Available online at www.sciencedirect.com
ScienceDirect
Geochimica et Cosmochimica Acta 72 (2008) 87–98
Geochimica et Cosmochimica Acta
www.elsevier.com/locate/gca

Microbial release of potassium from K-bearing minerals by thermophilic fungus *Aspergillus fumigatus*

Bin Lian [a,1], Bin Wang [a,2], Mu Pan [a], Congqiang Liu [a], H. Henry Teng [b,*]

[a] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, 550002, People's Republic of China*
[b] *Department of Chemistry, the George Washington University, Washington, DC 20052, USA*

Received 17 April 2007; accepted in revised form 2 October 2007; available online 13 October 2007

Abstract

A strain of thermophilic fungus *Aspergillus fumigatus* was cultured with K-bearing minerals to determine if microbe–mineral interactions enhance the release of mineralic potassium. Experiments were carried out in two settings, one with the mineral grains and the fungal cells in direct contact, and the other employing a membrane (pore size 0.22 μm) to separate the two. Measurements over a period of 30 days showed that, irrespective of the experimental setup, the concentration of free K in the culture was drastically higher than those in any of the control experiments where no living organism was present. Moreover, the occurrence of mineral–cell physical contact enhanced potassium release by an additional factor of 3 to 4 in comparison to the separation experiments. For contact experiments, Electron Probe Microanalysis revealed the formation of mycelium–mineral aggregates, and Atomic Force Microscopy imaging further indicated the possible ingestion of mineral particles by the fungus cells. Contrasting to what was observed and expected in control experiments, the potassium solubilization rate showed a positive dependence upon pH when fungi and minerals were mixed directly, and exhibited no correlations with solution acidity if cell–rock contact was restrained. These results appear to suggest that *A. fumigatus* promoted potassium release by means of at least three likely routes, one through the complexation of soluble organic ligands, another appealing to the immobile biopolymers such as the insoluble components of secretion, and the third related to the mechanical forces in association with the direct physical contact between cells and mineral particles.

Keywords: Microbe–mineral interaction; Mineral weathering and dissolution; Thermophilic fungus; *Aspergillus fumigatus*; K-feldspar; Illite; Potassium solubilization and release

1. INTRODUCTION

Microbial participation in mineral–water interactions is ubiquitous and often critical to the geochemical cycles of nutrients. It is no exception for potassium, a rock forming element and an essential soil nutrient that performs a multitude of important biological functions to maintain plant growth and health (Cassidy, 1970; Besford and Maw, 1975; Leeper and Uren, 1993). The amount of potassium required by plants is much greater than any other soil-supplied nutrient except nitrogen. However, plants cannot directly use mineralic potassium unless it is released by weathering or dissolved in soil water.

Numerous studies have documented the release of K during the degradation of silicate minerals by bacteria (Chen and Chen, 1960; Monib et al., 1984; Avakyan, 1984; Rozanova, 1986; Groudev, 1987; Malinovskaya, 1988, 1990; Mel'nikova et al., 1990; Friedrich et al., 1991; Welch et al., 1999). For instance, Chen and Chen (1960) observed 25–87% in-

* Corresponding author.
E-mail address: hteng@gwu.edu (H.H. Teng).
[1] Current address: Department of Bio-Engineering, Henan Institute of Science and Technology, Xinxiang, 453003, People's Republic of China.
[2] Current address: School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei, 230009, People's Republic of China.

0016-7037/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.gca.2007.10.005

creases of potassium concentrations in growth media when certain bacteria were cultured with K-bearing minerals. Monib et al. (1984) and Groudev (1987) further confirmed that bacteria indeed can solubilize K and Si from silicate minerals such as orthoclase and mica. Another study by Lian (1998) reported an 8% and 16% increase of aqueous K when feldspar and illite, respectively, were cultured with a soil bacterium. It was postulated that the reactions responsible for microbially promoted potassium solubilization may involve acidolysis, enzymolysis, capsule absorption, and complexation by extracellular polysaccharides (Avakyan, 1984; Rozanova, 1986; Malinovskaya, 1988, 1990; Mel'nikova et al., 1990; Friedrich et al., 1991; Welch et al., 1999). For example, after studying K release from feldspar and illite by silicate bacteria, Lian (1998) and Lian et al. (2002) proposed a staged model suggesting first the formation of bacterium-mineral complexes upheld by extra-cellular polysaccharides, followed by mineral dissolution and potassium solubilization in the microenvironments within the complexes where lower pH and the presence of organic ligands promote interfacial reactions. The formation of bacterium-mineral complexes may also facilitate the contact between microbial cells and mineral crystals to increase reaction surface area and time. The authors further suggested that, for phyllosilicate such as illite, smaller organic ligands may force into the interlayer spacing to drive out the potassium.

The occurrence of bacterially promoted potassium release suggests that a similar effect should also come about in fungus-mineral interactions (Blum et al., 2002; Weiner and Dove, 2003). Although the geo-microbiological potential of fungi has not been fully appreciated (Sterflinger, 2000), a number of studies have already documented the active roles of fungi in the weathering of minerals such carbonates (Staley et al., 1981; Verrecchia, 1990; Wollenzien et al., 1995; Verrecchia and Dumont, 1996), phosphates (Kucey, 1983, 1988; Cunningharn and Kuiack, 1992; Nahas, 1996; Whitdaw, 1999; Reyes, 1999; Reyes et al., 1999; Narsian and Patel, 2000; Ademola and Geoffrey, 2005), and silicates (Hallbauer and Jahns, 1977; Mehta et al., 1979; Rossi, 1979; Barker et al., 1997; Banfield et al., 1999). Accelerated potassium release from aluminosilicate minerals by fungi was observed as well (Wallander and Tonie, 1999; Yuan et al., 2000; Glowa et al., 2003; Yuan et al., 2004). For example, Wallander and Tonie (1999) found that the availability of soluble potassium from biotite and feldspars was correlated to the biomass of fungus in a community composed of *Paxillus involutus*, *Suillus variegates*, and the seedlings of *Pinus sylvestris*. Yuan et al. (2000) reported that *ectomycorrhizas* could mobilize potassium from clay minerals and thus speed up the progress of potassium uptake by plants. Yuan et al. (2004) further studied the effect of four fungal strains, *Pisolithus* XC1, *Pisolithus* sp., *P. microcarpus*, and *Cenococcum geophilum* SIV collected from the roots of eucalyptus, on the degradation of phlogopite and vermiculite. Their results showed that all four strains were able to weather the mineral phases and release elemental potassium. Glowa et al. (2003) compared the ability of fungus *Piloderma* in extracting potassium from biotite, microcline, and chlorite and found this species was able to acquire potassium from all three minerals with biotite being the more biodegradable.

In this study, we report potassium release from a mineral assembly of K-feldspar and illite by thermophilic fungus *Aspergillus fumigatus*. This strain was chosen because, among several others cultivated and isolated from a compost sample, it grew fast and showed a particularly strong physical affinity toward K-bearing minerals (see experimental section for details). Unlike true thermophiles whose growth conditions need to be above 20 °C, *A. fumigatus* can grow at temperatures as low as 12 °C (Hudson, 1986). Commonly, this species is found in composts of various types, but it is also one of the most abundant fungi colonizing rock surfaces (See review by Sterflinger, 2000 and reference therein) and a frequent, although not dominant, component of fungal flora in soils (Tansey and Jack, 1976). It is shown that *A. fumigatus* is able to degrade almost all components of organic waste including sugars, fatty acids, proteins, cellulose, pectin, and xylan (Fogarty, 1994). Although little literature data are available (to the best of our knowledge) to document its importance in geochemical processes, the wide occurrence, broad temperature tolerance, and strong ability of biodegrading suggest a possible potent role of this species in mineral weathering. In the present study, we investigate its potential to solubilize potassium from K-rich minerals. The goal is to determine if this species is able to accelerate potassium release and the possible reaction pathways responsible for the biological impact if a positive effect is observed.

