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# Bioleaching of Fe, Cu and Ca from Ore-bearing Rocks by Fermentation Liquors of some Fungi

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POTENTIALITY of the fermentation liquors of three filamentous fungi *i.e. Aspergillus niger, A. carbonarius* and *Penicillium purpurogenum* as bioleaching agents for the excess iron of kaolin, used in ceramic and paper industries, as well as solubilization of copper from the low-grade ore pyrite was investigated in this work. Influence of these metabolic solutions upon limestone, greatly used in construction of the ancient Egyptian monuments and in many of the recent seaside resorts, was also ascertained. Excreted metabolites of the two aspergilli were found to have good solubilizing effects on iron from kaolin and calcium from limestone. This influence was beneficial in the first case and detrimental in the other. Mechanism involved in this bioleaching process was related to the synergistic acidolysis effect of both oxalic and citric acids produced by the experimented fungi which was not sufficient enough for pronounced solubilization of copper from pyrite.

**Keywords**: Bioleaching, Metals, Filamentous fungi .

Many investigators allover the world directed their efforts in the last decades to study the interactions of microorganisms with metals that could be beneficial or harmful. Microorganisms are increasingly used beneficially in different biotechnological scopes such as bioleaching of metals from low-grade ores (Toro *et al.*, 1992; Sukla *et al.*, 1993; Muller *et al.*, 1995; Strasser *et al.*, 1995; Reeza *et al.*, 1997; Gadd, 1999; Rawlings, 2002; Mulligan and Kamali, 2003; Santhiya and Ting , 2005 and Mikkelsen *et al.* 2006), bioaccumulation of metals from dilute solutions (Perkins and Gadd, 1995; Simmons *et al.*, 1995; Tolley *et al.*, 1995; Abdel-Razek and Hafez, 1997; Tashirev *et al.*, 1998 and Tsekova and Ilieva, 2001 and bioremediation (Philips *et al.*, 1995; Rittmann and McCarty, 2001 and Matlakowska and Sklodowska, 2007). In contrast, the detrimental effect of microorganisms includes the microbial weathering of building stone such as limestone, leading to defacement or structural changes (Smith, 1996; Warscheid and Braams, 2000 and Mulligan and Kamali, 2003).

A number of microorganisms with an extraordinary capability in dissolving metals directly from minerals and the surface of rocks had been reported. Bacteria in the genera *Bacillus* and *Pseudomonas* and fungi in the genera *Aspergillus* and *Penicillium* were the most active in this regard (Toro

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*et al.*, 1992; Muller *et al.*, 1995; Perkins and Gadd, 1995; Cameselle *et al.*, 1995; Hamdy, 2000 and Reeza *et al.*, 2001).

Bioleaching is a process by which metals are dissolved from ore-bearing rocks using microorganisms. Metal solubilization may be an indirect process, due to microbial production of certain metabolities that have potential leachability (Burgastaller and Schinner, 1993; Cameselle *et al.*, 1995; Muller *et al.*, 1995; Dodge and Francis, 1997; Reeza *et al.*, 2001, Mulligan and Kamali, 2003 and Santhiya and Ting, 2005). Organic acids are recommended by some workers as suitable leaching agents (Cameselle *et al.*, 1995; Muller *et al.*, 1995; Dodge and Francis, 1997; Reeza *et al.*, 2001, Mulligan and Kamali, 2003 and Santhiya and Ting, 2005).

In this work, the potentiality of *Aspergillus niger*, *A. carbonarius* and *Penicillium purpurogenum* to leach some metals from ore-bearing rocks is investigated. The possible role of certain metabolites in the same regard is also ascertained.

## Material and Methods

*Ore-bearing rocks*

Rock samples were collected from Egyptian localities near Cairo i.e. Kattamia, Obour City and Suez, and were thoroughly washed with tap water, followed by double distilled deionized water. The rock samples were then powdered by a manual hummer and were sieved where only particles of 0.63 mm or less were used in this work after being thoroughly washed with double distilled deionized water and air, then oven-dried at $60^0$C before use.

*Microorganisms*

Two species of *Aspergillus* i.e. *A. carbonarius* (Bainier) Thom and *A. niger* Van Tiegh in addition to *Penicillium purpurogenum* Stoll were used throughout this work. These fungi are members of our Lab collection that were grown on Czapek's-agar medium and subcultured whenever required.

