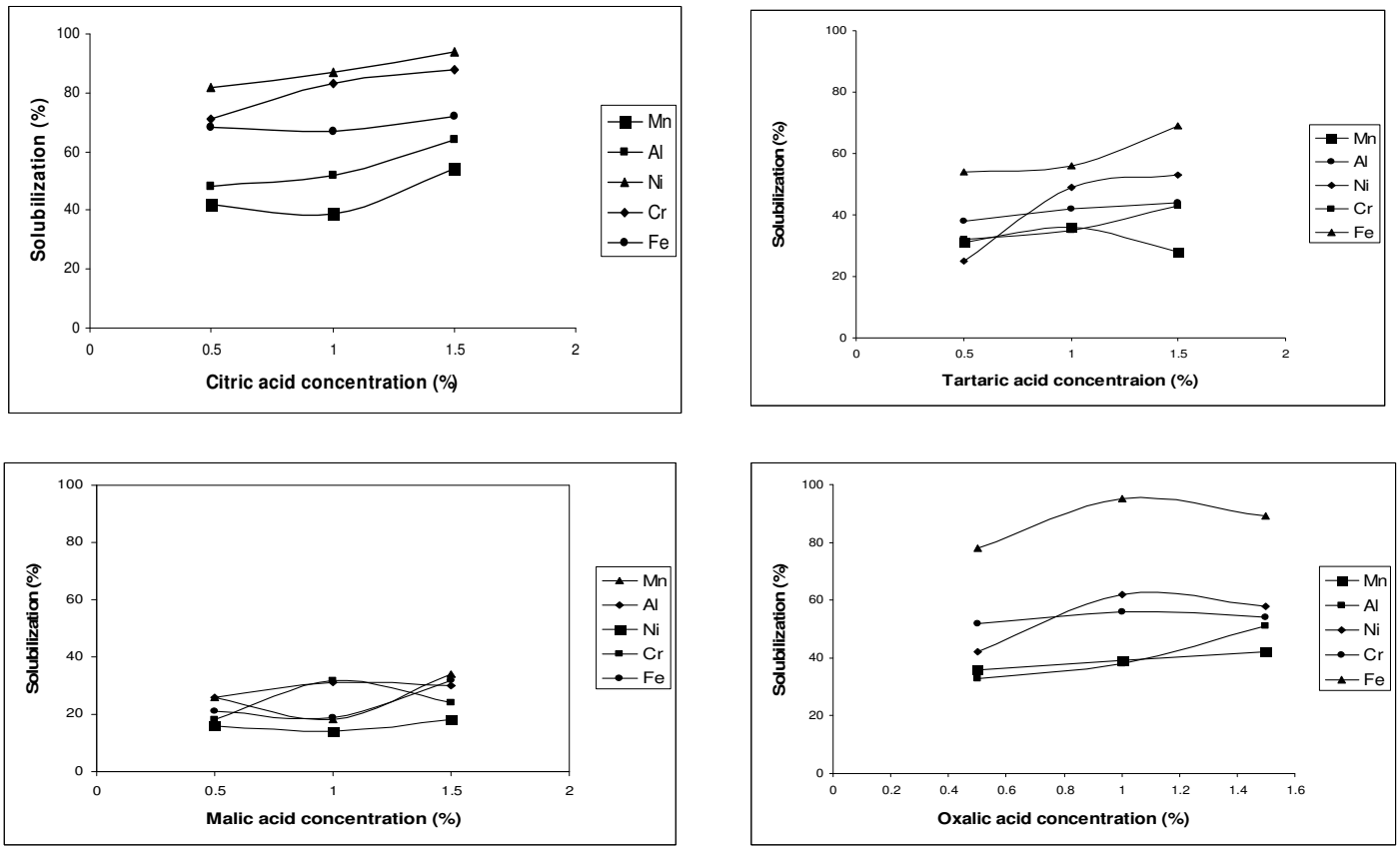

Figure 1. Metal solubilization at different concentrations of (a) citric acid (b) tartaric acid (c) malic and (d) oxalic acid.

Similar result was also reported by Dodge and Francis (1997).

In case of Mn solubilization, citric acid showed significant potential for solubilization (54%) at 1.5% concentration. These results suggest that optimal metal solubilization require controlled hydrogen ion concentration. Increased H^+ ion concentration resulted in less effective metal dissolution as in case of oxalic acid solubilization (38%) as reported by Tang and Valix (2006); whereas, Mn dissolution by tartaric acid is stronger than citric acid and weaker than oxalic acid. It seems to depend on H^+ concentration as solubilization in this case was only 31% at an acid concentration level of 0.5%.