2. MATERIALS AND METHODS

2.1. Isolation, selection, and identification of fungal species

Ten grams of compost sample collected from a waste disposal center were first immersed in 100 mL sterile saline solution (0.8% NaCl). An aliquot of 0.1 mL of the suspension was then pipetted into a Petri dish containing a modified potato dextrose agar (PDA). The Petri dish was subsequently incubated at 45 °C for 48–72 h. The PDA (natural pH, autoclaved at 121 °C for 20 min) was made by dissolving 200 g potato extract, 20 g glucose, 5 g peptone, and 18 g agar in a liter distilled deionized water (DDW). Upon completing the incubation, a number of large colonies were separated and cultured individually with 5 g mineral powder in 100 mL liquid medium (PDA less agar) on a shaker at 45 °C and 120 r/min for 7 days. Visual inspection afterward revealed that the strain TH003 grew the most rapidly. In addition, no bare mineral grains remained at the bottom of the flask containing TH003, indicating the occurrence of a strong physical interaction between the mycelia and mineral particles. For this reason, TH003 was selected as our experimental strain for this study.

The working strain was identified through its colony, cell morphology, and genomic sequence. The colonies of TH003, showing a bulging center and fluffy surface and emitting a moldy odor, reached 60 to 66 mm in diameter within 5 days of growth. The color of the colonies was olivaceous with a gray tint, and the back of the plate was yellowish-brown. Microscopic observations (Fig. 1) revealed the presence of septas in the hyphae. The conidiophore is relatively long and appears to have a vesicle at the top. The ves-

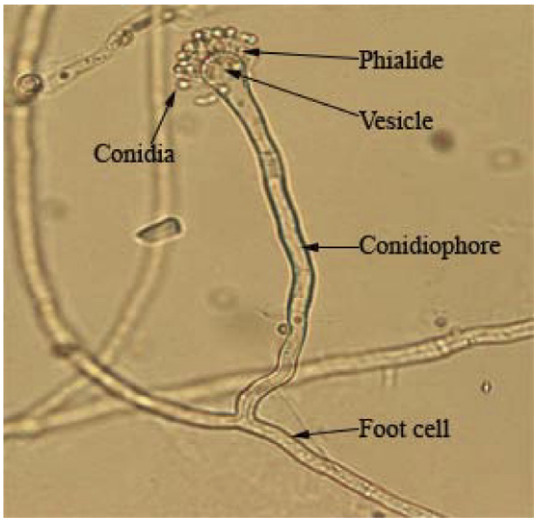

Fig. 1. A microphotograph of the conidial fructification of the strain TH003 (400×).

icles, 20–30 μm in diameter, have smooth walls and monostratal conidial fructifications. The conidium looks crude, having a spheroidal or globular shape with an approximate dimension of 2.5–3 μm across. These morphological characteristics and the color scheme of the colonies seem to fit the description of species *A. fumigatus* (Qi, 1997).

The genomic DNA of TH003 was extracted using a commercially available extraction kit (Genomic DNA Purification Kit 713, Shenergy Biocolor Biotech Ltd., Shanghai) following previously published methods (Bruns et al., 1991; Donnell, 1992). The PCR (polymerase chain reaction) amplification of a segment of the 18S-28S rDNA was carried out using a forward primer (5′-GGAAGTAAAA GTCGTAACAAGG-3′) and a reverse primer (5′-TCCTCCGCTTATTGATATGC-3′) by a thermal cycler (MJ Research PTC-100). The PCR products were purified and sequenced commercially. The resultant sequence was submitted (Accession No. DQ459328) to the GenBank database (www.ncbi.nlm.nih.gov) and analyzed following the method described by Altschul et al. (1997). The results showed that the sequence of TH003 matched 100% to the strain SRRC of *A. fumigatus*.

2.2. Mineral phases

The mineral assembly used in this study was a K-rich shale (Table 1) collected from a Late Cambrian formation in Hunan, China. The rock sample was crushed and sieved to collect grains <400 mesh (approximately 37 μm). The prepared rock powder had a surface area of approximately 0.86 m^2/g measured by a laser particle analyzer (Jingxin JL-1155).

2.3. Experimental settings for microbe–mineral interactions

Three setups were constructed to explore different aspects of the fungus–mineral interactions. One allowed the mineral phases to have direct contact with the fungal cells (contact experiments) and the other to interact only with the fungi's metabolic product (separation experiments). The third one was aimed at determining the maximum amount of mineral grains that could be aggregated by the mycelia.

All experiments were carried out in 250 mL triangular flasks containing 100 mL liquid medium (PDA less agar). Contact experiments were conducted by mixing 5 g of minerals into the medium, sterilizing at 121 °C for 20 min, followed by inoculation of 10 mL of spore suspension (1.0×10^6/mL). Separation experiments followed the same procedure except that the mineral powder was enclosed in a membrane bag (0.22 μm pore size). The flasks containing the final mixtures were incubated on a shaker at 45 °C and 120 r/min for 30 days. A total of 45 flasks were prepared for each type of experiment. The third set of experiment was similar to the first except that different amounts of minerals (1 through 12 g) were used. The mixtures were incubated at the same conditions as in the other two settings until no visible changes in the flasks were observed (about two weeks).

Three control experiments were carried out parallel to the experimental runs. Control I was identical to contact experiments but with autoclaved spores. Controls II and III used DDW instead of the liquid medium, with and without autoclaved spores, respectively. To be consistent with the conditions of the experimental runs, the pH values of the control experiments were adjusted continuously to match the measurements of the contact experiments obtained at the times of each sampling.

2.4. Experimental sample analysis

Mineral grains collected at the end of experiments were air-dried and analyzed by EPMA (electron probe microanalysis, Shimadu-1600, 25 kV, 0.25 nA), EDS (energy disperse spectroscopy, Genesis EDAX, 25 kV, 0.45 nA, and 0.1 μm beam spot diameter), and TEM (transmission electron microscopy, JEOL JEM-2000FX II, 180 KV,

Table 1
Mineralogical and chemical compositions of the shale

Mineralogical composition (%) by XRD[a]									
K-feldspar		Plagioclase		Quartz		Montmorillonite		Illite	
65.50		23.90		6.02		2.38		2.18	
Chemical composition (%) by XRF[b]									
SiO_2	Al_2O_3	K_2O	Na_2O	Fe_2O_3	CaO	TiO_2	MgO	MnO	H_2O
67.80	18.76	9.00	3.28	0.17	0.16	0.04	0.03	0.02	0.25

[a] X-ray diffractometry, Rigaku D/Max-2200, Cu$K\alpha$ at 40 kV and 30 mA, and 3°/min scan rate.
[b] X-ray fluorescence spectrometry, Panalytical Axios PW4400.

0.6 μA). Mycelia grown 30 days in the presence of minerals were examined by ContactMode™ AFM (atomic force microscope, DI NanoScope IIIa, J scanner, Si_3N_4 probe).

