

Bioleaching of heavy metals from a low-grade mining ore using *Aspergillus niger*

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Abstract

The main concern of this study is to develop a feasible and economical technique to microbially recover metals from oxide low-grade ores. Owing to the significant quantities of metals that are embodied in low-grade ores and mining residues, these are potential viable sources of metals. In addition, they potentially endanger the environment, as the metals they contain may be released to the environment in hazardous form. Hence, mining industries are seeking an efficient, economic technique to handle these ores. Pyrometallurgical and hydrometallurgical techniques are either very expensive, energy intensive or have a negative impact on the environment. For these reasons, biohydrometallurgical techniques are coming into perspective. In this study, by employing *Aspergillus niger*, the feasibility of recovery of metals from a mining residue is shown. *A. niger* exhibits good potential in generating a variety of organic acids effective for metal solubilization. Organic acid effectiveness was enhanced when sulfuric acid was added to the medium. Different agricultural wastes such as potato peels were tested. In addition, different auxiliary processes were evaluated in order to either elevate the efficiency or reduce costs. Finally, maximum solubilization of 68%, 46% and 34% were achieved for copper, zinc and nickel, respectively. Also iron co-dissolution was minimized as only 7% removal occurred.

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1. Introduction

Bacterial leaching of metals from mining ores, also called bioleaching, is a full scale process that can be performed by slurry reactors or heap leaching. Mining wastes include low grade ores, mine tailings, sediments from lagoons or abandoned sites. Low pH values lead to solubilization of the metals in the mining ores. Elemental sulfur or ferrous iron may be added as bacterial substrates. Reactors such as Pachuca tanks, rollings reactor or in propeller vessels have been used [1]. Heap leaching is more common since it allows the large volume wastes to be treated in place [2]. To enhance this process, aeration can be forced through the pile or hydrophilic sulfur compounds can be added [3]. *Thiobacilli* bacteria are responsible for the oxidation of inorganic sulfur compounds. Applications include metal dissolution in

low-grade sulfide ores, generation of acidic ferric sulfate leachate for hydrometallurgical purposes, and removal of gold by oxidation of pyrite by bacterial sulfide production. The extraction of metals from low grade metals ores and refractory gold ores is a multi-billion dollar business worldwide [4]. Bacterial solubilization by oxidation of the sulfide minerals, pyrite and arsenopyrite, enhances gold extraction by the traditional method of cyanidation. The solubilization mechanisms have been debated extensively, however.

Biohydrometallurgical processes are efficient and cause less environmental problems than chemical methods [5]. For slurry processes, oxidation rate per reactor volume, pH, temperature, particle size, bacterial strain, slurry density, ferric and ferrous iron concentrations need to be optimized. Bioleaching is very effective for recovery of gold from refractory gold pyrite and copper from chalcopyrite.

One of the most significant applications of biohydrometallurgy is in low-grade ore recovery. For instance, Hoffmann et al. [6] elaborated on a process in which ferric iron was biologically reduced to ferrous iron by *Pseudomonas sp.*

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strain. This method has been applied in steel production. Economical recovery of gold has been the main focus of some bio-leaching processes. Kleid et al. [7] illustrated the use of cyanide-producing microorganisms in refractory gold ore. In 1994, Hahn [8] described a biological method for recovering copper from solid wastes containing 5% of copper. He utilized a microbial culture fed continuously with saccharose. After 18 days 99% copper had been extracted.

Bioleaching of fly ash by *A. niger* was first reported in 1996 [9]. The experiments were conducted in shake flasks and the leaching of different metals such as Al, Cd, Cr, Cu, Zn and Mn present in fly ash was determined. Gadd et al. [10] endorsed employing *A. niger* in bioremediation of radionuclide pollution. They also mentioned that, the process commercially is not yet feasible.