Biological leaching of metal ions using different substrates

The results regarding the solubilization of manganese (Mn) from laterite ore are shown in Figure 2 It is obvious from the graph, that maximum Mn recovery (72.53%) was recorded with leaching sample containing 5 g molasses and 3 g ore under shaking condition keeping substrates as constant. The enhanced solubilization of Mn might be due to the production of citric acid which has more affinity for Mn as compared to other organic acids produced as metabolites. However, minimum solubilization of Mn (31.44%) was observed in flasks containing 5 g potato peel and 3 g ore under shaking condition, as shown in Figure 2a.

In case of Mn solubilization under shaking condition keeping sample as constant, maximum recovery of Mn (77. 61%) was obtained with 6 g of molasses and 1 g of sample while minimum Mn was solubilized by potato peel (that is, 37.67%) in flasks having 2 g of substrate and 1 g of sample as shown in Figure 2b. While maximum recovery of Mn (81.54%) was obtained for grapefruit peel in sample (substrate 5 g and sample 5 g) and minimum Mn (33.56%) was solubilized by black chickpeas bran with 5 g substrate and 2 g sample under non-shaking conditions as shown in Figure 2c. Maximum recovery of Mn (88.56%) was obtained with grapefruit peel as substrate (substrate 10 g and sample 1 g) while minimum amount of manganese was 38.63% with black chickpeas bran (substrate 4 g and sample 1 g) under non- shaking condition as shown in Figure 2d.

Maximum recovery of aluminum (66.43%) was observed with 5 g molasses as substrate and 6 g sample of ore under non shaking conditions while minimum (23.76%) was obtained with potato peel (substrate 5 g and