Potassium contents in the growth media were analyzed by AAS (atom absorption spectrometry, Perkin-Elmer PE-5100). Three flasks were taken out from the incubator every other day and aliquots of 10 g of the culture-mineral mixture were sampled from each one of them. The samples were centrifuged at 8000 rpm for 10 min to collect the supernatant. The remaining solid mycelium–mineral residues were then transferred into a triangular flask containing 100 mL of 1 M ammonium acetate solution. The flask was shaken at 120 r/min for 30 min at 25 °C, followed by centrifugation at 8000 rpm for 10 min to collect the second supernatant. The first and second supernatants were mixed and filtered by 0.45 μm filter paper before AAS analysis. The average values of the three samples were reported as the final K concentrations.

3. EXPERIMENTAL RESULTS

3.1. Release of K during fungus–rock interactions

The amount of potassium released in the presence of *A. fumigatus* at any given time in the 30-day incubation period was one to two orders of magnitude higher than that in any of the control experiments (Table 2 and Fig. 2). A comparison of contact and separation experiments indicated that direct interaction between mycelia and minerals further enhanced potassium solubilization by a factor of 3 to 4 (Fig. 2). In both experimental settings, the potassium concentrations increased steadily with time in the first 16–20 days, and then flattened out afterwards. The overall increase in the amount of accumulated potassium from day 2 to day 30 was about 400–500%. Meanwhile, the pH of the culture trended in an opposite direction, decreasing from a physiological value of ~6.5 to an acidic condition of close to 3. In contrast to the parabolic-like behavior of K release, the pH change was sigmoidal irrespective of the experimental setup. That is, pH moved slowly in the beginning and the ending weeks but dropped rapidly between day 10 and day 20. It appeared that the fastest decrease in pH, from ~6 to ~ 4, took place between day 10 and 16.

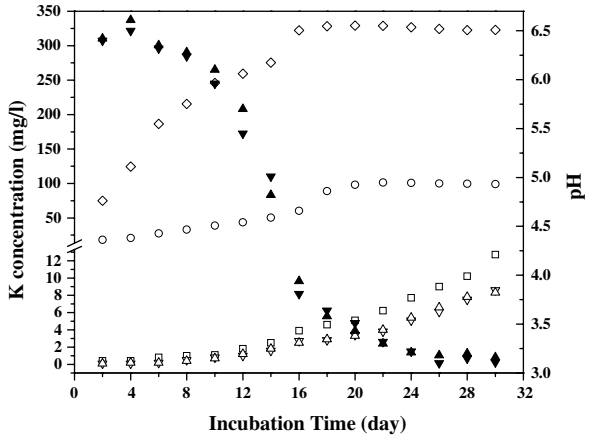

Fig. 2. Experimental measurements of K concentration (♦: contact experiments; ○ separation experiments; □: control 1; △: control 2; ▽: control 3) and pH (▲: contact experiments; ▼: separation experiments) during a 30-day period of incubation.

While no significant changes occurred for the total amount of potassium in the presence *A. fumigatus* in the last 10 days of incubation, the K concentration continued to increase in all three control experiments (Fig. 2). More potassium seemed to be released in control experiments I (liquid medium with autoclaved fungi) relative to II (DDW with autoclaved fungi) and III (DDW only). The difference between control II and III was virtually unnoticeable.

Table 2
pH and potassium concentrations in experimental and control runs

Incubation (day)	Direct experiments		Indirect experiments		Control experiments			
	pH	K (ppm)	pH	K (ppm)	pH	K (ppm)		
						I	II	III
2	6.42	75.0	6.40	18.4	6.42	0.4	0.1	0.1
4	6.61	124.6	6.50	21.0	6.61	0.4	0.2	0.1
6	6.35	186.6	6.32	27.4	6.35	0.8	0.2	0.2
8	6.28	215.4	6.24	33.2	6.28	1.0	0.4	0.5
10	6.10	245.8	5.96	39.0	6.10	1.1	0.7	0.8
12	5.70	259.2	5.45	43.6	5.70	1.8	1.2	1.0
14	4.82	275.4	5.01	50.4	4.82	2.5	1.8	1.6
16	3.94	322.2	3.81	60.4	3.94	3.9	2.5	2.7
18	3.58	328.1	3.64	89.0	3.58	4.6	2.9	2.8
20	3.43	329.0	3.51	98.2	3.43	5.1	3.3	3.4
22	3.30	328.8	3.32	101.4	3.30	6.2	4.0	3.8
24	3.21	326.6	3.22	100.8	3.21	7.7	5.4	5.1
26	3.18	324.4	3.10	100.0	3.18	9.0	6.6	6.1
28	3.20	322.6	3.15	99.6	3.20	10.2	7.8	7.5
30	3.16	323.0	3.11	99.0	3.16	12.7	8.3	8.6

3.2. Formation of mycelium–mineral aggregates

Individual mycelia with a light yellowish color and a spherical shape reached a size of approximately 0.2–1.0 cm in diameter in two weeks. It looked as if the entangled hyphae served as a porous and adhesive matrix, into which the rock grains were embedded to form mycelium–mineral aggregates. It appeared that the maximum amount of minerals that could be taken in by the mycelia was approximately 10 g as any additional grains were detected as bare particles on the bottom of the flasks by visual inspection.

Microscopic analysis by EPMA further revealed the adhesion of minerals on cell surfaces and the tendency of increasing attachment with longer incubation time (Fig. 3). After 7 days of growth, the surfaces of hyphae were completed covered by mineral grains (Fig. 3B). At the end of the 30-day incubation, the spaces between hyphae were almost completely filled with minerals (Fig. 3C). Meanwhile, any remaining hyphae looked thin and undernourished, presumably due to the dearth of nutrients and the deterioration of environmental conditions inside the flasks. EDS analysis showed that, in comparison to the pristine minerals (Fig. 4C), surfaces of the aggregated mineral particles contained additional elements in P, S, and Cl (Fig. 4A and B), indicating the presence of organic substances. Further examination of the minerals by TEM (Fig. 5) showed the presence of surface coatings as well as the smoothening of sharp corners on the grains, signifying the accumulation of biopolymers and the occurrence of dissolution on the minerals.

A close-up view (Fig. 6) of the mycelia unveiled that the surfaces of the hyphae were rough with submicron-sized spherical or ellipsoidal knobs. EDS analysis of the protrusions showed the presence of mineral components Si and