Mulligan et al. [11,12] conducted various experiments with *A. niger* in the presence of different concentrations of low-grade ore and determined that 10% pulp density was optimal. This research is, however, focused on the evaluation of different substrates and their pretreatment in order to develop an inexpensive leaching process using *A. niger* for low-grade ores, to recover metals such as copper.

2. Materials and methods

2.1. Fungal strain and growth conditions

A. niger strain ATCC 6275 was obtained from American Type Culture Collection (ATCC), in a freeze dried state. The medium utilized for growth of the culture was based on the method of Bousshard et al. [9] in which a culture was inoculated on a potato dextrose agar (3.9% (w/v)). In order to obtain sufficient numbers of spores, the culture was kept for 7 days at 26 °C in this medium. Afterward, sodium dodecyl sulfate (0.2% (w/v)) was used to recover the spores. The spores were counted using a Petroff-Hausser counting chamber where 1 mL of spores (approximately 3×10^7 spores) was obtained. The spores were then added to a 500 mL flask containing 150 mL of prepared medium.

Table 2
Composition of various media used in the experiments

Medium no.	Substrate	Pre-treatment
1	100 g/L sucrose	Autoclaving
2	100 mL/L molasses (80% diluted)	Autoclaving
3	40 g/L potato peels	Autoclaving
4	40 g/L potato peels and 1 g/L sucrose	Autoclaving; no yeast extract
5	40 g/L potato peels and 1 g/L sucrose	Not autoclaved; no yeast extract
6	40 g/L sawdust passing through sieve no. 16	Sulfuric acid (pH 2) not autoclaved; no yeast extract
7	40 g/L leaves passing through sieve no. 10	Sulfuric acid (pH 2) not autoclaved; no yeast extract
8	1 L/L potato chips waste	Sulfuric acid (pH 2) not autoclaved; no yeast extract
9	40 g/L potato peels	Sulfuric acid (pH 2) not autoclaved; no yeast extract
10	20 g/L corn kernel and 20 g/L corn husk	Sulfuric acid (pH 2) not autoclaved; no yeast extract

Table 1
Heavy metal characterization of the mining ore

Metal	Concentration (mg/kg residue)
Copper	7,245
Iron	26,470
Zinc	201
Nickel	27

2.2. Characterization of the mining residues

An oxidized sample of mining residues was obtained from a mine in the Gaspé region (Canada). Because of the large size of these residues, they were crushed. The crushed ore residue (obtained by passing crushed ore through sieve no. 4 and remaining on sieve no. 8) was selected. The quantity of metals was determined after nitric acid/hydrogen peroxide digestion of the residues, as described by Environment Canada [13]. The analysis of metals was performed by Perkin Elmer Atomic Absorption Spectrophotometer Model A Analyst 100. The results of the analysis are shown in Table 1.

2.3. Chemical leaching experiments

Preliminary chemical leaching tests were carried out in order to evaluate the effectiveness of oxalic and citric acids with various concentrations for nickel, zinc, iron and copper leaching. Four different concentrations 0.01% (w/v), 0.05% (w/v), 0.1% (w/v), 0.5% (w/v) of citric and oxalic acids with 10% (w/v) of residue in duplicate were shaken for 48 h. Liquid samples were taken at the end, filtered, and analyzed for metals dissolved in each sample by atomic absorption.

2.4. Preparation of substrates

ACS reagent grade sucrose was obtained from Sigma-Aldrich Canada Ltd. (Oakville, Canada) and used for medium no. 1 (Table 2). Molasses was obtained from a local grocery. A quantity of 20 mL of molasses was diluted with 80 mL of distilled water and then the 100 mL/L of molasses solution was added per flask for medium no. 2. For the experiments with sawdust, sawdust was obtained from a local

workshop and passed through sieve no. 16. The fraction passed through the sieve was used in all experiments with sawdust. Leaves (maple leaf variety) were collected from a local yard and left to air dry. The leaves were then crushed by hand and passed through sieve no. 10. The sieved fraction was then used for further experiments. Locally grown potatoes were peeled to remove the skins. The skins were collected, air dried and crushed by hand. Experiments were carried out with sulfuric acid by soaking the substrate in dilute sulfuric acid (pH 2) for 24 h. The substrate was then weighed and added to the mineral salts medium and the ore.

