

## Bioleaching of zinc and nickel from silicates using *Aspergillus niger* cultures

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### Abstract

In this work, we investigated the role of bacteria from the genera *Bacillus* and *Pseudomonas* and fungi from the genera *Aspergillus* and *Penicillium* in the leaching process of two different silicates (calamine and garnierite). Since the results obtained with *A. niger* were better than those with different bacteria, a more detailed investigation of the leaching process with this microorganism was conducted. Moreover, although it is clear that the citric acid generated by fungi could be an important leaching agent acting in the solubilization of the used silicates, other products of metabolism could be involved. Related to this, the results obtained with chemical leaching using low concentrations of citric acid (lower than 10 mM) showed, for both calamine and garnierite, that the respective dissolution of zinc and nickel was much lower when compared to those processes in which cultures or supernatant liquor of *A. niger* cultures were used and in which the maximum concentration of citric acid was 8 mM. The results obtained also suggest that the type of mineral (and/or the metal present in it) presents a different susceptibility to the bioleaching process and also demonstrate that depending of the situation, the presence of the fungi cells seem to improve the leaching process. From a practical point of view, the high yield rate of extracting metals from silicates obtained by using for example, supernatant liquors of *A. niger* cultures, is noteworthy. This bioleaching process present two advantages as compared to conventional chemical leaching processes: (a) the very low concentrations of organic compounds present in such a situation represent a lower ecological risk; and (b) even with a lower final yield, the economical cost of a such process. Both characteristics could facilitate its industrial application. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* *Aspergillus niger*; Zinc; Nickel

### 1. Introduction

The world's nickel and zinc reserves are about 71 million and 90 million tons, respectively. Despite this apparent abundance, the treatment of silicate

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minerals will become increasingly important because the escalating depletion of high-grade ores will make it necessary to obtain metals from the abundant low-grade silicate minerals. Microorganisms may contribute to the extraction of metals from carbonaceous low-grade ores and mining wastes, as well as from metals containing secondary raw materials and industrial intermediate products like slags [1]. Among the advantages of extracting metals from industrial wastes by using microorganisms is that these methods are less polluting and less energy-consuming than conventional methods [2].

Three groups of microorganisms are used for the leaching process: autotrophic bacteria [3], heterotrophic bacteria [4,5] and fungi [4,6]. In the bioleaching of silicates, different groups of microorganisms have been used to extract metals, such as magnesium, zinc, copper, aluminum. Among active organisms, *Aspergillus niger* (aluminosilicates), *Thiobacillus ferrooxidans* (sulfide minerals), *Penicillium* sp. (nickel and zinc silicates). Bacteria from *Pseudomonas*, *Erwinia* and *Bacterium* genus produce 2-ketogluconic acid when grown on a glucose medium. It was demonstrated that the dissolution of silicates by these microorganisms is a consequence of the complexation of the cationic elements present in different silicated minerals [7,8]. At the same time, complexation often reduces the toxicity of metals ions [9,10]. A second example of biosolubilization of silicates is obtained by the utilization of the bacteria *Sarcina ureae*. Grown in the presence of urea, this species produces ammonia, which results in the high alkalization of the media. The siloxan binding is disrupted in this condition. It has been demonstrated that silicon can be released from nepheline, plagioclase or quartz by *S. ureae* grown on peptone and urea [11]. It is also known that *Bacillus mucilaginosus* are able to prompt the release of silicon from quartz. The data obtained suggest an indirect mechanism in which the siloxan binding is disrupted by the action of exopolysaccharides [11].

Besides bacteria, fungi from genus *Aspergillus*, *Botrytis*, *Mucor*, *Penicillium* and *Trichoderma* are able to solubilize Ca, Mg and Zn from silicates. This is due to the fact that these species produce organic acids (oxalic and citric). In other silicates — such as in the case of aluminum-silicates (plagioclase), the solubilization of silicon is not the result of the direct

action of acids, but occurs through the complexation of metals. As a consequence, there is a reorganization of the mineral's structure with the release of silicon [12].

The purpose of this investigation was to determine the ability of a number of microorganisms, specially *A. niger*, to extract Ni or Zn from silicate minerals. First, we tried to isolate heterotrophic microorganisms from the ore sample. Inorganic and organic acids (oxalic and citric acids) were also used to study the extraction of metals and the results were compared with those of microbial leaching.

## 2. Materials and methods

### 2.1. Strains

The strains used throughout the present work are: *A. niger* ATCC 1015, *B. megaterium* ATCC 21916, *B. circulans* (a kind gift from S. Groudev, Sofia, Bulgaria), *Pseudomonas* sp. (ATCC 21025) and *Sporosarcina ureae* ATCC 6473. Stock cultures were kept at 4°C in malte extract/peptone/sucrose/agar (MPSA) agar slants (*A. niger*), potato/dextrose/agar (PDA) slants (*B. megaterium* and *B. circulans*) or nutrient agar slants (*Pseudomonas* sp. and *Sporosarcine ureae*). They were subcultured every month. Spores or conidias of 5- to 7-day-old cultures incubated at 30°C were used for the preparation of inocula to be added to the sterile medium.