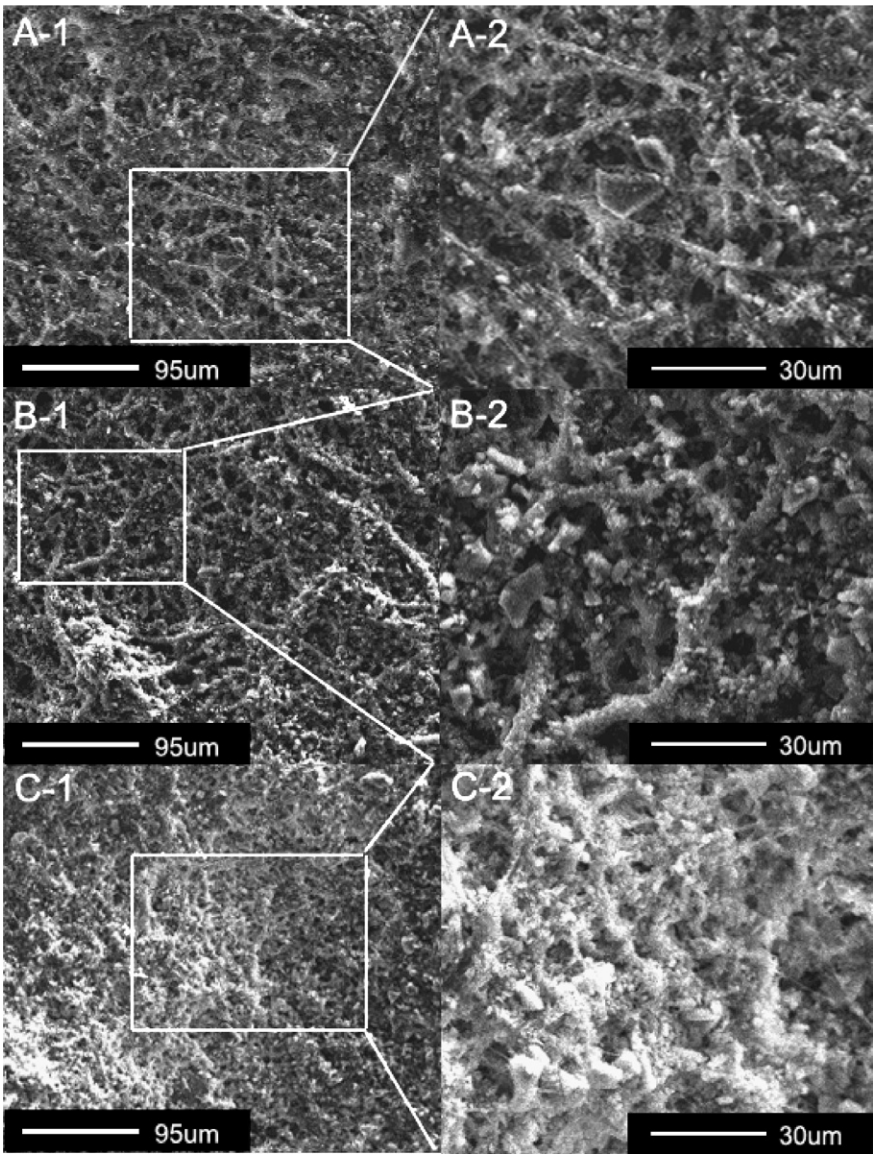

Fig. 3. EPMA micrographs showing the adhesion of mineral particles on the mycelia of TH003 as well as the changes in the size of hyphae after 2 (A), 7 (B), and (30) days of incubation.

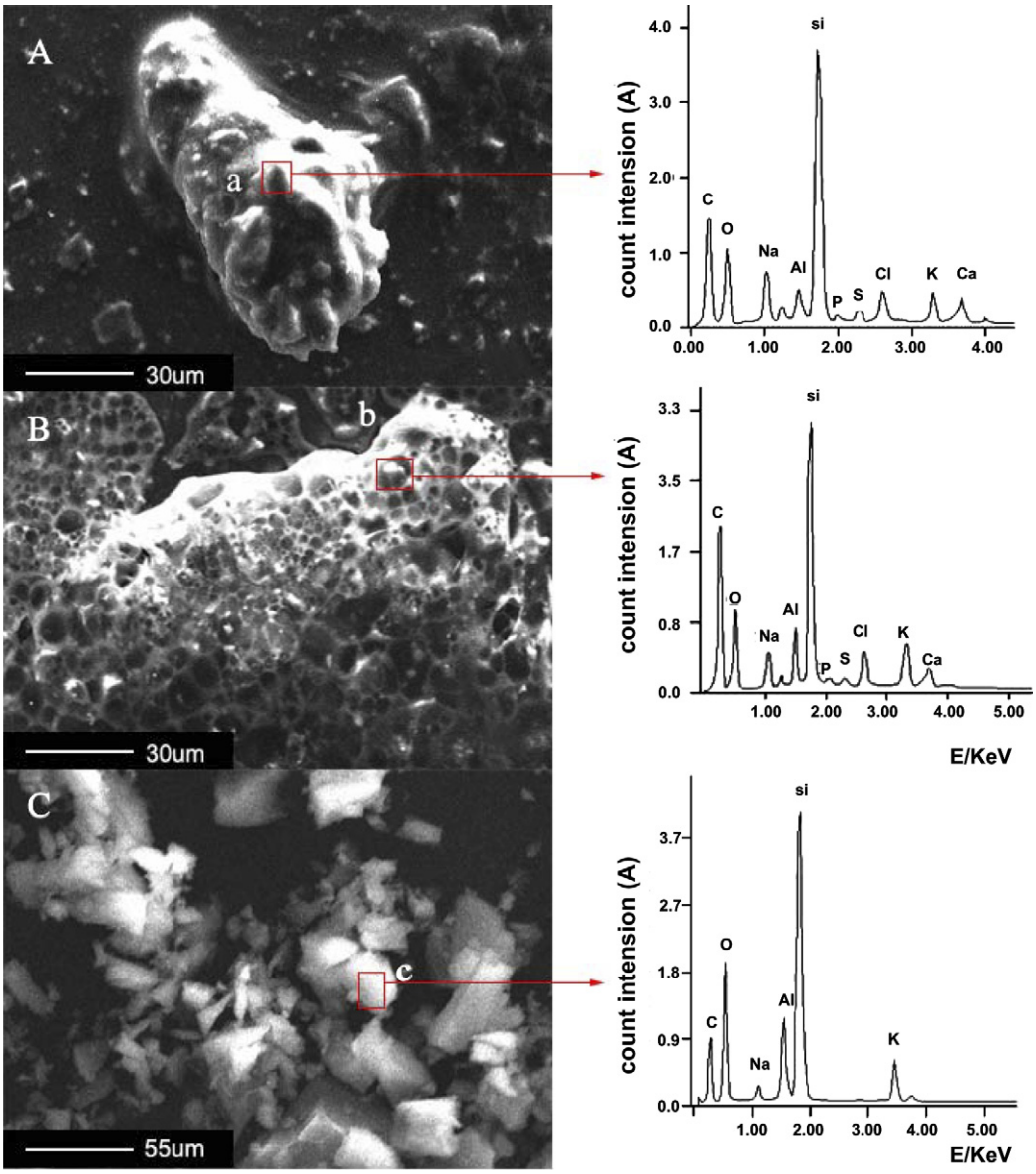

Fig. 4. EPMA micrographs and EDS analyses of the mineral grains incubated with TH003 for 30 days (A and B) and the pristine rock sample (C). Notice that the reacted minerals are covered by a layer of porous substance presumably derived from the secretion and decomposition of the organisms.

Al, but the spectra differed from those of the aggregated mineral grains (Fig. 4) in that the intensities of these elements were much weaker, suggesting that these bulging features may be mineral fragments enclosed in the cells. Contact Mode™ AFM scanning (Fig. 7) was not able to move the protrusions, further indicating that it is unlikely they were attached to the exterior surfaces of the cell walls.

4. DISCUSSION

The measurements of K concentration (Table 2) unambiguously indicate that the presence of *A. fumigatus* strongly enhances the release of potassium from the mineral phases. However, entangled with the increase of K concentration is a consistent decrease of pH in the culture media throughout the experiments. While it is reasonable to assume that the pH decrease results largely from the accumulation of secreted organic acids by the organism, it is not immediately clear how important the falling pH is relative to other microbe–mineral interactions for potassium solubilization. It is therefore necessary to make an effort to separate the pH effect from the microbial impact to the experimental observations.