2.5. Leaching of the mining residue by *A. niger*

All assays were carried out in a 150 mL volume. Medium, ore samples (100 g/L) and salts (1.6 g/L yeast extract, 1.5 g/L NaNO₃, 0.5 g/L KH₂PO₄, 0.025 g/L MgSO₄·7H₂O, 0.025 g/L KCl) were sterilized by autoclaving for 20 min at 121 °C before spores were added. Afterwards, all flasks were sealed with removable cotton. Temperature was kept at 20 °C during the experiment. Each flask was shaken by a Burrell Wrist Action shaker during the period of the experiment in order to keep everything in a homogenous slurry form. Samples were collected by disposable sterilized pipettes and filtered. Metals and organic acid quantities were then measured in the supernatant after filtration through 0.45 µm filters. Each experiment was done in triplicate and was repeatable to within 5–10%, and a control flask, one without the microorganisms, was used maintaining the same conditions. All media compositions are summarized in Table 2.

2.6. Analytical methods

2.6.1. Determination of organic acids produced by *A. niger*

Samples were analyzed for organic acids after centrifugation with ICE HN-S II centrifuge at 3500 rpm for 10 min. Then the samples were filtered using a 0.45 µm microfilter. The method of Bosshard et al. [9] was employed, in which the concentration of organic acids such as citric acid, oxalic acid, gluconic acid, malic acid and tartaric acid were determined by a Beckman Coulter System GOLD Model HPLC (high pressure liquid chromatography). In this method an A-312 YMC-Pack column (6.0 mm × 150 mm in length) at a flow rate of 5 mL/min (room temperature), with a mobile phase of 50 mM monoammonium phosphate adjusted to pH 2.4 with H₃PO₄ was utilized. Concentrations of organic acids were measured based on a standard solution made with 0.1% citric acid, 0.1% malic acid, 0.1% tartaric acid, 0.01% oxalic and 0.1% phytic acid. Organic acid concentrations were measured and were plotted versus time.

2.6.2. Heavy metal analysis

At the end of each set of experiments, residue samples were washed with distilled water three times. Thereafter, the

residues were air-dried for 24 h. Five grams of residue from assay were digested by the nitric acid/hydrogen peroxide method, illustrated by Environment Canada [13]. All samples were filtered and the metal concentrations in all samples were determined by a Perkin Elmer Atomic Absorption Model A Analyst 100.

Copper concentrations over time period were plotted for all media and in each graph, copper concentrations in the control media were indicated as well. Since zinc, nickel and iron concentrations in some of the media were very low, graphs were plotted only for some of the media.

3. Results and discussion

3.1. Chemical leaching

The effects of oxalic and citric acid addition were compared for the removal of heavy metals from the mining residue. The results are shown in Figs. 1 and 2. Oxalic acid is a stronger acid than citric acid and its pH is lower for the same concentration. In our experiments, the oxalic acid pH was between 1.9 and 2.8, and the citric acid pH was between 2.7 and 4.5. Citric acid showed more potential than oxalic acid to solubilize copper. As illustrated in Figs. 1 and 2, at lower concentrations, both acids worked equivalently, whereas at higher acid concentrations, citric acid was more effective.

Since zinc solubilization is more dependent on the presence of H⁺ ions, acids with lower pH dissolve more zinc. Therefore, oxalic acid solubilized more zinc than citric acid. On the other hand, oxalic acid is a very ineffective agent for nickel solubilization. The removal of nickel for the mining residue was also evaluated. In nickel solubilization, oxalic acid is the least desired acid, as nickel oxalate has a very low solubility and makes the leached nickel precipitate. However, combination of oxalic acid with other organic acids would make it more effective as shown by Tzeferis et al. [14].