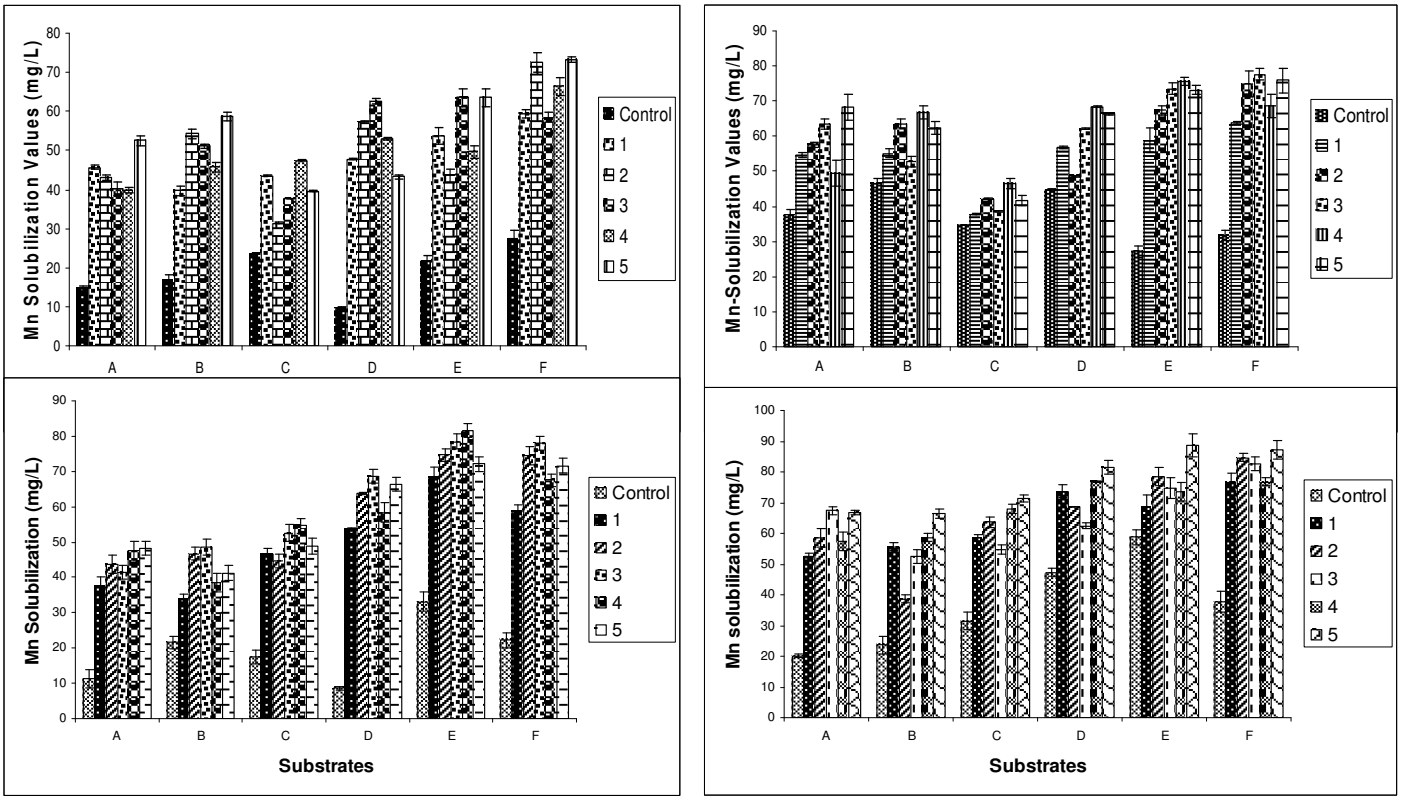

Figure 2. Manganese solubilization at shaking condition (a) keeping substrates as constant; (b) keeping sample as constant, and Manganese solubilization at non-shaking condition (c) keeping substrate as constant and (d) keeping sample as constant.

sample 3 g) in contrast to the results of Tang (2004). Molasses was viewed with maximum recovery of aluminum under shaking condition keeping sample as constant, that is, 86.78% (substrate 8 g and sample 1 g) and minimum solubilization of aluminum was observed with glucose (substrate 2 g and sample 1 g) which was 37.34%. Maximum recovery of Al might possibly be due to the maximum production of citric acid which showed more potential to solubilize Al than other organic acids produced. Among substrates, grapefruit peel showed maximum solubilization of aluminum (83.54%) with 5 g substrate and 4 g of sample under non-shaking condition keeping substrate as constant while minimum (38.67%) with black chickpeas bran containing 5 g substrate and 3 g of sample. In case of sample as constant, maximum recovery of Al was 81.43% with molasses as substrate (substrate 6 g and sample 1 g) while minimum (37.84%) with black chickpeas bran (substrate 2 g and sample 1 g) under non- shaking condition as shown in Figure 3.

It can be seen from the nickel (Ni) solubilization graph, that maximum recovery of Ni (57.31%) was observed in flasks having molasses (substrate 5 g and sample 2 g) under shaking condition keeping substrates as constant. With sample concentration of 6 g and substrate 5 g, under shaking condition, minimum Ni was solubilized (21.42%) in flasks receiving black chickpeas bran used as substrate as shown in Figure 4a. In case of Ni solubilization under shaking condition keeping sample as constant, maximum (54.36%) and minimum (34.77%) recovery of Ni was observed with grapefruit peel and glucose respectively as shown in Figure 4b, in contrast to Catherine et al. (2004), while maximum (43.43%) and minimum (27.56%) recovery of Ni was obtained with grapefruit peel (substrate 5 g and sample 3 g) and potato peel (substrate 5 g and sample 6 g) under non-shaking conditions as shown in Figure 4c. In case of sample as constant, maximum recovery of Ni was 46.53% with molasses (substrate 4 g and sample 1 g) while minimum (21.24%) potato peel (substrate 10 g and sample 1 g) under non- shaking condition as shown in Figure 4d.

The results of Cr solubilization using different substrates and sample size under shaking and non-shaking conditions are depicted in Figure 5a to d. It can be observed from the chromium solubilization graph, that maximum Cr (34.32%) recovery was observed in flasks having glucose substrate (substrate 5 g and sample 3 g) under shaking condition keeping substrates as constant and minimum (11.43%) in case of potato peel (substrate 5 g and sample 4 g), as shown in Figure 5a. In case of Cr solubilization under shaking condition keeping sample as constant, maximum (32.54%) and minimum (11.78%) recovery of Cr was recorded with molasses (substrate 4 g

