Effect of Environmental Parameters on the Efficiency of Biodegradation of Basalt Rock by Fungi

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Summary

The biodegradation of an aluminum-bearing (basalt) rock by *Penicillium simplicissimum* has been investigated. This organism grows on a sugar substrate and releases organic acid compounds. These acids interact with the mineral matter and cause their partial decomposition. The dissolved metals are then complexed by the excess organic acids. The activity of the fungi was found to be optimum at an initial pH 7 and in the presence of 5% (w/v) substrate concentration. In 30 days of leaching almost 20% of the aluminum in the rock was solubilized and the pH was decreased from 7 to less than 3.5 in the inoculated flasks. The controls showed less than 1% of the aluminum solubilized and the final pH dropped to only 6.8. A surface characterization study performed by scanning electron microscopy indicated that the specific mineralogical phases containing aluminum and iron within this host rock were preferentially corroded. The mineral phases containing olivine and plagioclase were found to be least resistant, while phases containing titanium were most resistant to the acids released by the fungi.

INTRODUCTION

Bioleaching of aluminosilicate rocks by fungi has occurred in nature since antiquity. Microorganisms were found in 2×10^9 year-old precambrian rocks of the Canadian and Australian shield.[1-4] Limited data have been published on the weathering of rocks and the solubilization of metals from those rocks by heterotrophic microorganisms.[5] It has been reported that fungi can dissolve 94% of the silicon from calcium silicate, 76% from magnesium silicate, and 96% from zinc silicate.[6] The bioleaching of copper and uranium is well established and those are practiced at a commercial scale using autotrophic microorganisms under acidic conditions and in the presence of sulfur and iron.[7,8] Wherever these conditions are not present and silicate- and carbonate-bearing materials have to be leached,

Biotechnology and Bioengineering, Vol. XXI, Pp. 875-885 (1979)
 0006-3592/79/0021-0875$01.00

the application of heterotrophic microorganisms is found to be successful.[9–11] The chalcocite and native copper deposits in Michigan's upper peninsula have been leached by fungi. The amount of copper solubilized apparently depended on the ability of a fungus to adapt to a particular sugar supplied as a substrate.[10] The citric acid-producing strain of *Aspergillus niger* leached 80% of the copper from low-grade copper ore containing carbonates and silicates from a low-grade ore in Israel.[11] There have been few papers published on bioleaching of metals by *Aspergillus* and *Penicillium* species.[12–17] A study[12] of solubilization of metals from rocks such as basalt, granite, granodiorite, rhyolite, andesite, peridotite, and quartzite indicated that after seven days of leaching, 31% of the silicon, 11% of the aluminum, 64% of the iron, and 59% of the magnesium solubilized from some of the rocks in the presence of *Penicillium simplicissimum*. In these processes the decomposition of rocks was achieved by the organic acids that were released by the fungi. A similar mechanism was observed for the action of *P. simplicissimum*, which solubilized up to 80% of the titanium from granitic rocks.[13] Biodegradation of biotite by *A. niger* showed that the organism produced both oxalic and citric acid from glucose; however, the alteration in biotite was more closely related to changes caused by oxalic than by citric acid.[14] Vermiculite has been formed from mica, when three trioctahedral and one dioctahedral mica was used as a source of K^+ for seven species of fungi. The mechanism involved was an exchange of Na^+ for K^+ in mica.[15] The possibilities and limitations for potassium recovery through leucite bioleaching have been investigated. It was found that the strains of *A. niger, S. brevicaule*, and *P. expansum* leached between 21 and 27% of the available potassium in 150 days.[16] The reduction of ferric to ferrous ion by fungi was reported to be accelerated by increasing the amount of nitrate added to the medium.[17]

The present work deals with the biodegradation of aluminum-bearing rock by fungi.

MATERIALS AND METHODS

Microorganisms

A culture of *Penicillium simplicissimum* (American type culture collection (ATCC) No. 10495) was grown on Sabourauds dextrose agar for seven days. It was then routinely maintained in our laboratory on Pope and Skerman's basal mineral salt medium[18] that

contained glucose as an energy source and 0.01% yeast extract. The culture flasks and nutrient media were previously sterilized in an autoclave at 121°C for 15 min while the glucose and yeast extract were sterilized separately and then added to these flasks under aseptic conditions. The culture flasks were incubated at 30°C and transferred to the fresh nutrient medium after every 15 days, while a 10-day-old culture was used as the inoculum.