For iron, oxalic acid was five times more effective than citric acid. Tzeferis et al. [14] postulated that oxalic acid was capable of complexing and reducing iron. Citric acid leaching of iron, however, can lead to iron (II) citrate and iron (III) citrate that are stable and dissolve slowly. However, since the levels of these two acids were not high, leaching of iron was insignificant.

3.2. Biological leaching

After the experiments with oxalic and citric acid addition, fungus was added to the mining ore to determine if the acids could be produced by the fungus and enhance metal leaching. In the sucrose medium (Fig. 3), up to 60% of copper was solubilized 14 days after inoculation. The pH was approximately six at the beginning and decreased to 2.9 after 5 days which implies organic acid production. The high

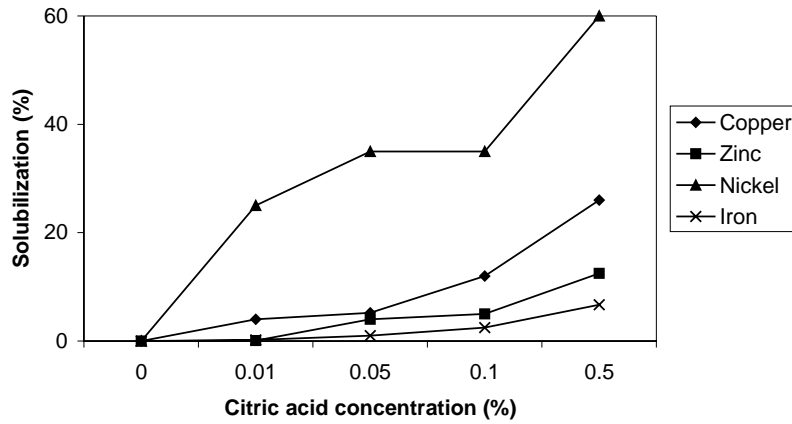


Fig. 1. Metal solubilization by citric acid.

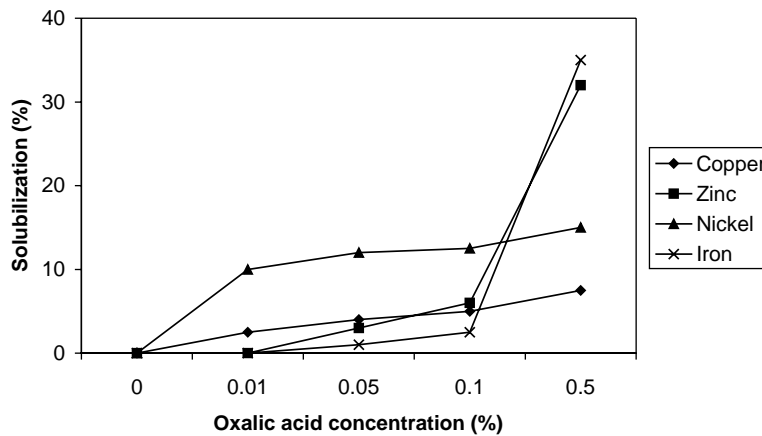


Fig. 2. Metal solubilization by oxalic acid.

copper recovery may be the result of the acidic affect of the environment and the chelating capacity of organic acids.

In many of the assays conducted in this research, no significant amounts of zinc were dissolved. Therefore only the significant ones are discussed here. In medium no. 1 and medium no. 2 as indicated in Fig. 3 (sucrose medium) and Fig. 4 (molasses medium) 28% and 20% of zinc were, re-

spectively, solubilized. These results are considered reasonable compared to previous literature reported by Muller et al. [15] as maximum amounts of zinc will be solubilized after the maximum rates of copper leaching. Organic acid production was higher in medium no. 2 (Table 3) than medium no. 1 at day 14, but more copper was removed in the sucrose medium.