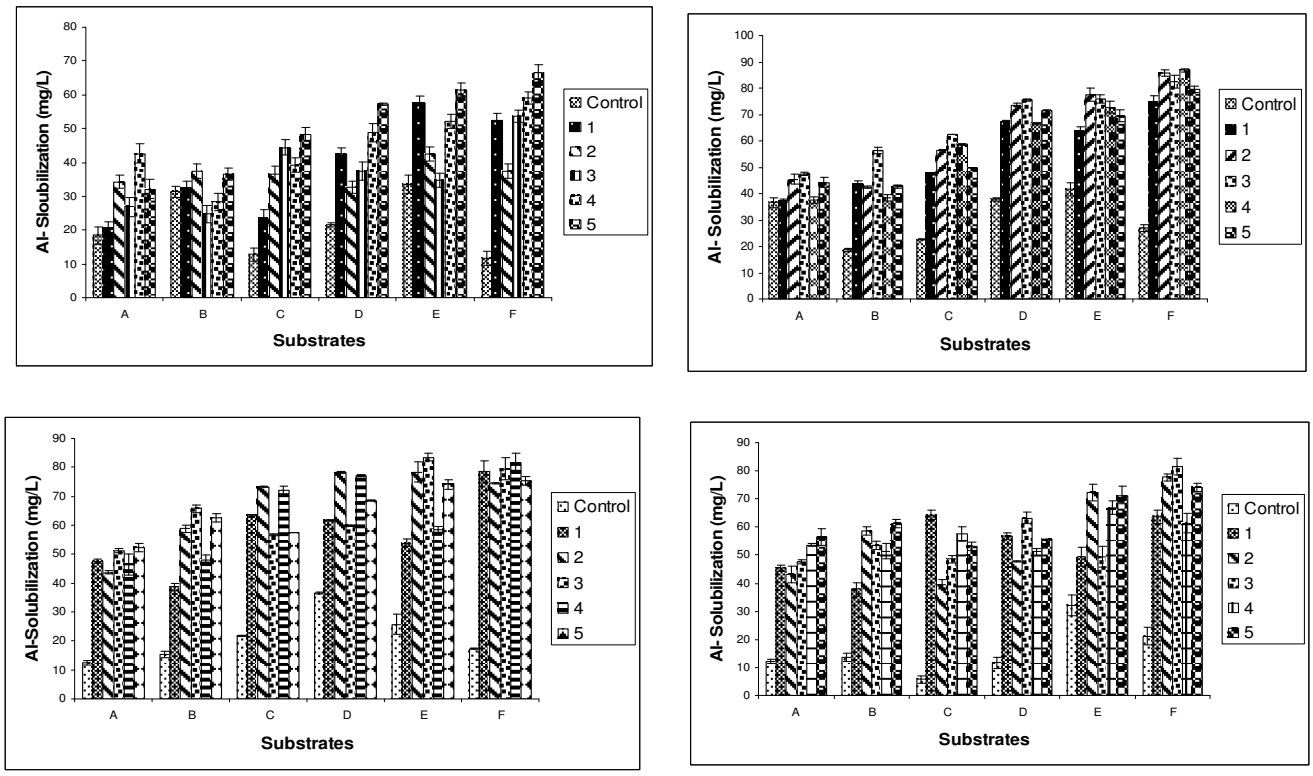

Figure 3. Aluminum solubilization at shaking condition (a) keeping substrate as constant (b) keeping sample as constant, and at non-shaking condition (c) keeping substrate as constant (d) keeping sample as constant.