*Fermentation*

The fermentation medium was that described by Ghareib (1978) having the following composition (g/100 ml): sucrose, 14; $NH_4NO_3$, 0.2; $KH_2PO_4$, 0.1 and $MgSO_4.7H_20$, 0.023. The medium was distributed in 250 Erlenmeyer flasks, each containing 50 ml and sterilized at 121 $^0$C for 15 minutes. After cooling the initial pH value was adjusted to 5 and the flasks were inoculated with 1 ml of spore suspension (contained ~ $4x10^6$ spores) obtained from 7 day-old cultures and incubated at $30^0$C on a rotary shaker at 150 rpm. The metabolic solutions were then filtered to remove the biomass and the filtrates served as leaching solutions.

*Leaching process*

The crushed ore-bearing rocks were subjected to action of the metabolic solutions. Flasks of 250 ml capacity containing 15 g of fine powdered rock and 95 ml of a leaching solution were used as recommended by Cameselle *et al.* (1995). The process was carried out on a rotary shaker at a speed of 200 rpm and 45$^0$C. Autoclaved fermentation medium was used in the same manner as control leaching solution.

*Analyses*

The liquids containing the leached metals were separated from the suspension of the ores by centrifugation and the solubilized metals were determined by Atomic Absorption Spectrophotometer (Perkin Elmer-3100).

Organic acids produced by the three investigated fungi were separated and identified adopting the descending paper chromatography technique. The identified acids were quantitatively estimated by Knauer HPLC system using Luna 5u $C_{18}$ (2) column.

## Results and Discussion

*Effect of the fermentation liquors on kaoline, pyrite and limestone*

In this study, kaolin, pyrite and limestone were subjected to the action of fermentation liquors of *Aspergillus niger, A. carbonarius* and *Penicillium purpurogenum* grown in submerged cultures. The results (Table 1) show the fluctuating potency of the three liquors in leaching iron (from kaolin), copper (from pyrite) and calcium (from limestone). Maximum solubilization of iron from kaolin was achieved by the metabolic solution of *A. niger* followed by that of *A. carbonarius* amounting to 105.52 and 72.80 ppm, respectively. These results are important from the economic point of view since kaolin used in ceramic and paper industries should be of good whiteness. Kaolin clay is frequently contaminated by certain amounts of iron oxides that reduce its whiteness giving a brownish coloration makes kaolin useless for industrial uses. Removal of the excess iron from kaolin renders it suitable for industrial applications that require a clear whiteness. This method of bioleaching of kaolin was recommended by other investigators as an alternative to the high expensive classical abiotic bleaching methods either physico-mechanically or chemically (Toro *et al.*, 1992 and Cameselle *et al.*, 1995).

**TABLE 1. Solubilization of metals from the ore-bearing rocks by metabolites of the three investigated fungi**

| Treatment with metabolite of: | Solubilized metals (ppm)* | | |
|---|---|---|---|
| | Iron (from kaolin) | Copper (from pyrite) | Calcium (from limestone) |
| *A. niger* | 105.52 | 2.50 | 404.73 |
| *A. carbonarius* | 72.80 | 2.15 | 284.31 |
| *P. purpurogenum* | 8.13 | 1.22 | 108.84 |

* After 6 hr of treatments.

. No solubilization effect has been detected in the control leaching solution.

*Bioleaching of copper from pyrite*

The results recorded here show that copper was dissolved from the low grade ore pyrite at a very low rates amounting to 2.5, 2.15 and 0.82 ppm by action of the metabolities of *A. niger, A. carbonarius* and *P. purpurogenum*, respectively. *A. niger* has been recorded previously as capable of bioleaching copper from low grade ore (Mulligan and Kamali, 2003).

*Effect of the bioleaching on limestone*

The results also demonstrate the detrimental effect of the bioleaching on the limestone that used in construction of the temples of ancient Egyptian as well as buildings in many recent seaside resorts. Calcium was greatly solubilized from limestone by the fermentation liquors of *A. niger* and *A. carbonarius* which have the potentiality to dissolve 404.73 and 284.31 ppm calcium from the studied limestone, respectively. The microbial weathering of stone was studied extensively by Warscheid and Braams (2000). They found that microflora improves the nutrient and moisture-restricted growth conditions on building stones by the formation of surface-covering biofilms. Acidolytic and oxido-reductive biocorrosion process complete the biodeterioration attack of stone acting as a preliminary precursor for the latter formation of detrimental crusts. Protective treatments as well as biocidal application have to be considered.