Rock Samples

The basalt rock used in this work was collected from a region eight miles southwest of Socorro, New Mexico. The chemical and mineralogical compositions of this rock are shown in Table I.

For the experiments to be described, samples less than 37 μm particle size were prepared by first washing, cleaning, and crushing the rocks in a jaw crusher (The Mine and Smelter Supply Co.), and then in a Bico pulverizer (type UA, Bico Inc.), and finally in a Bleuler rotary mill (Willy Bleuler, Switzerland). The crushed rocks were passed through a 325 size screen, using a Tyler Ro-Tap sieve

TABLE I
Chemical and Mineralogical Composition of the Aluminum-Bearing Rock

Chemical	Percent (%)
Al_2O_3	16.34
CaO	8.18
Fe_2O_3	10.65
K_2O	0.92
MgO	7.91
MnO	0.14
Na_2O	5.15
SiO_2	48.78
TiO_2	1.57
	100

Mineral	Composition
Plagioclase	labradorite ($Ab_{47}An_{53}$); Ab-albite,[a] An-anorthite[b]
Pyroxene group	augite $(Ca, Mg, Fe, Ti, Al)_2 (Si; Al)_2O_6$
Olivine	$(Mg, Fe)_2SiO_4$
Pyrite	FeS_2
Ilmenite	$FeTiO_3$

[a] Albite: $Na(AlSi_3O_8)$.
[b] Anorthite: $Ca(Al_2Si_2O_8)$.

shaker. For the surface characterization of biodegraded rocks by scanning electron microscopy (SEM), 0.5 cm pieces of rock were cut and then smooth, uniform surfaces were prepared by polishing the rocks on 150, 240, 320, 400, and finally on 600 grit size paper.

Culture Technique

All experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml nutrient medium,[18] 1–8 g glucose, 0.01% yeast extract, 4 g less than 37 μm size sample (or 5–10 g of the 0.5 cm polished rock specimens) and 10 ml antibacterial solution containing 5 ml 2% thymol in methyl alcohol plus 5 ml 10% formaldehyde in methyl alcohol. The initial pH of these suspensions was varied from 3 to 10. The flasks were incubated on a rotary shaker (Junior Orbit Shaker, Lab Lines) agitated at 225 rpm and 30°C for four to six weeks. The water loss due to evaporation was periodically adjusted with distilled water.

At defined intervals of time 10 ml samples were collected, then filtered, and the filtrate was analyzed for soluble aluminum and iron on an atomic absorption spectrophotometer.[19]

Scanning Electron Microscopy

The rock specimens from both inoculated and sterile control flasks were collected at the end of each week for a period of up to six weeks. These specimens were cleaned, dried, and mounted with silver paste on aluminum stubs, then coated with 300–400 Å Au/Pd in a sputtering unit (minicoater) and finally examined in the SEM (Hitachi–Perkin Elmer HHS-2R). X-ray mapping (energy-dispersive x-ray spectrometry) techniques were employed in the identification of the specific mineralogical phases.[19]

The fungi specimens were prepared with special care. The pieces of fungi were collected from the pure colonies of *P. simplicissimum* grown on Sabourauds dextrose agar, then dried in a dessicator for 15 to 25 min, mounted with silver paste on aluminum stubs, and then observed in the SEM. In a later experiment this specimen was prepared by using a critical point dryer.[19]

RESULTS AND DISCUSSION

Effect of Nutrients

These series of experiments were carried out using four media (M_1 to M_4), whose compositions are shown in Table II. In all the

TABLE II
Composition of Nutrients Media[a]