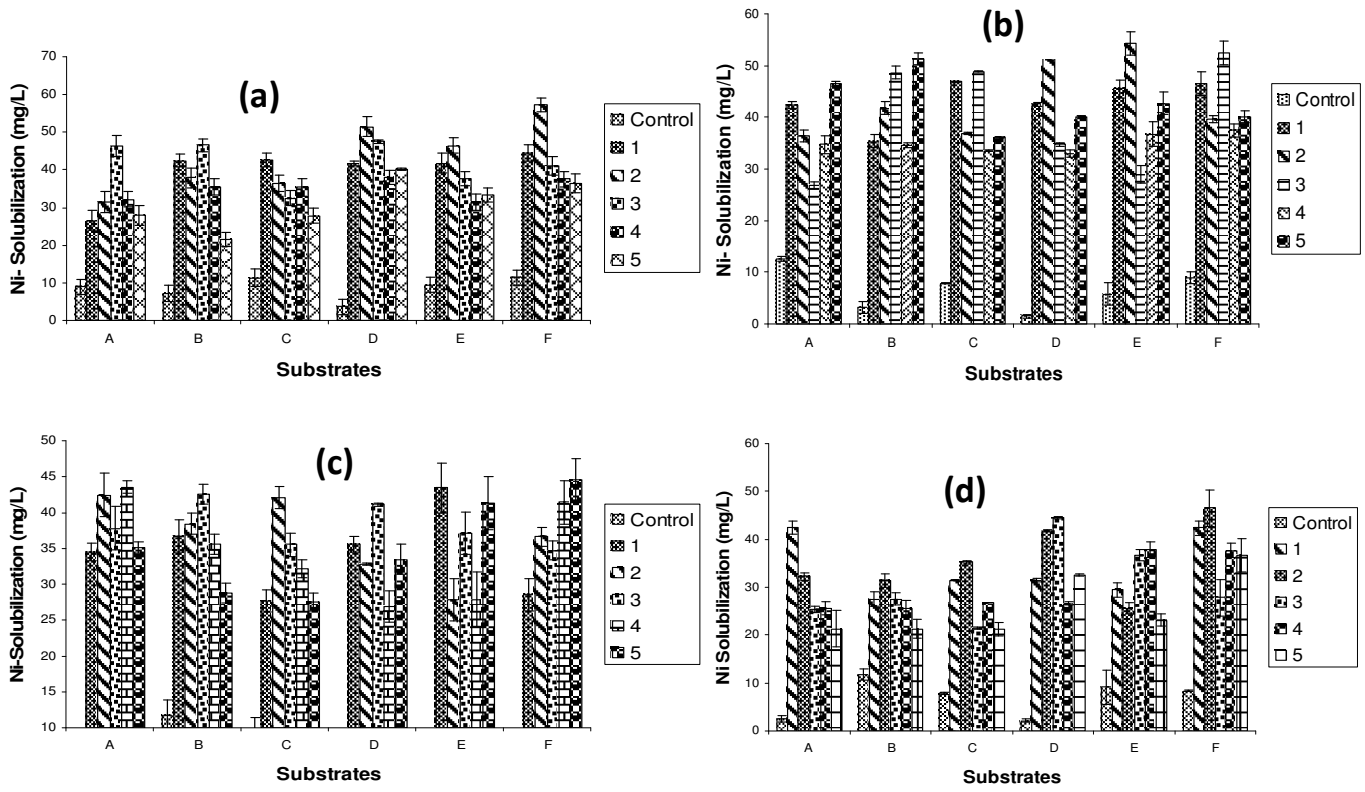

Figure 4. Nickel solubilization at shaking condition (a) keeping substrate as constant (b) keeping sample as constant and non-shaking condition (c) keeping substrate as constant, (d) keeping sample as constant.