*Effect of the leaching period*

Leachability of the three fermentation liquors was monitored after different periods of treatment (Figs. 1-3). It is evident that the leach capacity of all solutions was increased with increasing the leaching period although after 8 h it was a declining increase. Such phenomenon was markedly obvious in case of A. *niger* metabolic solution and least in that of *P. purpurogenum*. So, this period is recommended as the shortest economic leaching period.

*Role of organic acids*

Chromatographic analysis was carried out for the organic acids contained in the leach solutions. The results (Table 2) reveal the dominance of citric and oxalic acids in fermentation liquors of the two aspergilli. In addition, moderate level of gluconic acid was detected in that of *A. niger*. On the other hand, *P. purpurogenum* produced moderate yields of gluconic, succinic and citric acids. Superiority of the metabolic solutions of the two aspergilli in the bioleaching of iron and calcium to that of *P. purpurogenum* may be due to the relatively high acidity of these solutions as a result of their high content of both citric and oxalic acids. Low pH of the previous metabolic solutions provides an excellent condition for the leaching process where it prevents the reoxidation of the solubilized metals (Cameselle *et al.*, 1995). These results throw the light on the possibility that citric and/or oxalic acids may contribute to the bioleaching of iron and calcium from their low grade ores. Citric acid was found by Muller *et al.* (1995) to be the main leaching agent at the time that oxalic acid was recommended by other workers (Cameselle *et al.*, 1995, Strasserr *et al.*,1995 and Santhiya and Ting, 2005). To realize this assumption, different concentrations of citric and oxalic acids- either separately or in combination- were used as leach solutions. The results (Table 3) show that both acids had good leaching capacity although oxalic acid was superior. This may indicate that both acids play a role in this process in a synergistic manner. This is confirmed by achieving a high leaching of all metals when the rocks were treated with mixtures of citric and oxalic acids rather than either of the two acids. The weak leaching capacity of the metabolic solution of *P. purpurogenum* confirmed this assumption where oxalic acid was lacking

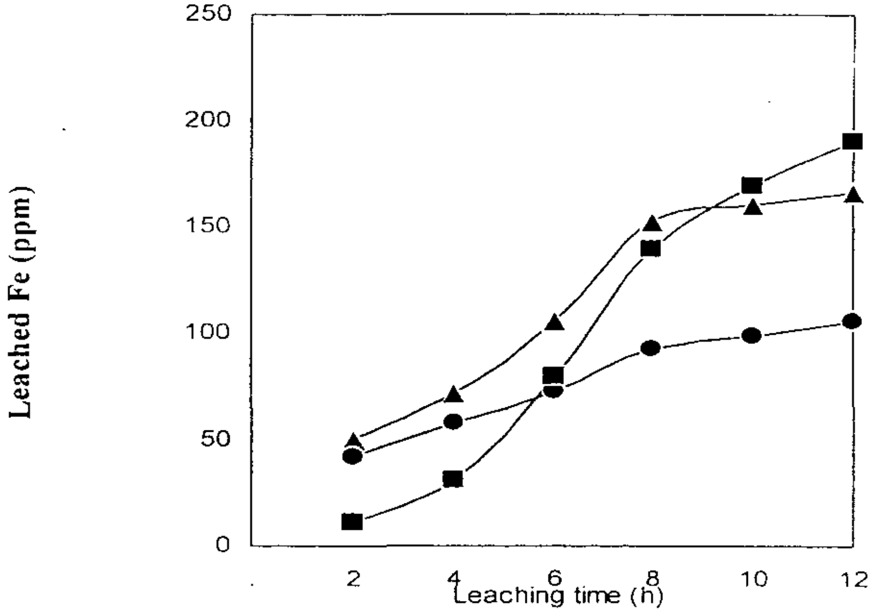


**Fig. 1. Solubilization of iron from kaolin after different leaching periods by metabolites of *A. niger*, —▲—, *A.carbonarius*,—●—, and *P. purpurogenum* (X 10), —■—.**