### 2.2. Growth conditions

The standard medium for *A. niger* contained 5–15% (w/v) sucrose, 0.025%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.25%  $(\text{NH}_4)_2\text{CO}_3$ , 0.25%  $\text{KH}_2\text{PO}_4$ , 0.06 mg/l  $\text{ZnCl}_2$ , 1.3 mg/l  $\text{FeCl}_3$  or  $\text{Fe}_2(\text{SO}_4)_3$ . The pH in the medium was adjusted to 7.5 by adding NaOH. *B. megaterium*, *B. circulans* and *Pseudomonas* sp. were grown in a medium containing 2% (w/v) peptone, 0.2% yeast extract, 0.075%  $\text{KH}_2\text{PO}_4$ , 0.03%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 2% glucose. *Sporosarcina ureae* was grown in 2% (w/v) peptone, 0.2% yeast extract, 0.075%  $\text{KH}_2\text{PO}_4$ , 0.03%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 2% urea.

### 2.3. Substrates

The Zn silicate (calamine-SiO<sub>2</sub>) and the Ni silicate (garnierite-SiO<sub>2</sub>) were supplied by the mining companies: Cia Mineral de Metais (Vazante, MG) and Acesita (Timóteo, MG, Brazil), respectively. These silicates were ground to 100 mesh.

The chemical analysis of the silicates used in this work showed the following compositions: calamine — 40.7% SiO<sub>2</sub>, 10.9% ZnO, 6.7% Fe<sub>2</sub>O<sub>3</sub>, 8.65% CaO, 4.13% MgO; garnierite — 43.2% SiO<sub>2</sub>, 0.90% Ni.

### 2.4. Microbial leaching technique

Two different types of experiments were carried out. In one of them (direct process), the leaching was carried out in 250-ml Erlenmeyer flasks containing 5 g of ore and 100 ml of the growth medium inoculated with microbial culture. Prior to leaching, media and ore samples were sterilized at 121°C for 20 min. The flasks were incubated on a rotary shaker at 30°C at an operating speed of 200 rpm. At regular intervals, samples were withdrawn for pH measurements and filtered through 0.45- $\mu$ m-pore-size membrane filter for elemental analysis. Subsequently, the volume was made up by sterile culture medium. In a second set of experiments, the supernatant liquor of a microbial culture was collected after the cellular growth and used as the leaching agent (indirect process).

### 2.5. Chemical leaching with organic and inorganic acids

To determine the effect of organic acids (intermediate or final products of the microbial metabolism) as well as of inorganic acids on the extraction of nickel and zinc from silicates, experiments were carried out using citric acid, oxalic acid, H<sub>2</sub>SO<sub>4</sub>, at a pulp density of 5 g/100 ml, in the presence of sodium azide 10 mM, to inhibit microbial growth.

### 2.6. Analytical methods

Zinc and nickel were measured by atomic absorption spectrophotometry using standard conditions, after the filtration of the medium through a 0.45- $\mu$ m-pore-size membrane filter to remove biomass.

Organic acids were determined by two different methods. (a) High-pressure liquid chromatography (HPLC): The separation of citric acid and oxalic acid was carried out in an Aminex HPX 87 H cation exchanger column and an Aminex Micro Guard Cation H precolumn; mobile phase, 5 mM H<sub>2</sub>SO<sub>4</sub>; flow rate, 0.5 ml/min<sup>-1</sup>; temperature, 30°C; detection, UV detector at 213 nm. (b) Enzymatic assay in a Beckman spectrophotometer, Model DU 68- Citric acid was measured in a reaction system containing 50 mM triethanolamine/HCl pH 7.4, 10 mM MgSO<sub>4</sub>, 5 mM EDTA and 0.2 mM NADH. The NADH oxidation after the addition of citrate liase

Table 1

Yield of zinc and nickel extractions from the respective substrates by different groups of microorganisms

In the direct process, the silicate pulp density was 5%. The corresponding times (h) for maximum dissolution are enclosed by parentheses.