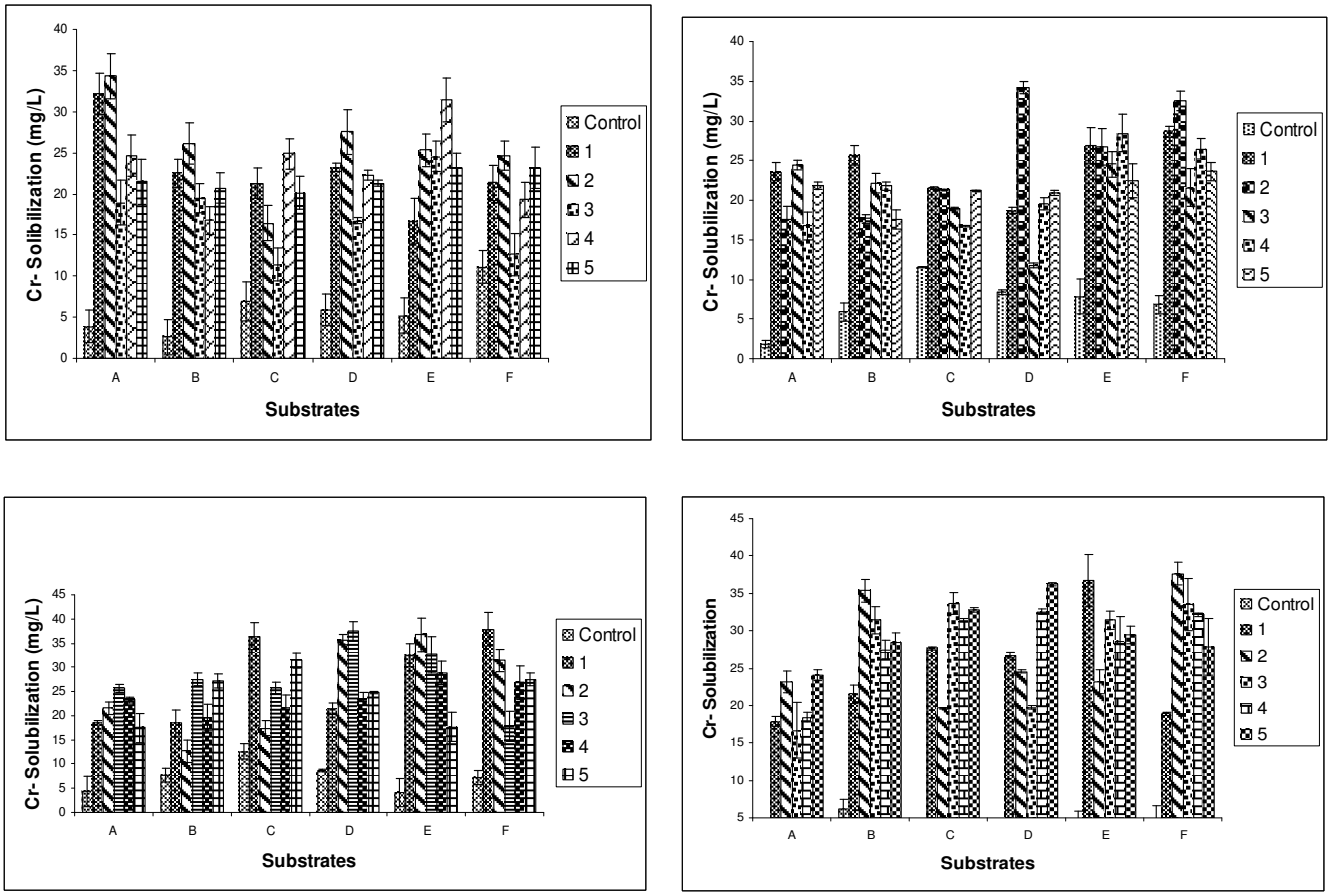

Figure 5. Chromium solubilization at shaking condition (a) keeping substrate as constant (b) keeping sample as constant and non-shaking condition (c) keeping substrate as constant (d) keeping sample as constant.

and sample 1 g) and musambi peel (substrate 6 g and sample 1 g) as shown in Figure 5b. While maximum recovery of Cr (37.82%) was obtained with molasses (substrate 5 g and sample 2 g) and minimum (12.65%) with black chickpeas bran (substrate 5 g and sample 3 g) under non-shaking conditions as shown in Figure 5c. Keeping sample constant, maximum recovery of Cr was 37.62% using molasses as substrate (substrate 4 g and sample 1 g) while minimum amount (16.67%) of Cr was leached with glucose (substrate 6 g and sample 1 g) under non- shaking condition as shown in Figure 5d. These results are in accordance with those of McDonald (2008).

The results regarding the effect of substrate and sample size on solubilization of iron (Fe) are shown in Figure 6a to d. The results indicate that maximum Fe recovery (97.54%) was achieved in flasks receiving grapefruit peel as substrate (substrate 5 g and sample 3 g) and minimum (73.51%) with black chickpea (substrate 5 g and sample 4 g) under shaking condition keeping substrates as constant as shown in Figure 6a. In case of Fe solubilization under shaking condition keeping sample as constant, maximum and minimum recovery of Fe was 96.21 and 74.26% using molasses (substrate 4 g and sample 1 g) and glucose (substrate 6 g and sample 1 g) as substrate, respectively (Figure 6b). Maximum recovery (94.54%) of Fe was achieved with grapefruit peel (substrate 5 g and sample 2 g) while minimum (68.42%) with black chickpeas bran (substrate 6 g and sample 1 g) as shown in Figure 6c. While keeping sample as constant, maximum and minimum recovery of Fe was 88.31 and 16.67% with molasses (substrate 4 g and sample 1 g) and black chickpeas bran (substrate 4 g and sample 1 g) under non- shaking condition as shown in Figure 6d.

pH trends in shaking condition

The results regarding the pH trends are shown in Table 3. In shaking conditions, it can be observed from the trends of pH, that after 24 days, the maximum pH monitored was 8.4 with sample 1 g (constant) and substrate concentration 2 g (variable) whereas minimum pH observed was 4.3 in the sample with substrate 2 g (variable) and sample 1 g (constant) in case of glucose.