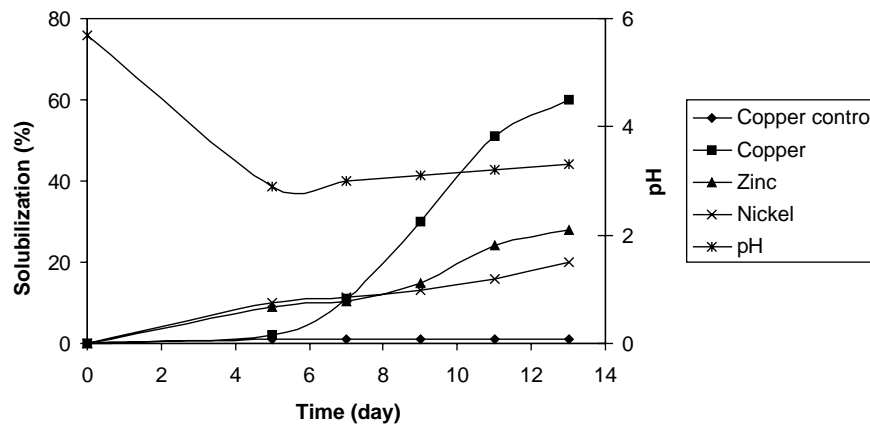


Fig. 3. Copper, zinc and nickel solubilization in medium no. 1 (sucrose) by *A. niger*.

Table 3
Maximum concentration of organic acids produced in various media (day 14)

Medium no.	Oxalic acid (% (w/v))	Tartaric acid (% (w/v))	Malic acid (% (w/v))	Citric acid (% (w/v))	Phytic acid (% (w/v))	Total acid yield (g/g substrate)
1	0.01 ± 0.005	0.05 ± 0.01	0.20 ± 0.02	0.10 ± 0.01	0.70 ± 0.07	0.11 ± 0.01
2	0.10 ± 0.01	0.40 ± 0.04	0.80 ± 0.08	0.20 ± 0.02	0.80 ± 0.08	0.23 ± 0.02
3	0.20 ± 0.01	0.02 ± 0.005	<0.01	0.01 ± 0.005	<0.01	0.06 ± 0.005
5	0.20 ± 0.01	0.05 ± 0.005	<0.01	<0.01	0.60 ± 0.06	0.22 ± 0.01
6	0.20 ± 0.01	0.01 ± 0.005	0.02 ± 0.005	<0.01	0.70 ± 0.07	0.16 ± 0.01
9	0.01 ± 0.005	0.01 ± 0.005	0.23 ± 0.02	0.01 ± 0.005	1.02 ± 0.10	0.31 ± 0.02
11	0.05 ± 0.01	0.01 ± 0.005	0.15 ± 0.01	0.05 ± 0.005	0.80 ± 0.08	0.26 ± 0.02

Research on the effect of different organic acids on zinc oxide leaching by Golab and Orłowska [16] shows that among citric, gluconic, oxalic and tartaric acids, tartaric acid is the most effective in zinc solubilization. Tartaric acid is among the chelating agents produced by *A. niger* as shown in Table 3 but it is not produced in large quantities.

Leached nickel in the sucrose medium (medium no. 1) as is shown in Fig. 3 was about 20% in a 13-day period. Considering the graph's slope, it is obvious that over a longer period of time, more nickel solubilization would have been achieved as endorsed by Tzeferis [17].

Molasses (medium no. 2), an inexpensive by-product of sugar, showed very good potential for copper solubilization (up to 48%). The escalating slope of the curve indicates this media is capable of solubilizing more copper over a longer period of time as indicated in Fig. 4. In this medium, the pH dropped less than that in the sucrose medium, reaching a final pH of approximately 4.5. In medium no. 2, 15% of nickel in 13 days was solubilized as it can be seen in Fig. 4, which is considered satisfactory. Overall, iron solubilization was also determined and found to be minimal, reaching a maximum of 7% in medium no. 2. Iron solubilization is undesirable since it interferes with the copper recovery during purification of copper.