Microorganism	Yield of extraction (%)			
	Zinc		Nickel	
	Direct process	Indirect process	Direct process	Indirect process
<i>B. megaterium</i>	7.8 (185)	4.2 (230)	5.1 (60)	10.3 (230)
<i>B. circulans</i>	5.6 (212)	2.5 (230)	4.5 (31)	6.3 (182)
<i>Pseudomonas</i>	4.3 (162)	1.2 (230)	3.3 (119)	4.7 (67)
<i>Sporosarcina urea</i>	8.0 (162)	11.1 (20)	22.3 (114)	5.0 (02)
<i>A. niger</i> cultivated in different sucrose concentrations	5%	36 (120)	14 (04)	11 (71)
	10%	66 (148)	41 (146)	54 (120)
	15%	62 (300)	54 (43)	78 (325)
Control		0.3 (200)		0.1 (200)

and malate dehydrogenase to the reaction system was taken as the control. The reaction, monitored at 340 nm, was started by addition of supernatant samples of the medium, filtered through Whatman filter paper. Oxalic acid was measured using a Sigma Diagnostic Kit for oxalate. Oxalate was oxidized to carbon dioxide and hydrogen peroxide by oxalate oxidase. The hydrogen peroxide reacts with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-(dimethylamine) benzoic acid (DMAB) in the presence of peroxidase to yield an indamine dye, which has a maximum absorbance at 590 nm.

All experiments were performed at least three times. The representative results are shown in this paper.

### 3. Results and discussion

Before testing the ability of selected microorganisms to solubilize zinc and nickel from the respective silicates, attempts were made to isolate bacteria and fungi from the ore samples. Since the screening that was carried out did not lead to the discovery of any microorganism usually used for the leaching process, we focused on the knowledge of biohydrometallurgy accumulated up to date, viz., the ability of a number of bacteria and fungi to solubilize large amounts of metals from solid materials. Since bacteria from the genera *Bacillus* and *Pseudomonas* and fungi from the genera *Aspergillus* and *Penicillium* are capable of reducing and/or dissolving heavy metals from

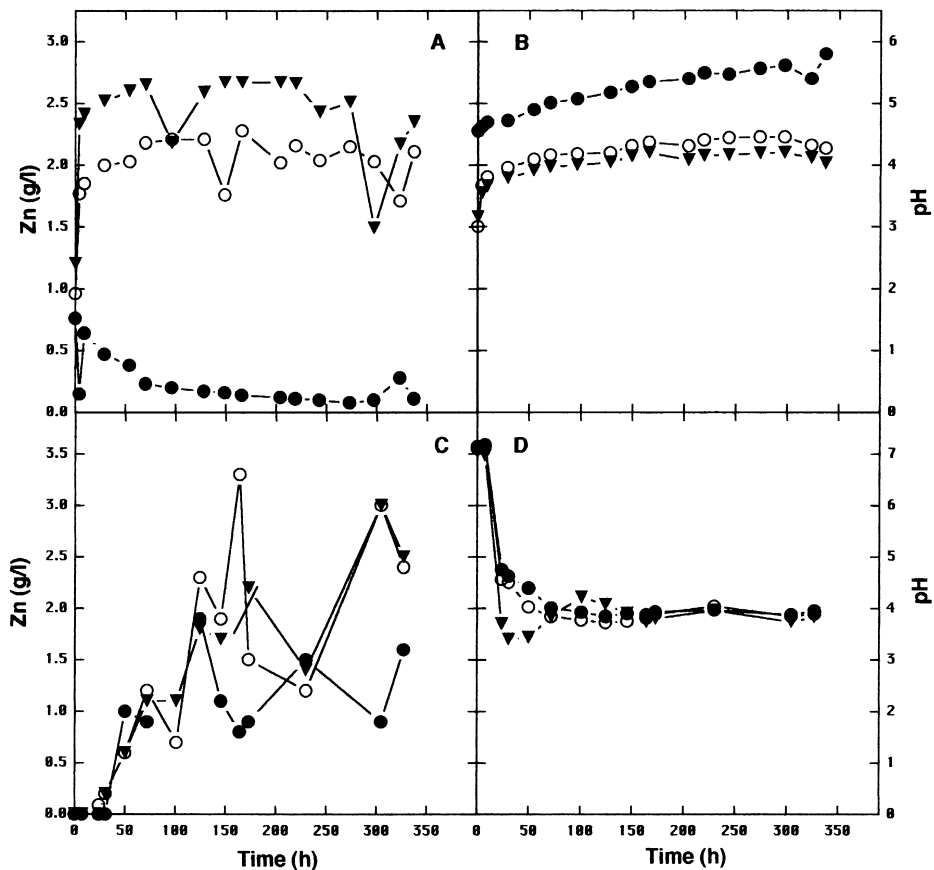


Fig. 1. Amounts of zinc extracted from calamine by the supernatant liquor of cultures of *A. niger* via the indirect process (A) and the direct process (C) and the pH variation in the indirect process (B) and direct process (D) as a function of time. Sucrose concentrations: (●) 5%, (○) 10% and (▼) 15%.

minerals and the surface of the rocks [12], we investigated the role of five of these microorganisms in the leaching process.