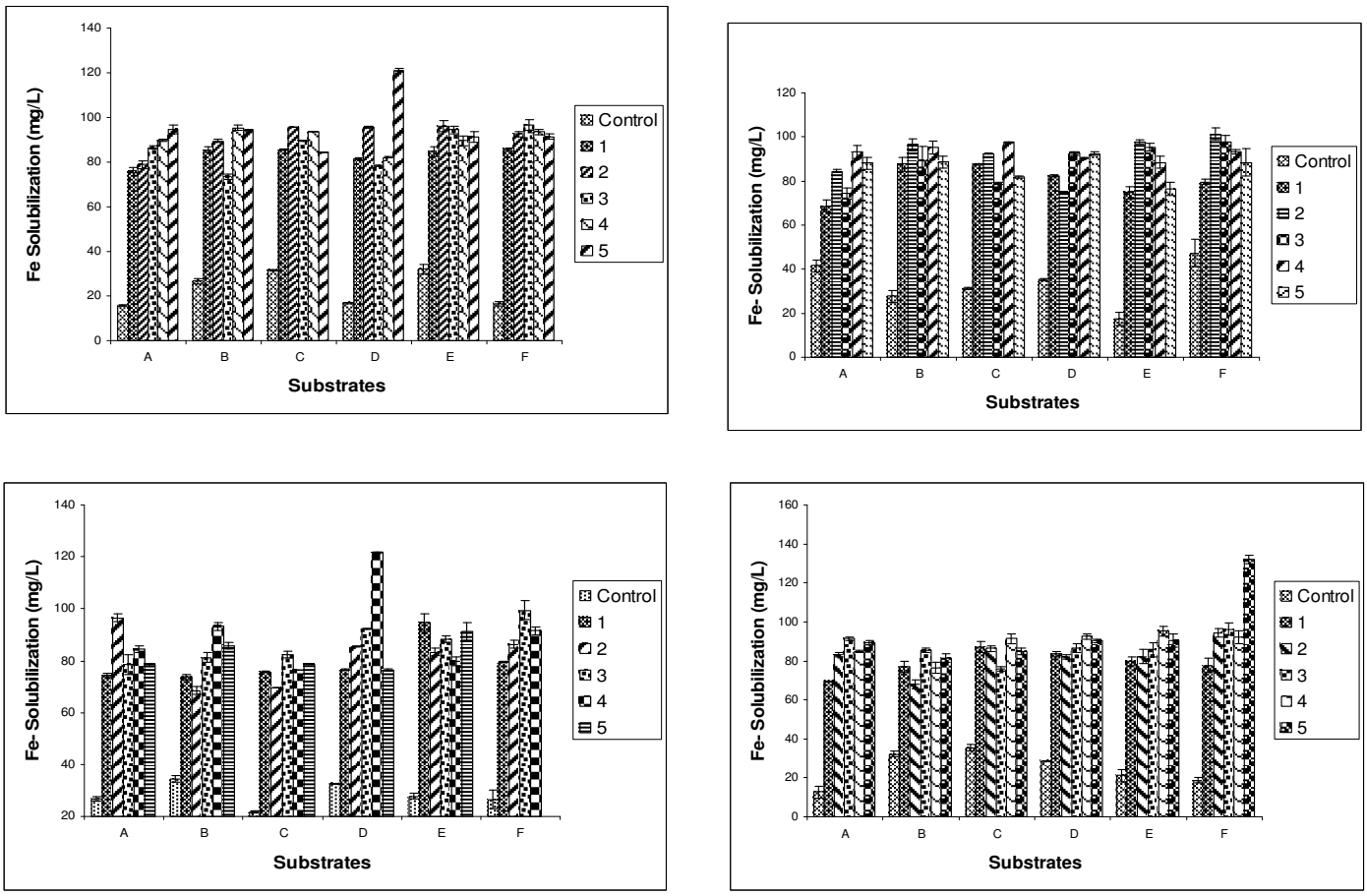

Figure 6. Iron solubilization at shaking condition (a) keeping substrate as constant; (b) keeping sample as constant non-shaking condition (c) keeping substrate as constant and (d) keeping sample as constant.

Table 3. pH trends during bioleaching studies in shaking and non shaking conditions**.**