4.1. Dependence of K solubilization kinetics upon pH and time

Given the mineralogical makeup of the shale used in this study (Table 1), it is conceivable that the reactions responsible for the release of potassium involve primarily K-feld-

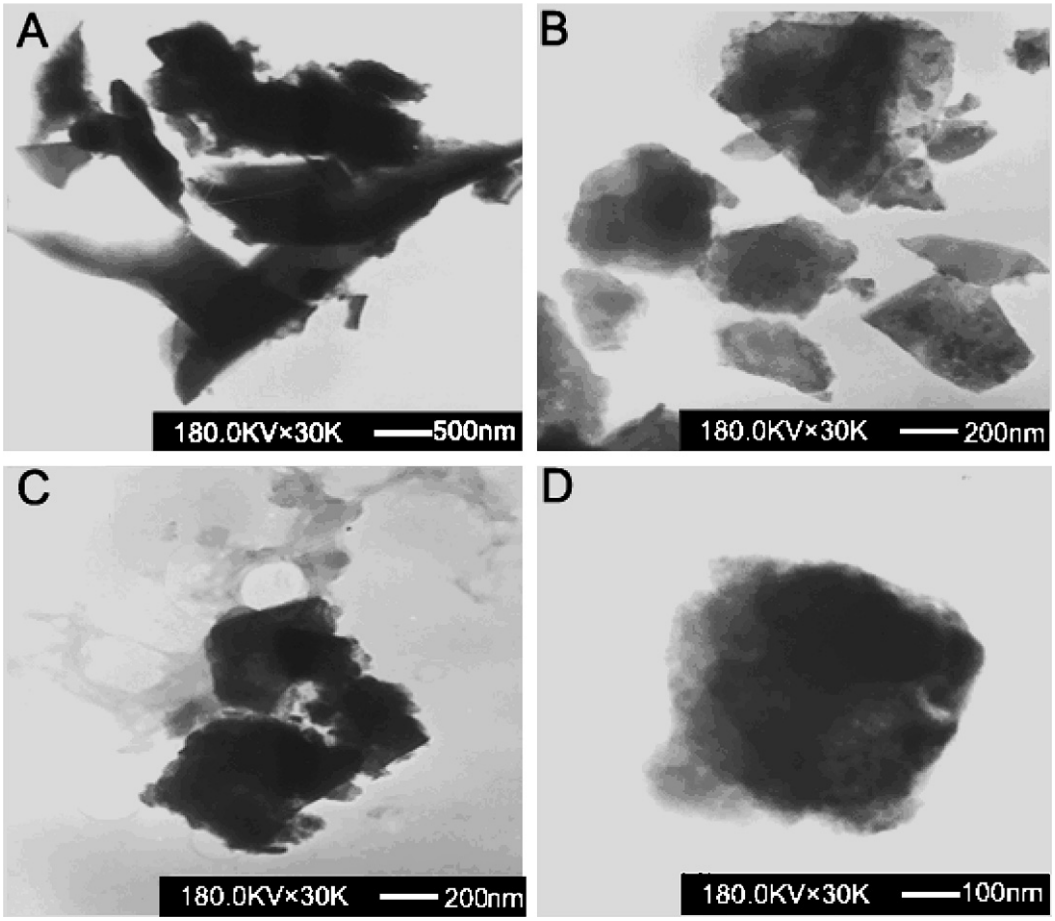

Fig. 5. TEM images of the fresh mineral grains (A) and the samples incubated with TH003 for 2 (B), 7 (C), and 30 (D) days. Notice the surface coatings and the rounded corners on the reacted particles.

spar and illite. Incidentally, the following discussion will be focused on the dissolution of these two minerals.

The overall dissolution of potassium feldspar in acidic environments may be described by simplified reactions such as

$$2KAlSi_3O_8 + 2H^+ + 9H_2O = Al_2Si_2O_5(OH)_4 + 4H_4SiO_4(aq) + 2K^+ \quad (1)$$

or

$$KAlSi_3O_8 + H^+ + 7H_2O = Al(OH)_3 + 3H_4SiO_4(aq) + K^+, \quad (2)$$

depending upon the solution chemistry. Without defining elemental reactions, Eqs. (1) and (2) offer no indication of the dissolution mechanism. For example, no information can be derived from these expressions in terms of where on the mineral surfaces the H^+ attacks and how the Si(Al)–O framework of feldspar is disrupted throughout the reaction. However, voluminous observations consistently indicate that the release of potassium is one of the first steps (if not the first) of the overall dissolution process. For instance, surface titration data suggest that alkali metals in feldspars are released from the crystal lattice through an ion exchange process (e.g., H^+ or H_3O^+ for Na^+ or K^+) during dissolution (see review by Blum and Stillings, 1995 and references therein), and the cation exchange mechanism appears to be reversible and to affect the outermost 2 or 3 unit cells under most experimental conditions. Such understanding can be readily rationalized in light of the crystal structure of feldspars which may be viewed as pairs of zigzag chains of Si(Al)–O tetrahedra that run parallel to the *a* axis. Looking into the *a*-axis (i.e. on the (100) face), one sees that each individual pair constitutes a four-membered ring in which two adjacent corners are occupied by tetrahedra pointing upward and the other two downward. These rings are arranged in such a way that an elongated voids form between four of them and two cations such as K^+ and Na^+ are caged in the open space as interstitial ions. In the presence of aqueous cations such as H^+, rapid ion exchange can take place to result in the release of the interstitial metals. Available experimental data suggest that the cation exchange is relatively rapid (within minutes) and is thought to precede the disruption of the Si(Al)–O framework. However, how the cations below the uppermost surface layers are transported through the feldspar lattice is not well understood.

Illite is a typical 2:1 phyllosilicate mineral with metal ions such as K^+ (along with Na^+ and Ca^{2+}) functioning