Nutrients	Solvent (mg/100 ml)			
	M_1	M_2	M_3	M_4
1) NaCl	3000	3000	3000	–
2) $(NH_4)_2SO_4$	6600	6600	6600	6600
3) $LiCl_2$	21	21	–	–
4) $CuSO_4 \cdot 5H_2O$	80	80	–	–
5) $ZnSO_4 \cdot 7H_2O$	106	106	–	–
6) H_3BO_4	600	600	–	–
7) $Al_2(SO_4)_3 \cdot 18H_2O$	123	–	–	–
8) $NiCl_2 \cdot 6H_2O$	110	110	–	–
9) $CoSO_4 \cdot 7H_2O$	109	109	–	–
10) $TiCl_4$	60	60	–	–
11) KBr	30	30	–	–
12) KI	30	30	–	–
13) $MnCl_2 \cdot 4H_2O$	629	629	629	–
14) $MgSO_4 \cdot 7H_2O$	1400	–	–	–
15) $SnCl_2 \cdot 2H_2O$	36	36	–	–
16) $FeSO_4 \cdot 7H_2O$	300	–	–	–

[a] M_1 concentrations are from Ref. 18. 1–10, 13 = H_3PO_4; 11–12, 13–16 = distilled water. M_1 is Pope and Skerman mineral salts medium.[18]

flasks the initial pH was adjusted to 7 and the substrate concentration was 4 g glucose. Figure 1(b) shows that biogenic activity was maximum when all the nutrients were present in the medium. During 30 days of leaching about 16.4% of the aluminum was dissolved in medium M_1, while less than 3% of the aluminum solubilized in the nutrient medium M_4. In the sterile controls less than 0.5% of the aluminum was present in the solution. The amount of aluminum solubilized decreased in proportion to the removal of the nutrients from the leach media, as shown in Table II by M_1-to-M_4 nutrient concentrations. A similar observation was reported for the growth of fungi regarding its nutrients requirements.[20]

Investigations of copper ore leaching[10] indicated that the copper dissolution as well as the fungal activity were dependent upon the medium composition, but optimum nutrient concentrations were not reported. However, it has been reported that a simplified medium, which contained no phosphate, ammonium, iron, and magnesium sources, used in solubilization of metals from minerals[21] indicated no decrease in acid production by the organisms grown on glucose.

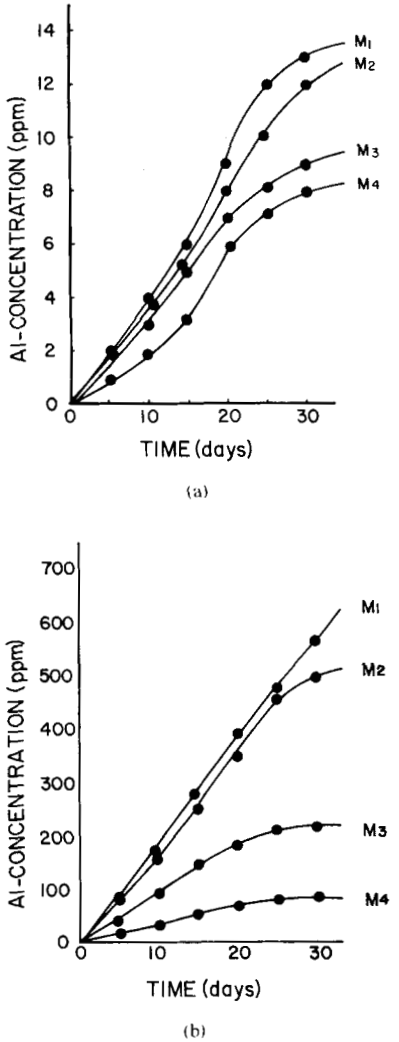

Fig. 1. Effect of the nutrients on fungal metabolic activity in terms of aluminum dissolution. M_1–M_4 nutrient media as described in Table II. (a) Sterile controls; (b) inoculated medium.