In medium no. 3, potato peels, (Fig. 5), up to 35% of copper was solubilized. Although supplementary amounts of sugar were used to promote fungus production (medium

no. 4), this effect was insignificant (Fig. 5). To determine the effect of less pre-treatment on potato peels, medium no. 5 was evaluated which was medium no. 4 without autoclaving. In this experiment the maximum copper leached was 14% as illustrated in Fig. 5.

Another experiment was performed to sterilize the media without autoclaving. To accomplish this, sulfuric acid was used since it may also promote hydrolysis processes and enhance organic acid production. Sawdust and leaves, two cellulosic wastes, were soaked in sulfuric acid for 2 h and then autoclaved. The sawdust medium (Fig. 6) didn't show potential as substrate and the maximum copper leached was only 8%.

As indicated in Fig. 7, copper solubilization in medium no. 7 (leaves) was about 30% which is considered a high amount. The amount of copper solubilized in the control medium was also high which could be due to the low pH of the control medium. However, the effect of the sulfuric acid alone on the residue wasn't the only factor leading to the enhanced copper removal since the copper solubilized in the control medium was lower.

Potato starch, a waste from a potato chips company, was evaluated as another medium. The maximum solubilized copper in this medium (medium no. 8) did not exceed 25% as it is indicated in Fig. 7, which was almost the same as the copper solubilized in the control. It was concluded that this waste without any pre-treatment would not be a good alternative.

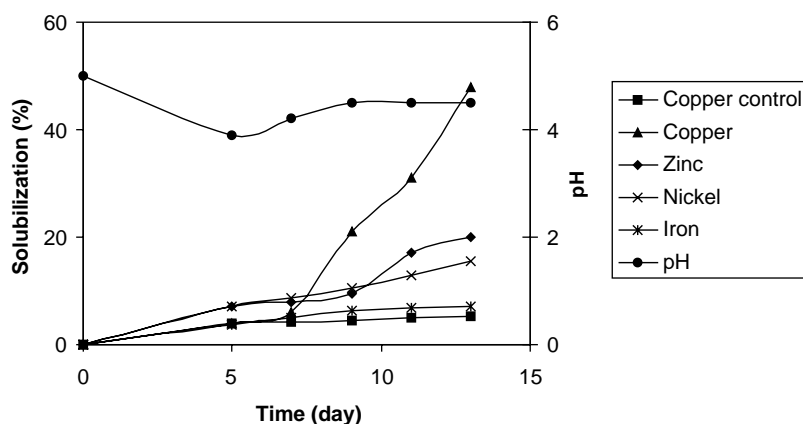


Fig. 4. Copper, zinc, nickel and iron solubilization in medium no. 2 (molasses) by *A. niger*.

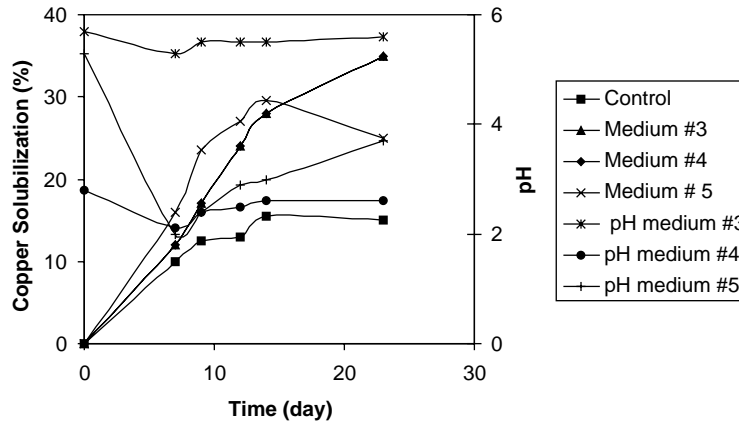


Fig. 5. Copper solubilization in medium no. 3 (potato peels), 4 (potato peels with sucrose and autoclaving) and 5 (potato peels with sucrose and no autoclaving) by *A. niger*.