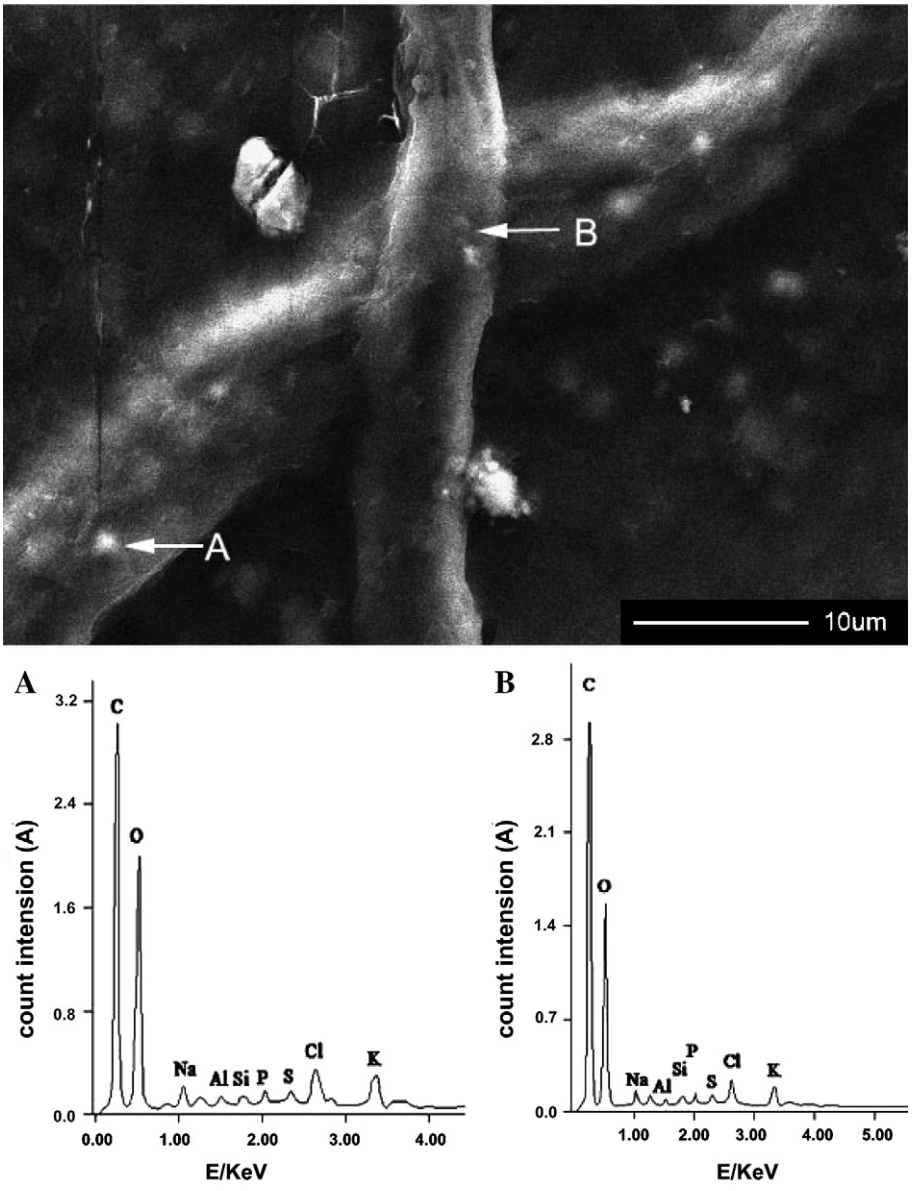

Fig. 6. An EPMA micrographs of individual hyphae of TH003 (top) and the EDS analyses of two knobs on the cell surface (A and B).

as charge balancing interlayer cations, analogous to the alkali metals in feldspars. Accordingly and similar to feldspars, the release of K is not regarded as a rate limiting step for illite dissolution. Simplified expressions such as

$$KAl_2(Si_3Al)O_{10}(OH)_2 + H^+ + 9H_2O = 3Al(OH)_3 + 3H_4SiO_4(aq) + K^+ \quad (3)$$

may be considered a representation for illite dissolution under acidic conditions. Although not carrying as high a capacity as other clay minerals such as montmorillonite and vermiculite do (Garrels and Christ, 1965), ion exchange is an important reaction in the overall dissolution process of illite (see review by Nagy, 1995 and references therein). In fact, ion exchange associated with illite dissolution has long been suggested as a mechanism for potassium release in soils (Feigenbaum and Shainberg, 1975).

Assuming metal cation-proton exchange is the primary mechanism responsible for potassium release from K-feldspar and illite at acidic conditions without biological participation, we may use the following rate expression (to a first degree of approximation) to describe the potassium solubilization:

$$d[K^+]/dt = k[H^+]^n \quad (4)$$

where $[K^+]$ and $[H^+]$ are the concentrations of potassium and proton, t is time, k is rate constant, and n is the reaction order with respect to proton. The logarithmic form of Eq. (4) gives

$$\log(d[K^+]/dt) = -n\mathrm{pH} + \log k. \quad (5)$$

Eq. (5) indicates that the releasing rate of K should be inversely related to pH if no parallel reactions other than proton exchange are in play.

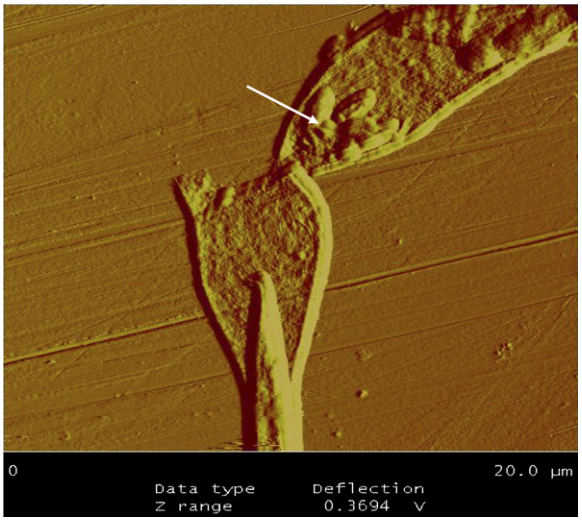

Fig. 7. A deflection AFM image of individual hyphae of TH003 showing the protrusions (pointed to by the arrow) on the cell surfaces.

Plots of $\log(d[K^+]/dt)$ vs. pH for the control experiments (Fig. 8, top) convincingly show the anticipated inverse relationship. It appears that the average rates (i.e., mean rates at the times of each sampling, $d[K^+] = [K^+]_i$ ppm and $dt = i$ days) are less scattered than the instantaneous rates (i.e., rates between successive samplings, $d[K^+] = [K^+]_{i+2} - [K^+]_i$ ppm and $dt = 2$ days). The same relationship manifests also

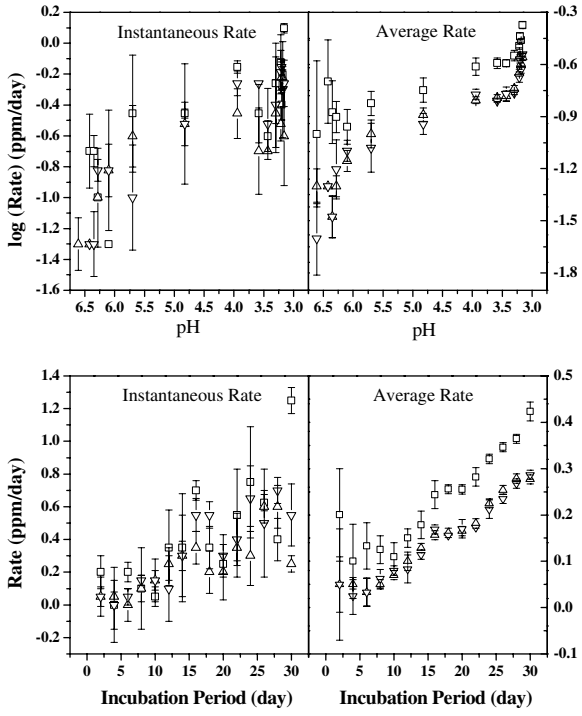

Fig. 8. Dependence of potassium releasing rate upon pH and time in control experiments (□: control I; △: control II; ▽: control III). Error bars indicate the standard deviations (one σ) of the calculated rates.

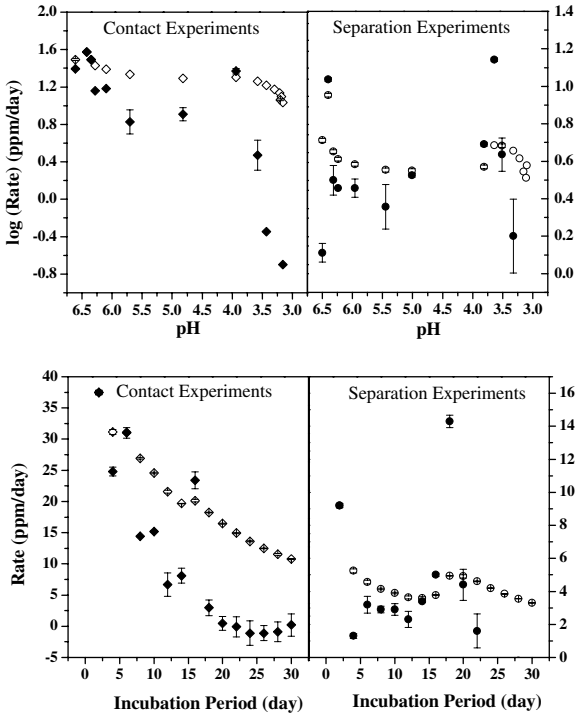

Fig. 9. Dependence of potassium releasing rate upon pH and time in contact and separation experiments (solid symbols: instantaneous rate; open symbols: average rate). Error bars indicate the standard deviations (one σ) of the calculated rates.

on the plot of $d[K^+]/dt$ vs. the length of incubation (Fig. 8, bottom) in view of the decreasing pH in the course of experiments. In a sharp contrast, the potassium releasing rate in the contact experiment (Fig. 9, top left) reveals a positive dependence upon pH, although the instantaneous rate seems to decrease faster with falling pH relative to the average rate. In the case of separation experiments, however, no dominant trend is visible, indicating that the rate has a much weaker dependence upon pH if at all (Fig. 9, top right). The similar differences between control, contact, and separation experiments are well exhibited in the relationship between $d[K^+]/dt$ and the length of incubation (Figs. 8 and 9, bottom). For example, the rates increase with time in all three control experiments and decrease in the course of the contact experiments, but do not correlate with time in any easily identifiable fashion in the separation experiments.