Effect of Initial pH

These series of experiments were carried out with the M_1 nutrient medium containing 4 g glucose as substrate (Fig. 1). The initial pH of the suspensions were varied from 3 to 10. The results are presented in Table III. As can be seen, the highest aluminum extraction

TABLE III
Effect of Initial and Final pH on the Aluminum Solubilization after 30 Days of Leaching

Initial pH	Final pH	Al (ppm)
3	3.95	281
4	4.20	278
5	5.61	259
6	4.00	329
7	3.43	568
8	3.61	500
9	3.45	521
10	3.82	459

was obtained with an initial pH 7 solution. At higher and lower initial pH values the aluminum dissolution was considerably decreased. The final pH was decreased in the solutions where the initial pH was 6 or higher. At lower initial pH values a slight increase was noticeable. The decrease in the final pH is due to the organic acid formation from glucose by the fungi. The organic acid dissolves the aluminum in the form of a chelated complex, similarly as mentioned by other investigators.[22,23] Only 8% of the aluminum was solubilized at initial pH 3, while more than double of this amount at the initial pH 7. In sterile controls the aluminum solubilization was less than 1%.

Effect of Substrate Concentration

These series of experiments were done with nutrient medium containing 1 to 8 g glucose at an initial pH 7. The results are summarized in Figure 2, which represents the amount of aluminum

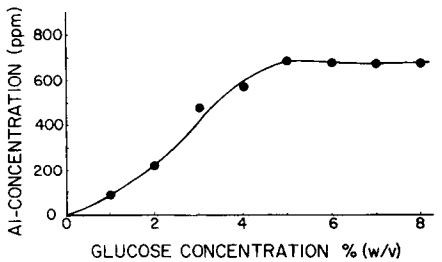

Fig. 2. Effect of the substrate concentration on fungal metabolic activity in terms of aluminum solubilization.

solubilized in 30 days as a function of glucose concentration. The aluminum solubilization increases in proportion to the substrate concentration up to about 5% of glucose. Beyond this concentration, the additional increase in the amount of substrate is not followed by an increase in the aluminum dissolution. In 30 days of

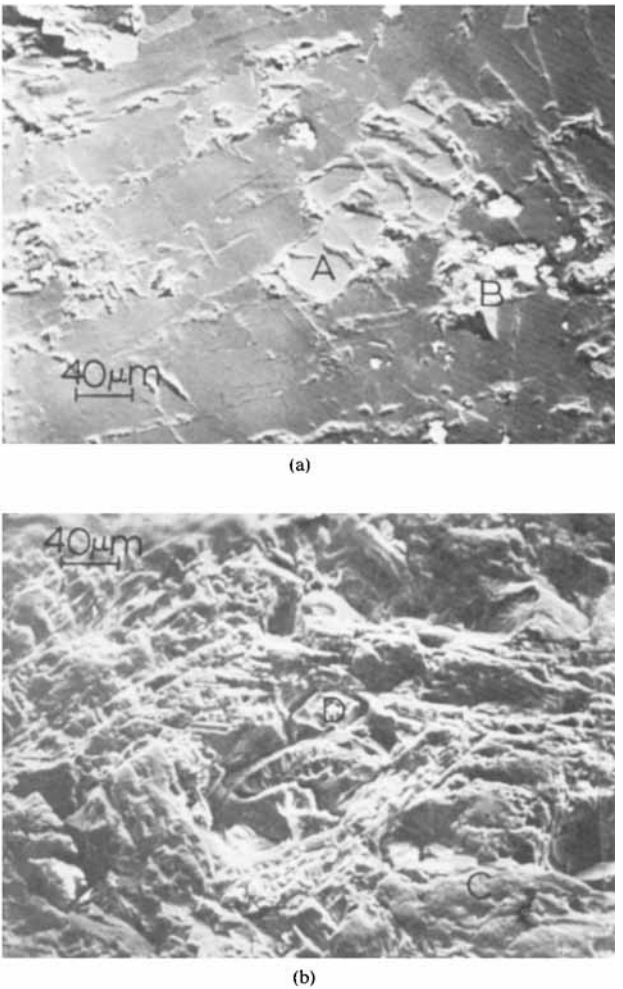

Fig. 3. Surface characterization of rock samples: (a) 3 weeks control; (b) 3 weeks in inoculated medium, showing the preferential corrosion of plagioclase (labradorite) and olivine grains within the basalt matrix. (A) pyroxene; (B) ilmenite; (C) olivine, and (D) plagioclase.

leaching about 19.8% of the aluminum solubilized in the inoculated flasks containing 5 g substrate while less than 0.5% of the aluminum solubilized in the respective sterile controls. From this presentation the optimum substrate concentration is approximated to be 5% (w/v) glucose.