Table 1 shows the maximum values of zinc and nickel extraction (and the corresponding time) from calamine and garnierite, respectively, both by direct process (experiments were carried out incubating the microorganisms in a medium containing ore samples) and indirect process (using supernatant liquor of the cultures as a leaching agent). The results of the atomic absorption spectrophotometric analysis show that the highest concentration of soluble zinc and nickel was reached by using *A. niger* in the leaching process. This is probably due to the nature of the leaching active metabolite excreted by *A.*

*niger* and not to the toxicity of heavy metal ions towards the other microorganisms that were used in this investigation. We also observed higher extraction rates with the increase of sucrose concentration in the growth medium of *A. niger* cultures. Control experiments (uninoculated media) were performed in the absence of microorganisms.

Analyzed altogether, the results expressed in Table 1 seem to suggest that the efficiency of the leaching process using *A. niger* may vary according to the metal being extracted and the leaching process. While the extraction of zinc from calamine appeared to be higher by the direct than the indirect process, no consistent differences were observed in the extraction of nickel from garnierite.

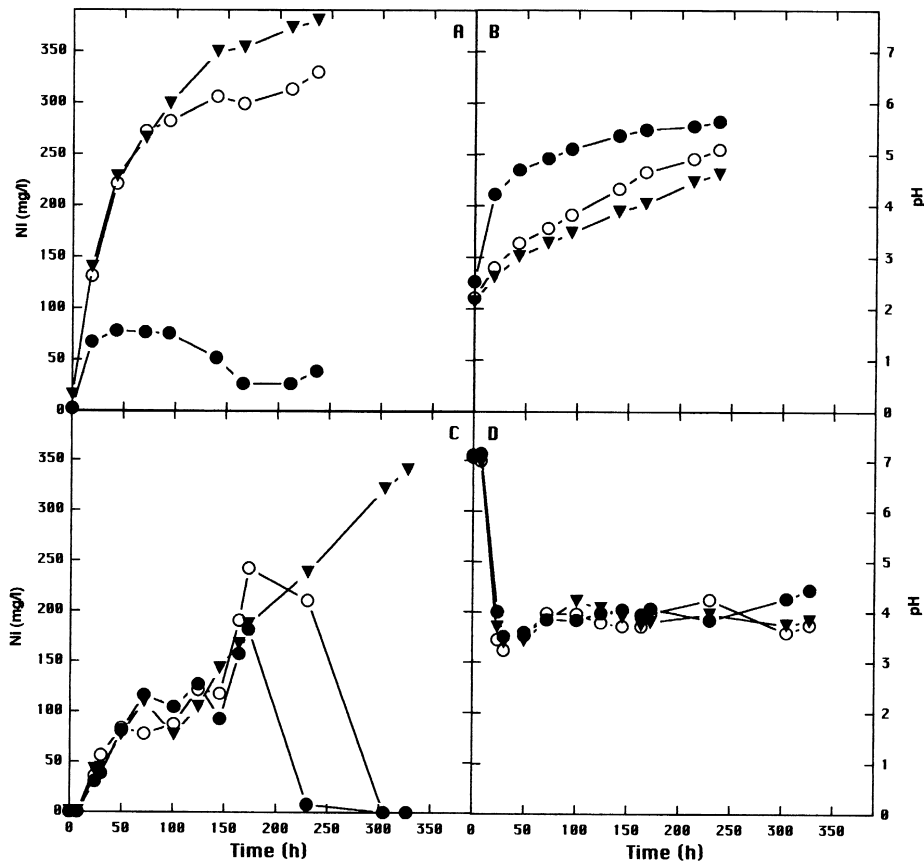


Fig. 2. Amounts of nickel extracted from garnierite by the supernatant liquor of cultures of *A. niger* via the indirect process (A) and the direct process (C) and the pH variation in the indirect process (B) and direct process (D) as a function of time. Sucrose concentrations: (●) 5%, (○) 10% and (▼) 15%.

Since the results obtained with *A. niger* were better than those obtained with different bacteria, we decided to investigate in more detail the process of leaching with this microorganism. Fig. 1A summarizes the results obtained by the action of the supernatant liquor from *A. niger* culture grown in 5%, 10% and 15% sucrose in the zinc solubilization process. A clear influence of sucrose concentration can be observed in the results of experiments where increased sucrose concentration exerted a noticeable improvement on the leaching process. The treatment was extended beyond 15 days in order to determine the maximum leaching capacity of the medium. In this condition, using the clear liquor from the fermentation (indirect process), there was an increase in the amount of dissolved zinc, reaching a plateau after the first day. In another study (Fig. 1C) the fungus was grown in the presence of the mineral (direct process). The maximum leaching capacity was obtained again with sucrose at higher concentrations (10–15%). Zinc dissolution shows considerable fluctuation with time in the direct process.