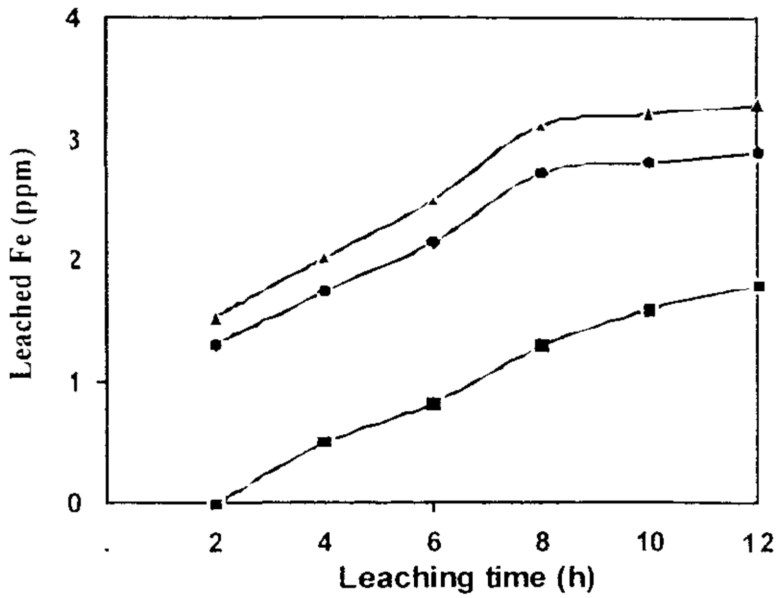


**Fig. 2. Solubilization of copper from pyrite after different leaching periods by metabolites of *A. niger*, —▲—, *A. carbonarius*, —●—, and *P. purpurogenum*, —■—.**

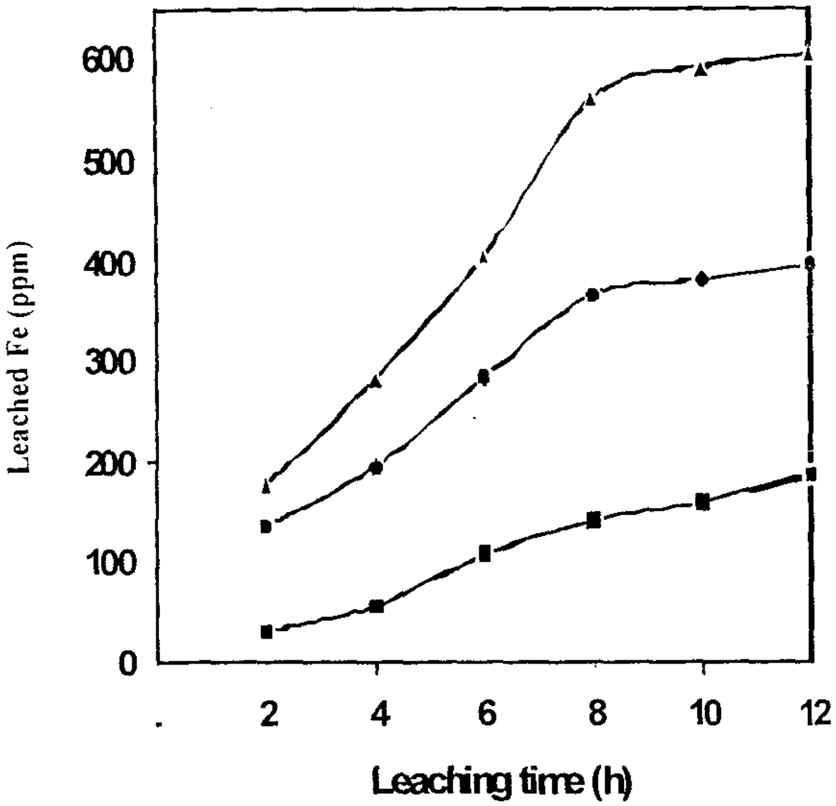


**Fig. 3. Solubilization of calcium from limestone after different leaching periods by metabolites of *A. niger*, —▲—, *A. carbonarius*, —●—, and *P. purpurogenum*, —■—.**

**TABLE 2 . Organic acid contents of the three investigated metabolites**

| Metabolite of: | Organic acids (mM) | | | | Titrable acidity* (Normality) | Final pH |
|---|---|---|---|---|---|---|
| | Citric | Oxalic | Gluconic | Succinic | | |
| *A. niger* | 108.9 | 11.6 | 10.2 | - | 2.9 | 1.4 |
| *A. carbonarius* | 76.9 | 7.0 | - | - | 1.0 | 1.8 |
| *P. purpurogenum* | 12.8 | - | 14.3 | 13.6 | 0.2 | 2.9 |

* Titrable acidity was calculated after titration against standard solution of NaOH.