Surface Characteristics of Rock Samples

Figures 3(a) and 3(b) illustrate the surfaces of specimens collected from the sterile control and the inoculated flask, respectively. There is very little alteration on the surface of the sterile control rock while corrosion is considerable on the surface of the specimen collected from the inoculated flasks. Figure 3(b) also illustrates the selective corrosion of plagioclase and olivine grains within the basalt matrix. The ilmenite and augite (pyroxene group) mineral phases were not altered. Thus, corrosion is observed to occur preferentially at mineral inclusions containing Na, Ca, K, Al, Mg, Si, and Fe, i.e., at plagioclase (labradorite), olivine, and pyrite. These observations are in good agreement with earlier investigations[12,13] that were carried out with similar aluminum-bearing rocks and *P. simplicissimum*.

CONCLUSIONS

The present study demonstrated the biodegradability of an aluminum-bearing rock by *P. simplicissimum*. This microorganism produces organic acid from a sugar substrate, which is the leaching agent in the above process. As a class, fungi, under optimum conditions are more efficient synthesizers of cell material than most bacteria.[20] The effect of different parameters on fungal metabolic activity in terms of the aluminum solubilization has shown that at initial pH 7, the maximum solubilization of metal was obtained. In 30 days of leaching the final pH was less than 3.5 in the presence of *P. simplicissimum*, which indicates the formation of acid by the metabolic activity of fungi. The nutrients have exercised profound effects on the metabolic processes of fungi. Best results of metal solubilization have been obtained in the complete Pope and Skerman's basal mineral salt medium (M_1). These results have led to the conclusion that although the nutrients are present in trace amounts, they play an important role in fungal metabolism. The available energy source and its consumption by microorganisms also have an important role. The aluminum concentration increases sharply in the solution up to 5% (w/v) glucose concentration. Beyond this

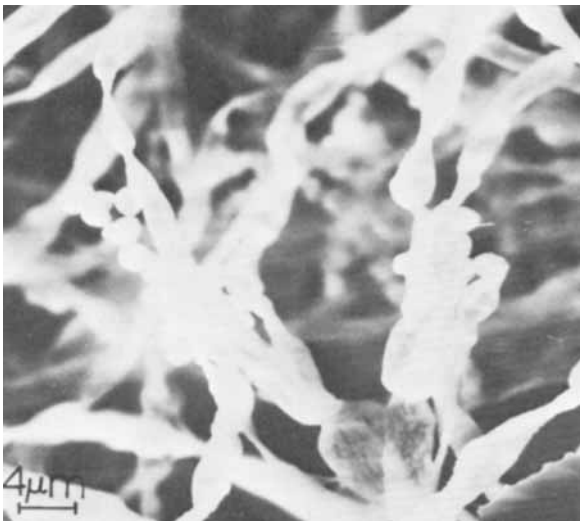

Fig. 4. *Penicillium simplicissimum* showing the spores and vegetative filaments. Specimen was observed in the SEM.

concentration and up to 8% (w/v) glucose the metabolic activity of fungi remained unchanged. Thus, the highest yield of the aluminum dissolution was almost 20% in the inoculated sample while it was less than 1% in the sterile controls, when the initial conditions were pH 7, 5% (w/v) glucose, and nutrient medium M_1.

The SEM examination (Fig. 4) of the leach residue indicated that the mineralogical phases containing aluminum and iron (labradorite, olivine, pyrite) were preferentially leached. This finding supports the observations reported by other investigators.[12–13]

This work was supported in part by the National Science Foundation (RANN) Grant No. AER-76-03758-A01. Our thanks are due to Dr. V.K. Berry for his help in the SEM work, and to Dr. Clay T. Smith of the Department of Geoscience, New Mexico Tech, for his help in the identification of the mineral phases in basalt rock by the thin section technique. Also sincere thanks are due to Mrs. Lynn Brandvold and Mr. Robert Blackmore for the analytical facilities in the New Mexico Bureau of Mines and Mineral Resources.

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Accepted for Publication August 4, 1978