Fig. 2 shows the microbial leaching of nickel using the supernatant liquor of the *A. niger* culture as the leaching agent (indirect process, Fig. 2A) and the leaching activity produced by *A. niger* grown in the presence of garnierite (direct process, Fig. 2C). Similar to the experiments carried out with calamine (Fig. 1A and C), the best leaching index was obtained with higher concentrations of sucrose (10–15%). However, when *A. niger* was grown in the presence of garnierite, after 170 h there was a strong reduction in the level of nickel solubilized even in 10% sucrose. On the other hand, with the 15% sucrose medium, the level of solubilization increased consistently until 330 h of culture.

Since the efficiency of the dissolution of both metals was higher when the sucrose concentration reached 10–15%, we decided to study in more detail some parameters of cellular growth of *A. niger* as a function of the sucrose concentration present in the growth medium. In Fig. 3 (Panel A), it can be seen that an increase in sucrose concentration resulted in a faster cellular growth rate (mainly with sucrose 15%) and in a higher amount of cells at the end of the experiment (10–15% sucrose). A higher production of citric acid as a function of sucrose concentration (Fig. 3, Panel B) was also observed. The optimum

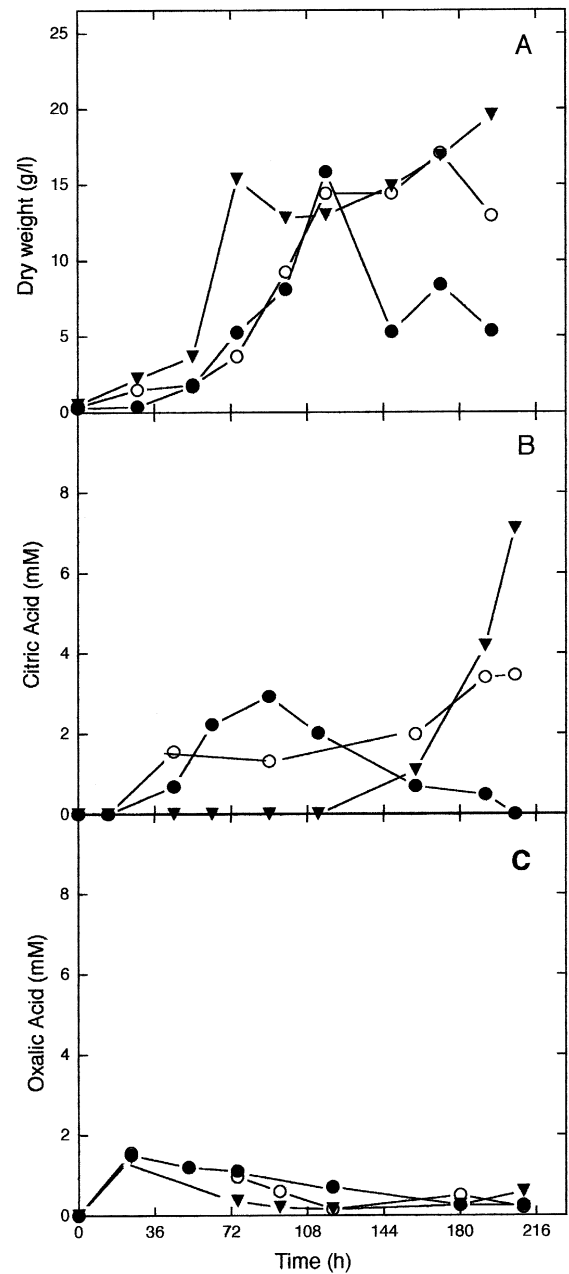


Fig. 3. Cellular growth (A), citric acid production (B) and oxalic acid production (C) by *A. niger* cultivated in different concentrations of sucrose: (●) 5%, (○) 10% and (▼) 15%.

initial concentration of sucrose for citric acid production was found to be between 10% and 15%, the same concentration reported by Xu et al. [13]. Oxalic

acid was produced in low concentrations only during the first hours of growth (Fig. 3, Panel C). Whatever the sucrose concentration, it was observed a strong external acidification was observed and the final pH reached was around 2 (data not shown).

Our results demonstrated that high concentrations of carbon sources are required for the fast growth and high citrate yields, raising the hypothesis that one of the leaching agents present in the fungi cultures is citric acid. However, since these cultures were carried out in the absence of ores, there is no evidence of the possible influence of the minerals on the organic acid production by *A. niger*. To test this possibility, we have measured the levels of citric and oxalic acids produced by *A. niger* grown in the presence of calamine (Fig. 4, Panels A and B) or

garnierite (Panels C and D). The maximum concentrations found for citric acid were about 4 mM (calamine) and 8 mM (garnierite), and about 2 and 4 mM oxalic acid in the presence of calamine and garnierite, respectively. These results are of the same order of magnitude as the ones shown in Fig. 3, suggesting that the presence of the silicates has no significant effect on the organic acid production.