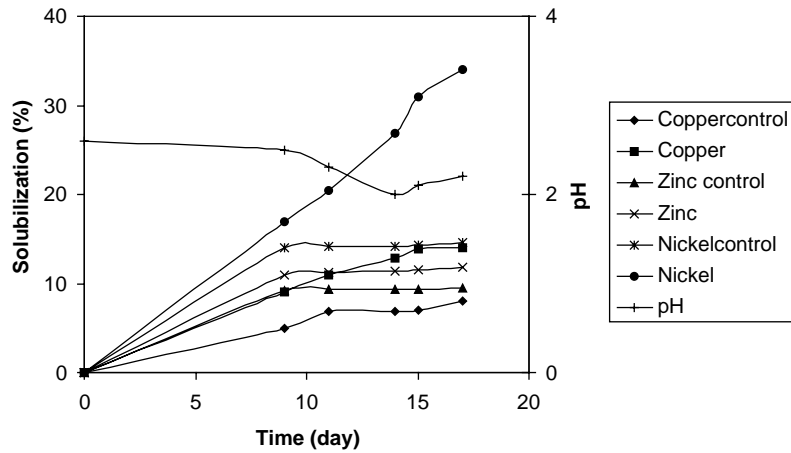


Fig. 6. Copper, zinc and nickel solubilization in medium no. 6 (sawdust treated with sulfuric acid, pH 2) by *A. niger*.

Another substrate, ground corn kernels and corn husks immersed in dilute sulfuric acid, was evaluated. The results are indicated in Fig. 7. It shows very good potential and that a maximum of 38% copper solubilization achieved. However,

its efficiency declined after 17 days, which could be due to substrate depletion for the fungi.

Potato peels were also soaked in sulfuric acid (medium no. 9). About 68% of copper was solubilized in this medium

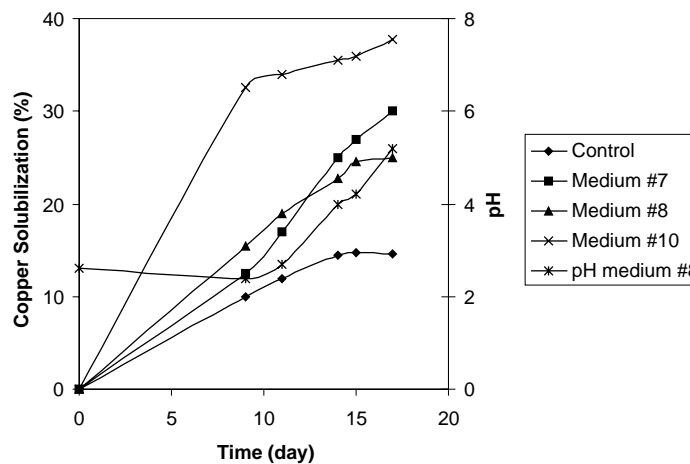


Fig. 7. Copper solubilization in media 7 (leaves), 8 (potato chip waste) and 10 (corn kernels and husks), all soaked in sulfuric acid (pH 2) by *A. niger*.

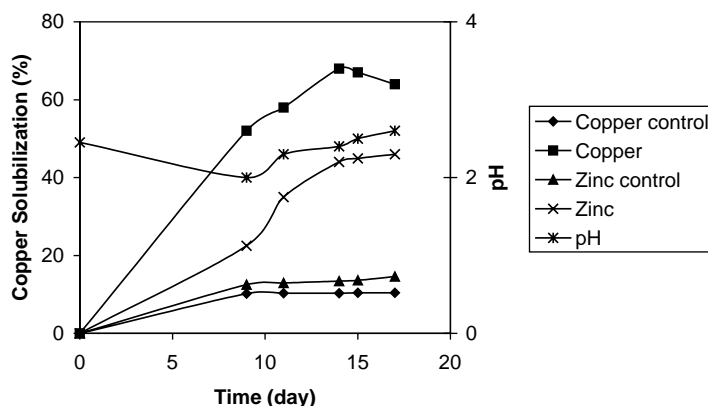


Fig. 8. Copper and zinc solubilization in medium no. 9 (potato peels soaked in sulfuric acid, pH 2).