4.2. Importance and implications of the fungus–mineral physical interactions

A compilation of literature data on the kinetics of K-feldspar dissolution in inorganic acids shows that the rate is linearly correlated with pH with a negative slope of 0.5 (Blum and Stillings, 1995). A review of illite dissolution studies indicates that a similar dependence is also in place with an average slope of −0.2 (sees Nagy, 1995, and reference therein). Along these lines, the negative dependence of potassium releasing rate upon pH observed in the control

experiments of the present study is well consistent with previous understanding of feldspar and illite dissolution.

Both the positive correlation and the lack of dependence between potassium solubilization kinetics and pH conditions seen in the presence *A. fumigatus* (Fig. 9, top) however warrant further discussion. We don't believe this suggests that neutral pH favors the release of potassium by biochemical reactions or that protons are not involved in the dissolution process. Rather, we suggest that processes other than direct attacks by H^+ on mineral surface sites begin to make bigger contributions once the microbe is involved. The experimental results obtained in this study alone may not be enough to help pinpoint these processes. Nevertheless, it is clear that multiple interactions concerning different aspects of microbial activities and metabolites are taking effect.

There are at least three possible pathways for fungi to enhance mineral dissolution. (1) Acid leaching. Organic acids secreted during metabolism or associated with the decomposition of metabolic wastes decrease the pH of the ambient environments. The higher acidity then leads to weakening chemical bonds in minerals and thus promotes mineral dissolution (Banfield et al., 1999; Harley and Gilkes, 2000). (2) Chemical degradation (Burford et al., 2003). Organic ligands in metabolites (Callot et al., 1987; Lee and Parsons, 1999; Adamo and Violante, 2000) attack mineral surfaces directly or form complexes with aqueous species to shift equilibrium (Barker et al., 1997). For *A. fumigatus*, these two reactions can proceed in association with a number of organic compounds such as acetic, fumaric, glyoxylic, and itaconic acids that are reportedly associated with the fungal metabolism (see review by Sterflinger, 2000 and references therein). (3) Mechanical fragmentation of rock particles (Jongmans et al., 1997). Mycelia find their way into the interior of minerals through the extension of hyphae (hence unique to fungal organisms) to acquire nutrition. The physical forces can fracture mineral grains to decrease particle sizes and generate fresh and more reactive surfaces. In the contact experiments, all three mechanisms can be operative. For separation experiments, however, only routes (1) and (2) are in action.

In the present study, it does not seem that metabolically induced pH decrease alone (i.e. route 1) makes significant contributions to potassium solubilization, given that the K contents in the absence of fungal activities are at least an order of magnitude lower than those in the presence of active fungi even though the pH conditions are maintained similar (Table 2, Fig. 2). This implies that, for separation experiments, smaller or soluble components of the extracellular biopolymers (i.e. those that are not limited by the membrane) are largely responsible for the K release. It is thus reasonable to suggest that the K solubilization therein is fulfilled primarily through route 2, ligand promoted mineral dissolution.

It is reported that the upper and lower pH limit for the growth of *A. fumigatus* is 3 and 8, respectively (Panasenko, 1976; Hung and Trappe, 1983). Given that the optimal pH range for majority of fungi is usually between 5 and 6 (Dix and Webster, 1995), we can assume that our working strain grows at a fast pace in the beginning of the experiments when the pH is around 6, but gradually loses the momentum of multiplication with decreasing pH, depleting nutrients, and accumulating toxic metabolites. The growth eventually comes to (or near) an end when the pH reaches the lower limit of 3. The fact that the acidity levels out near pH 3 in the experiments (Fig. 2) indicates in all likelihood that the input of organic acid to the system has come to a halt, a suggestive sign to the termination of the cells' metabolism.

If this is true, the lack of rate-pH correlation in the separation experiments (Fig. 9, top right) may suggest that potassium solubilization is not necessarily related to the well-being and the growth rate of fungi. This further indicates that the bio-molecules involved in chemical degradation do not have to come from metabolism. We hypothesize that, while a falling pH retards biological activity to lead to a reduction in cells' secretion, decomposition of extra-cellular biopolymers and the autolysis of the mycelia (Fig. 3C) regenerate organic acids to replenish the pool of ligands, sustaining the chemical degradation reactions and leaving the K releasing rate largely independent of the decrease in pH and bio-activity. The lack of active biological involvement thus implies that chemical degradation is primarily a "biologically induced" mineral dissolution process, parallel to the "biologically induced" mineralization defined by Lowenstam (1981) and Mann (2001).

In the meantime, the K concentrations in the contact experiments are three to four times higher than those in the separation experiments at any given pH (Table 2 and Fig. 2), and the solubilization rate exhibits a definitive positive correlation with pH. These indicate that the microbe–mineral physical interactions may have invoked additional processes to release K. The nature of the new processes can be both physical and chemical. Physically, the organism may disintegrate mineral grains (i.e. route 3) and ingest the smaller particles, as suggested by the EPMA (Fig. 6) and AFM observations (Fig. 7). It is also possible that physical motion of the cells enhances transport processes, such as diffusion, to move detached mineral components away from surfaces into solutions. Chemically, the microbe–mineral entanglement, as exemplified by the mycelium–mineral aggregates (Fig. 3), allows immobile bio-polymers, either membrane-bound proteins and lipids or the organic functional groups in the insoluble extra-cellular substances around the cells, to establish continual reactions with mineral surfaces. It is plausible that sustained reactions extract more K from the mineral phases. If these immobile bio-polymers contain macromolecules that carry enzymatic ability to selectively catalyze the chemical reactions that release K, the solubilization process essentially becomes a "biologically controlled" dissolution (see Mann, 2001).

The positive dependence of K solubilization rate upon pH in the contact experiments implies that the new processes may have a strong involvement of the bio-activity of the fungi. When the microbes slow down their metabolism with falling pH, the cells' bodily action and the supply to the immobile biopolymers begin to diminish. Such an occurrence would exert an adverse effect on both the physical and the chemical aspect of the established mycelium–mineral interactions. Consequently, on the assumption that

the release of K is facilitated by living cells, we should see a decreasing potassium releasing rate with descending pH (opposite to what takes place in inorganic environments), as demonstrated by the experimental measurements (Figs. 8 and 9).