In the leaching of zinc from calamine, using supernatant liquors, the situation is quite similar to the one observed for nickel dissolution from garnierite, reinforcing the idea that the intensity of metal dissolution is proportional to the amount of citric acid produced (see Figs. 1A–3) and confirming the results from the literature that the leaching efficiency of heterotrophic microorganisms depends on the ex-

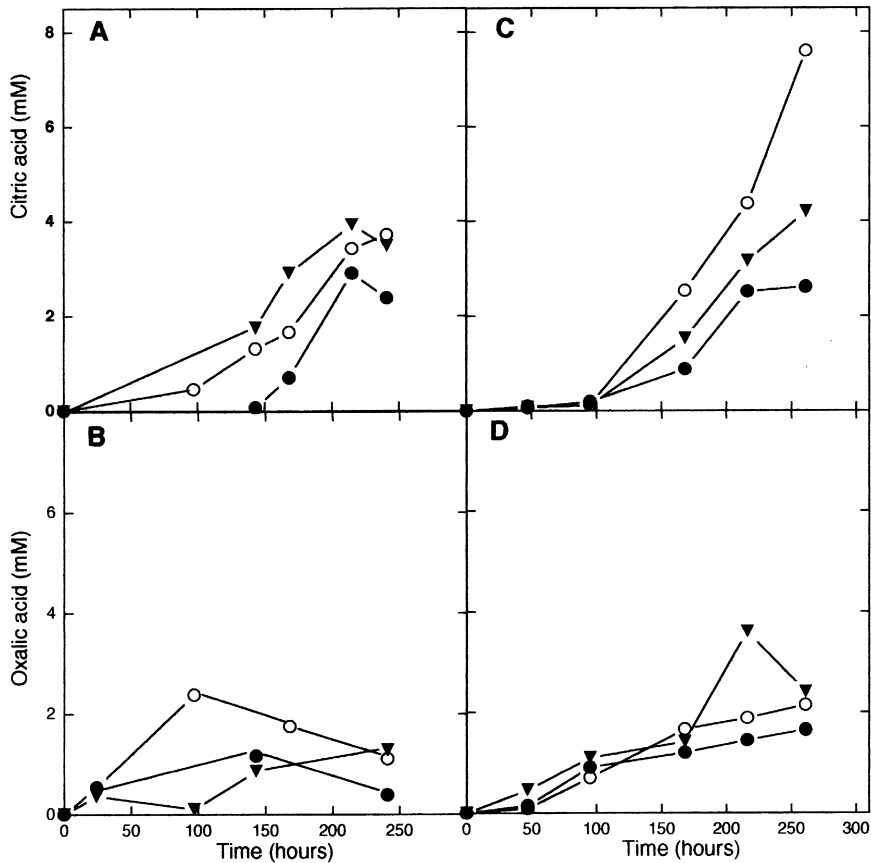


Fig. 4. Levels of citric (A and C) and oxalic (B and D) acids produced by *A. niger* grown in presence of calamine (A and B) or garnierite (C and D). Sucrose concentrations: (●) 5%, (○) 10% and (▼) 15%.

tent of the production of organic metabolites, which are excreted in the culture media and which then induce the dissolution of metals. Another aspect that has to be considered in the leaching process is the external pH variation. The incubation of both ores (calamine or garnierite) with supernatants from *A. niger* cultures showed a tendency of alkalization (Figs. 1B and 2B). On the other hand, when the ores were incubated with *A. niger* cells (direct process), there was a clear tendency of external acidification (Figs. 1D and 2D).

Considering that the organisms of the *Aspergillus* genus produce a rich variety of organic acids and are able to accumulate mainly citric and oxalic acids, we investigated the importance of organic acids normally produced by this fungus species in the leaching process of calamine and garnierite. The results (Table 2 and Fig. 5) show that chemical leaching using citric acid was more effective than with oxalic acid or even than a combination of both of them in the dissolution of zinc and nickel from calamine and garnierite, respectively. The yield obtained with citric acid for both silicates was around 76–78% of the yield obtained when sulfuric acid was used as a control. On the other hand, the kinetics shown in Fig. 6 suggest that, when oxalic acid is used as the chemical leaching for the solubilization of nickel from garnierite, after a very effective solubilization in the first hours of exposure to the acid medium, there is a decrease in the amount of metal extracted that probably results from a precipitation process of the metal ion already dissolved.