(Fig. 8), the medium effectiveness diminished after 14 days and solubilized copper started to decline. This evidence can be explained as a deficiency of available substrate for culture. It is suggested to terminate the experiment before approaching this point. It is also possible to supply continuous amounts of substrate.

As expected, sulfuric acid had an excellent ability to solubilize zinc. For instance, as is illustrated in Figs. 6 and 8, 10% of zinc was solubilized in control medium no. 6 and 15% of zinc was solubilized in control medium no. 9. Approximately, 45% of the zinc was solubilized in medium no. 9 which is considered a good result. Sulfuric acid augmented the capability of nickel solubilization and 35% nickel solubilization was achieved in medium no. 6 as shown in Fig. 6.

3.3. Mass balances

Finally, analysis of the residual solids after leaching was carried out to perform a mass balance for copper. The amount of copper was determined in the residual solids and then added to the amount of copper previously determined to be solubilized in the supernatant. This was then compared to the initial amount of copper in the residue. The following results were observed: On average, approximately 15% of the copper was not accounted for. It could then be concluded that most of missing copper was most likely absorbed in the fungal biomass [9]. Analysis on the fungi for mass balance determination of the copper was not possible due to the difficulty in separating the fungi from the mining ore.

4. Economic study

The operating costs for a potential full scale leaching process are shown in Table 4 using potato peels with dilute sulfuric acid as the substrate. They have been estimated using the study by McNulty and Thompson [18] for a bioleaching process, the operating costs from various mining operations taken from Biswas and Davenport [19] and the chemical costs for the nutrients from the Chemical Market Reporter

Table 4

Operating costs for a proposed full scale bioleaching process using potato peels and sulfuric acid as the substrate (6500 metric tons ore/day)

Process	Cost (US\$/kg copper)
Grinding	0.16
Bioleaching	
Nutrients	0.41
Electricity	0.45
Labour	0.19
Solvent extraction	0.02
Solvents for solvent extraction	0.03
Dewatering	0.03
Electrowinning	0.08
Total	1.37

[20] and Reisman [21]. The operating costs were then calculated per kg of copper produced. As seen in Table 4, the total cost to produce one kg of copper was estimated to be US\$ 1.37. Since the current price of copper is approximately US\$ 1455/metric ton or US\$ 1.46/kg, this process, at this point in its development, would be close to profitable. Replacement of the media components with less expensive alternatives such as agricultural wastes has reduced the cost of production significantly compared to a previous study using molasses as the substrate [12]. Electricity costs could be reduced if a heap leaching process is developed and used instead of the mixed tank one.

5. Conclusions

Our studies show that recovery of copper from a mining ore is feasible. A variety of organic acids were produced by *A. niger*. Organic acid effectiveness was enhanced when sulfuric acid was added to the medium. Various food and agricultural wastes were evaluated. Maximum copper dissolution of 68%, 46% for zinc, 34% for nickel and 7% for iron was obtained. The addition of sulfuric acid also enabled efficient metal extraction without the need for sterilization. As indicated in the table, the highest rate of removal was

achieved in the sucrose medium and the highest overall removal occurred in the potato peel medium with sulfuric acid pretreatment. The medium with the sawdust and sucrose showed also significant copper solubilization. Maximum zinc dissolution was 46% with sulfuric acid pre-treatment. Further efforts will be required to increase the rate of copper removal.

Acknowledgements

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