**TABLE 3. Effect of citric and oxalic acids as leach solutions for metals from ore-bearing rocks.**

| Treatment | pH | Leached metals (ppm)* Iron (from kaolin) | Copper (from pyrite) | Calcium (from limestone) |
|---|---|---|---|---|
| Citric | 50 mM 2.00 | 11.10 | 0.63 | 52.07 |
| | 100 mM 1.90 | 13.15 | 0.99 | 64.40 |
| | 150 mM 1.74 | 20.36 | 1.21 | 80.32 |
| Oxalic | 5 mM 2.40 | 41.05 | 0.95 | 107.95 |
| | 10 mM 2.10 | 64.75 | 1.34 | 162.66 |
| | 15 mM 1.95 | 107.85 | 1.59 | 191.09 |
| Citric + oxalic | 50 + 5 mM 2.16 | 55.15 | 1.41 | 116.42 |
| | 50 + 10 mM 2.08 | 84.95 | 1.87 | 185.89 |
| | 50 + 15 mM 1.99 | 113.80 | 2.13 | 220.69 |
| | 100 + 5 mM 2.20 | 68.45 | 2.10 | 266.31 |
| | 100 + 10 mM 2.00 | 126.91 | 2.22 | 382.78 |
| | 100 + 15 mM 1.95 | 152.17 | 2.54 | 411.55 |
| | 150 + 5 mM 2.06 | 72.70 | 2.02 | 281.85 |
| | 150 + 10 mM 1.90 | 135.08 | 2.39 | 392.00 |
| | 150 + 15 mM 1.86 | 148.40 | 2.56 | 435.68 |

* After treatment for 8 hr.

It is of interest to note that the fermentation liquor of *A. niger* produced a comparatively more solubilizing effect than that produced by any of the experimented mixtures. This can be attributed to the possible role of other metabolic components such as amino acids those proposed as solubilizing agents by other investigators (Golab and Orlowska, 1988 and Wenzl *et al.*, 1990).

The positive influence of organic acids as leaching agents was stated by some workers (Muller *et al.*, 1995; Cameselle *et al.*, 1995; Strasser *et al.*, 1995, Reeza *et al.*, 2001 and Santhiya and Ting, 2005). These acids were postulated to dissolve metals by direct displacement of metal ions from the ore matrix by hydrogen ion or by the formation of soluble metal complexes (Sukla *et al.*, 1993 and Dodge and Francis, 1997).

Leachability of copper differed from those of the two other metals. This phenomenon was recorded by Strasser *et al.* (1995) who reported that copper solubilization is caused not only by acidolysis but also by redoxolysis.

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## الإذابـة الحيويـة للحديـد والنحـاس والكالسيوم مـن الصـخور الحاملـة لخاماتها بإستخدام المحاليل التخمرية لبعض الفطريات

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تم دراسة إمكانية إستخدام المحاليل التخمرية لثلاث من الفطريات الخيطية هى أسبرجيللاس نيجر وأسبرجيللاس كربونيرياس وبنسيليوم بوربوروجينم كعوامل إذابة حيوية للحديد الزائد فى الكاولين المستخدم فى صناعتى السيراميك والورق ، وفى إذابة النحاس من صخور البيريت الحاملة له كما تم دراسة أثرتلك المحاليل الأيضية على الحجر الجيرى المستخدم فى المعابد المصرية القديمة وفى المنتجعات الساحلية الحديثة.

أثبتت الدراسة أن المحاليل الأيضية لفطرتى الأسبرجيللاس تمتلكان مقدرة جيدة على إذابة الحديد من الكاولين والكالسيوم من الحجر الجيرى ذلك التأثير الذى يكون مفيدا فى الحالة الأولى وضارا فى الثانية، وقد تم إرجاع تأثير تلك المحاليل الأيضية إلى الفعل التآزرى لكل من حمض الستريك وحمض الأوكساليك المنتجان بواسطة الفطريات المختبرة، وقد تبين أن تأثير تلك الأحماض العضوية لم يكن كافيا لإذابة النحاس من البيريت.