Substrate	Shaking condition	Non shaking condition	Maximum pH	Minimum pH
Glucose	+	-	8.4 (2 g substrate+1 g sample)	4.3 (2 g substrate+1 g sample)
Potato peel	+	-	7.92 (6 g substrate+4 g sample)	4.5 (5 g substrate+6 g sample)
Black chickpeas bran	+	-	8.2 (10g substrate+1 g sample)	4.3 (8 g substrate+1 g sample)
Grapefruit peel	+	-	8.3 (2 g substrate+1 g sample)	4.3 (2 g substrate+1 g sample)
Musambi peel	+	-	7.8 (5 g substrate+2 g sample)	4.4 (2 g substrate+1 g sample)
Molasses	+	-	8.4 (5 g substrate+4 g sample)	4.1 (2 g substrate+1 g sample)
Glucose	-	+	8.2 (8 g substrate+1 g sample)	4.2 (2 g substrate+1 g sample)
Potato peel	-	+	8.1 (8 g substrate+1 g sample)	4.5 (10 g substrate+1 g sample)
Black chickpeas bran	-	+	7.96 (2 g substrate+1 g sample)	4.2 (5 g substrate+2 g sample)
Grapefruit peel	-	+	8 (2 g substrate+1 g sample)	4.6 (2 g substrate+1 g sample)
Musambi peel	-	+	8.2 (8 g substrate+1 g sample)	4.7 (2 g substrate+1 g sample)
Molasses	-	+	8.1 (5 g substrate+6 g sample)	4.1 (5 g substrate+2 g sample)

The high pH value might be because of organic acids produced during bioleaching because glucose and sucrose were found to be suitable sources of carbon for inoculated fungi (Sati and Bisht, 2006).

In the case of black chickpeas bran, pH increases from 4.3 in sample with substrate concentration of 10 g

(keeping substrate variable) and sample 1 g (at constant) to 8.2 in sample with sample concentration of 1 g and substrate 8 g after 21 days. After 24 days, maximum pH observed was 7.92 in sample (4 g) keeping substrate (5 g) as constant while minimum pH observed was 4.5 in sample at constant substrate concentration (5 g) and sample concentration of 6 g in the case of potato peel in accordance to Uguru et al. (1998).

In case of musambi peel, maximum pH monitored was 7.8 in the sample containing substrate 5 g and sample concentration 2 g whereas minimum was 4.4 with same sample and substrate concentrations keeping substrate as constant. Subsequent to 24 days, the maximum pH was 8.3 to minimum pH 4.3 in sample containing substrate 6 g and sample of 4 g under constant sample condition in case of grapefruit peel.

After 24 days, pH 8.4 was scrutinized in sample containing sample concentration 4 g and substrate 5 g which decreased to 4.1 in a sample having substrate concentration 5 g and sample 2 g at substrate constant condition in case of molasses at shaking condition of the bioleaching experiment, in accordance to Pera and Callieri (1999). In case of control sample (without substrate) pH remained in the range of 5 to 6.48 under shaking conditions.

pH trends in non-shaking condition

Growth of the fungus in non-shaking conditions was observed to be lower than in shaking conditions in terms of pH. The maximum pH observed was 8.2 in sample containing 8 g substrate and 1 g of sample at constant sample condition whereas minimum pH observed was 4.2 in sample keeping substrate, that is, 2 g substrate and 1 g of sample, in case of glucose (Table 3). In case of black chickpeas bran, after 21 days, maximum pH 7.96 was monitored in sample with substrate concentration 2 g and sample 1 g under constant sample condition while minimum pH was 4.2 in sample (substrate 5 g and sample 2 g) keeping substrate constant.

Maximum pH 8.1 was observed for sample containing sample 1 g and substrate 8 g under sample constant but minimum pH was 4.5 in sample with substrate constant value (substrate concentration 10 g and sample taken 1 g) in case of potato peel after 18 days of incubation time. In case of musambi peel, maximum pH after 24 days was 8.2 in the sample (substrate 8 g and sample 1 g) with maintenance of sample as constant while minimum pH was 4.7 at the same conditions in sample containing 2 g of substrate and 1 g of sample. Maximum pH in case of grapefruit peel after 24 days was 8 in sample (10 g of substrate and 1 g of sample) keeping sample as constant but minimum observed pH was 4.6 in same sample and condition.

After 24 days, maximum pH, that is, 8.1 was monitored in sample with substrate constant (5 g) and sample varied (6 g) while minimum pH examined 4.1 in sample with substrate concentration of 5 g and sample 2 g keeping substrate as constant in case of molasses, at non-shaking condition of the bioleaching experiments as shown in Table 3. In case of control sample (without substrate), pH remained in the range of 5 to 5.8 under still conditions.

Mass balances

Analysis of residual solids after leaching was carried out to perform a mass balance for metal ions and this value was compared with the initial amount of metal ions in the raw sample. On the average, some amounts of Mn (15%), Ni (12.1%) and Al (7.5%) have not been recovered. During sample preparation and filtration some of the undissolved salts might be lost.

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