However, when lower concentrations of citric (10 mM) and oxalic (5 mM) acids were tested (data not

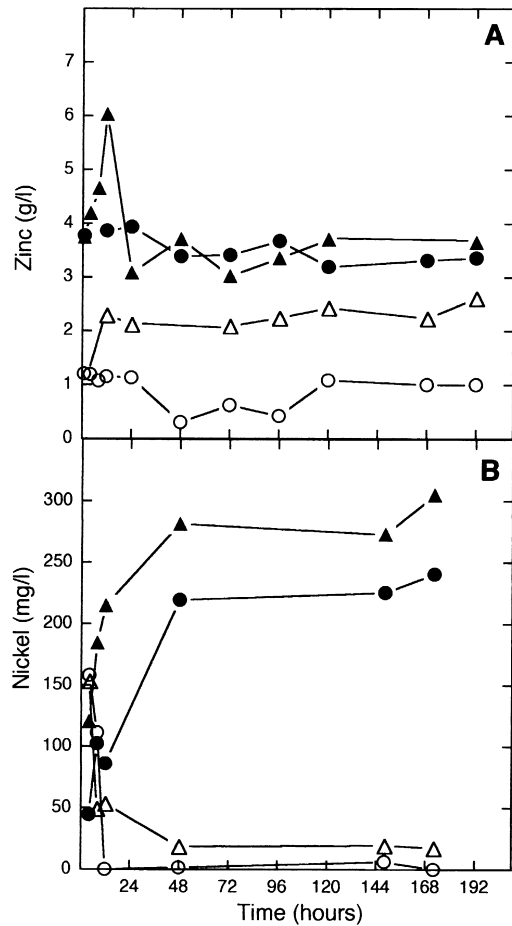


Fig. 5. Amounts of zinc (A) and nickel (B) extracted from the respective silicates by: (●) 100 mM citric acid; (○) 100 mM oxalic acid; (△) 100 mM citric + 100 mM oxalic acids and (▲) sulfuric acid.

Table 2

Yield of chemical leaching of zinc and nickel from the respective silicates by different acids

The silicate pulp density was 5%. The corresponding times (h) for maximum dissolution are enclosed by parentheses.

Leaching condition	Yield of extraction (%)	
	Zinc	Nickel
100 mM oxalic acid	22 (01)	24 (04)
100 mM citric acid	72 (24)	36 (172)
100 mM oxalic acid + 100 mM citric acid	48 (192)	23 (04)
Sulfuric acid (pH 1.0)	94 (08)	46 (172)

shown), the solubilization of zinc and nickel was drastically reduced when compared with the results presented in Figs. 1 and 2. This is an important point since under the conditions used in the direct process, the levels of citric and oxalic acids produced by *A. niger* reached only 8 and 4 mM, respectively (Fig. 4).

Thus, if we compare the effectiveness of extracting zinc from calamine expressed on Tables 1 and 2, we observe that the dissolution of metal was more effective when chemical leaching was performed with 100 mM citric acid than when using the super-



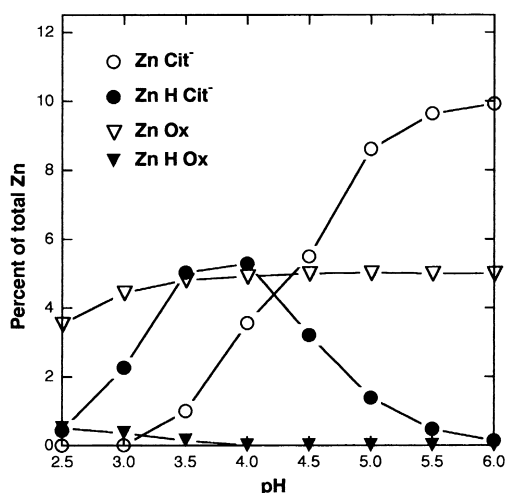


Fig. 6. Simulation for the chemical species distribution of Zn for a solution containing 40 mM  $Zn^{2+}$ , 4 mM citrate and 2 mM oxalate as a function of pH.

nant liquor of *A. niger* cultures (8 mM citric acid, Fig. 3B, 15% sucrose). However, comparison of the data obtained with chemical leaching and the direct process (4 mM citric acid; Fig. 4C, 10–15% sucrose) shows that the maximum yields reached are almost the same. On the other hand, the results reported in this work demonstrate that chemical leaching using citric acid was less effective in the extraction of Ni from garnierite compared to both the direct (8 mM citric acid; Fig. 4C) and the indirect process (8 mM citric acid; Fig. 3B).

In this way, our results seem to be in disagreement with those reported by Sukla and Panchanadikar [14]. They showed that chemical leaching, using oxalic and citric acids, was more effective in extracting the metal from the nickeliferous lateritic ore than the use of supernatants from *Aspergillus* sp. isolated from the same ore. Our work not only suggests that the type of mineral (and/or the metal present in it) presents a different susceptibility to the bioleaching process, but also indicates that the acid concentrations, the medium pH and the metal concentration are important.

In order to understand the leaching process, one has to consider in the first place that the external pH variation shows opposite tendencies, alkalization in the indirect process and acidification in the direct

one. Considering that the amount of organic acids present in both cases is the same, one can conclude that the pH increase in the indirect process is due to proton consumption while converting the oxides in each mineral into soluble metal salts, while the pH decrease in the direct process reveals that the amount of protons produced in this case exceeds the demand for this reaction. At the same time, even though the production of organic acids (citric or oxalic) increased as a function of sucrose concentration, sometimes the number of moles of metal ions dissolved exceeds the number of moles of organic acids produced. Therefore, the leaching process is mainly the reaction of an oxide with protons, even though citrate, which is an important metal coordinating agent, is certainly instrumental in keeping the metal ions soluble, once they have been leached from the respective mineral.

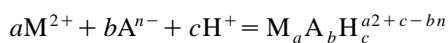
This can be understood, if it is considered that in aqueous media, the equilibria involving the metal ions  $Zn^{2+}$  or  $Ni^{2+}$  ( $M^{2+}$ ) with the anions citrate

Table 3

Stoichiometric coefficients ( $a$ ,  $b$  and  $c$ ) and equilibrium constants ( $\log \beta$ ) of the, chemical species resulting from the interaction of the anions citrate and oxalate with  $Zn^{2+}$  and  $Ni^{2+}$   
Data taken from A. Ringbom, "Complexation in Analytical Chemistry", Interscience, NY, 1963, pp. 323–324.

Compound	Anions		$M^{2+}$		$H^+$	$\log \beta$
	Cit <sup>3-</sup>	Ox <sup>2-</sup>	Zn <sup>2+</sup>	Ni <sup>2+</sup>		
HCit <sup>2-</sup>	1				1	6.1
H <sub>2</sub> Cit <sup>-</sup>	1				2	10.5
H <sub>3</sub> Cit	1				3	13.5
HOx <sup>-</sup>		1			1	4.0
H <sub>2</sub> Ox		1			2	5.1
NiHCit	1		1		1	9.0
NiCit <sup>-</sup>	1		1			4.0
Ni(Cit) <sub>2</sub> <sup>4-</sup>	2		1			5.1
NiOx		1	1			4.1
Ni(Ox) <sub>2</sub> <sup>2-</sup>		2	1			7.2
Ni(Ox) <sub>3</sub> <sup>3-</sup>		3		1		8.5
ZnHCit	1		1		1	8.7
ZnCit <sup>-</sup>	1		1			4.5
ZnHOx <sup>+</sup>		1	1		1	5.6
Zn(HOx) <sub>2</sub>		2	1		2	10.8
ZnOx		1	1			3.7
Zn(Ox) <sub>2</sub> <sup>2-</sup>		2	1			6.0

(Cit<sup>3-</sup>) and oxalate (Ox<sup>2-</sup>) or A<sup>n-</sup>, for brevity, can be represented by the generic expression:



for which  $a$ ,  $b$  and  $c$  represent the stoichiometric coefficients and the equilibrium constant ( $\beta$ ) is given by:

$$\beta = \left( \frac{[M_a A_b H_c^{a2+c-bn}]}{[M^{2+}]^a \cdot [A^{n-}]^b \cdot [H^+]^c} \right)$$

Evidently, according to this expression when “ $a$ ” is zero, we are simply considering the protonation of the citrate and oxalate anions. The species that can result from this equilibrium in an acidic medium are shown in Table 3.

We have calculated the chemical species distribution at different citrate and oxalate concentrations as a function of pH as well as metal ion concentrations. An example is shown in Fig. 6, for a solution containing 40 mM Zn<sup>2+</sup>, 4 mM citrate and 2 mM oxalate. These concentrations of Zn<sup>2+</sup>, citrate and oxalate are somewhat equivalent to the results of Zn dissolution from calamine by cultures of *A. niger* shown in Figs. 1 and 4. As shown in figure, the concentrations of zinc containing citrates or oxalate are below 10%, in the pH range of 2.5–6, which means that — under these conditions — zinc is present in the solution mainly as the bare ion Zn<sup>2+</sup>. There is also one important aspect to be noticed, i.e., the concentration of ZnOx (zinc oxalate) shown in Fig. 6 is of the order of 5% of 40 mM or 2 mM, which is well above the solubility limit of this compound. In other words, under these conditions, both the Zn<sup>2+</sup> extracted from the mineral and oxalate produced by the fungus would be only partially soluble. This fact can explain the fluctuation in the level of dissolution of metals in the direct process (Figs. 1C and 2C). Particularly in the case of the dissolution of nickel from garnierite, our results combined with the simulations, suggest that for a lower citric concentration (produced with lower sucrose concentration) and at pH values between 3 and 4 (Figs. 1D and 2D), there would be a predominance of these insoluble oxalates.

Finally, it should be mentioned that the supernatant liquors of *A. niger* cultures, containing very

low concentrations of organic compounds, produced maximal extractions of metals from silicates that represented 76–78% of the those obtained with sulfuric acid. It is obvious that the bioleaching process represents a lower ecological potential risk and that even with a lower final yield, the economical cost of a such process would be more attractive.

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