5. CONCLUSIONS

Interactions of *A. fumigatus* with K-bearing minerals release potassium through three different reaction pathways. The first involves the smaller and soluble components of the secretion, the second concerns the insoluble part of the macromolecules and membrane-bound biopolymers, and the third may be related to the physical activities of the cells including direct ingestion of mineral particles. While the interactions of free aqueous bio-molecules with minerals do not seem to require active involvement of fungal activities, the routes that entail the participation of immobile organic matrices and cell–mineral physical contact appear to have a strong dependence upon the vitality of the microbes and therefore are correlated to the optimal living conditions of the organisms.

The significance of microbe–mineral physical contact observed in the present study may have important implications. It suggests that microbially mediated mineral weathering cannot be approximated by simple dissolution reactions with the participation of organic or biochemical molecules. While the pH decrease resulting from metabolic activities and the complexation reactions involving organic ligands derived from metabolites and biomass decomposition are active participants in microbe–mineral interactions, the immobile biopolymers (either attached to the cell surfaces or insoluble secretion), combined with cell–mineral mechanical interactions, may be a more forceful agent, leading to an effective destruction and transformation of minerals in biogeochemical processes.

ACKNOWLEDGMENTS

This research was financially supported by the Ministry of Science and Technology of China (Grant No. 2006CB403200) and National Natural Science Foundation of China (Grant No. 40773069) to B.L., and the office of Basic Energy Science, US DOE (Grant No. DE-FG02-02ER15366) to H.H.T. The authors extend their gratitude to Dr. Hailiang Dong of Miami University for his helpful comments and Qiaona Hu of University of Michigan for her kind help in AFM imaging. We thank two anonymous reviewers for their insightful comments and suggestions.

REFERENCES

Adamo P. and Violante P. (2000) Weathering of rocks and neogenesis of minerals associated with lichen activity. *Appl. Clay Sci.* **16**, 229–256.

Ademola O. A. and Geoffrey M. G. (2005) Fungal degradation of calcium-, lead-, and silicon-bearing minerals. *BioMetals* **18**, 269–281.

Altschul S. F., Madden T. L., Schäffer A. A., Zhang J., Zhang Z., Miller W. and Lipman D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402.

Avakyan Z. A. (1984) Silicon compounds in solution bacteria quartz degradation. *Mikrobiologiya* **54**, 301–307.

Banfield J. F., Barker W. W., Welch S. A. and Taunton A. (1999) Biological impact on dissolution: application of the lichen model to understanding mineral weathering in the rhizosphere. *Proc. Natl. Acad. Sci. USA* **96**, 3404–3411.

Barker W. W., Welch S. A. and Banfield J. F. (1997) Biogeochemical weathering of silicate minerals. *Rev. Mineral* **35**, 391–420.

Besford R. T. and Maw G. A. (1975) Effect of potassium nutrition on tomato plant growth and fruit development. *Plant Soil* **42**, 395–412.

Blum A. E. and Stillings L. L. (1995) Feldspar dissolution kinetics. *Rev. Mineral* **31**, 291–351.

Blum J. D., Klaue A., Nezat C. A., Driscoll C. T., Johnson C. E., Siccama T. G., Eagar C., Fahey T. J. and Likens G. E. (2002) Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. *Nature* **417**, 729–731.

Bruns T. D., White T. J. and Taylor J. W. (1991) Fungal molecular systematics. *Annu. Rev. Ecol. Syst.* **22**, 525–564.

Burford E. P., Fomina M. and Gadd G. M. (2003) Fungal involvement in bioweathering and biotransformation of rocks and minerals. *Mineral. Mag.* **67**, 1127–1155.

Callot G., Maurette M., Pottier L. and Dubois A. (1987) Biogenic etching of microfractures in amorphous and crystalline silicates. *Nature* **328**, 147–149.

Cassidy N. G. (1970) The distribution of potassium in plants. *Plant Soil* **32**, 263–267.

Chen H. and Chen T. (1960) Characteristics of morphology and physiology and ability to weather mineral baring phosphorus and potassium of silicate bacteria. *Microorganism* **3**, 104–112.

Cunningharn J. E. and Kuiack C. (1992) Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. *Appl. Environ. Microbiol.* **58**, 1451–1458.

Dix N. J. and Webster J. (1995) *Fungal Ecology*. Cahpman & Hall, Cambridge, UK, p. 57.

Donnell K. (1992) Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Curr. Genet.* **22**, 213–220.

Fogarty W. M. (1994) Enzymes of the genus *Aspergillus*. In *Aspergillus* (ed. J. E. Smith). Plenum Press, New York, pp. 177–218.

Feigenbaum S. and Shainberg I. (1975) Dissolution of Illite—a possible mechanism of potassium release. *Soil Sci. Soc. Am. Proc.* **39**, 985–990.

Friedrich S., Platonova N. P. and Karavaiko G. I. (1991) Chemical and microbiological solubilization of silicates. *Acta Biotechnol.* **3**, 187–196.

Garrels R. M. and Christ D. L. (1965) *Solutions, Minerals, and Equilibria*. Harper & Row, New York (450).

Glowa K. R., Arocena J. M. and Massicotte H. B. (2003) Extraction of potassium and/or magnesium from selected soil minerals by Piloderma. *Geomicrobiol. J.* **20**, 99–112.

Groudev S. N. (1987) Use of heterotrophic microorganisms in mineral biotechnology. *Acta Biotechnol.* **7**, 299–306.

Hallbauer D. K. and Jahns H. M. (1977) Attach of lichens on quartzitic rock surfaces. *Lichenologist* **9**, 119–122.

Harley A. D. and Gilkes R. J. (2000) Factors influencing the release of plant nutrient elements from silicate rock powders: a geochemical overview. *Nutr. Cycl. Agroecosyst.* **56**, 11–36.

Hudson H. J. (1986) *Fungal Biology*. Edward Arnold, Baltimore, p. 160.

Hung L. L. and Trappe J. M. (1983) Growth variation between and within species of ectomycorrhizal fungi in response to pH in vivo. *Mycologia*, 234–241.

Jongmans A. G., Van Breemen N., Lundström U., Van Hees P. A. W., Finlay R. D., Srinivasan M., Unestam T., Giesler R.,

Melkerud and Olsson M. () Rock-eating fungi. *Nature* **389**, 682–683.

Kucey R. M. N. (1983) Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Can. J. Soil. Sci.* **63**, 671–678.

Kucey R. M. N. (1988) Effect of *Penicillium bilaii* on the solubility and uptake of Pand micronutrients from soil by wheat. *Can. J. Soil. Sci.* **68**, 261–270.

Lee M. R. and Parsons I. (1999) Biomechanical and biochemical weathering of lichen-encrusted granite: textural controls on organic-mineral interactions and deposition of silica-rich layers. *Chem. Geol.* **161**, 385–397.

Leeper G. W. and Uren N. C. (1993) *Soil Science: An Introduction*. Melbourne University Press, Carlton, Victoria, Australia.

Lian B. (1998) A study on how silicate bacteria GY92 dissolves potassium from illite. *Acta Mineral. Sin.* **18**, 234–238.

Lian B., Fu P. Q., Mo D. M. and Liu C. Q. (2002) A comprehensive review of the mechanism of potassium releasing by silicate bacteria. *Acta Mineral. Sin.* **22**, 179–183.

Lowenstam H. A. (1981) Minerals formed by organisms. *Science* **211**, 1126–1131.

Malinovskaya I. M. (1988) A method for the determination of the ability of bacterial polysaccharides to sorb silicic acid ions. *Mikrobiol. Zhurn.* **57**, 84–86.

Malinovskaya I. M. (1990) The role of Bacillus mucilaginosus polysaccharide in the destruction of silicate minerals. *Mikrobiologiya* **59**, 70–78.

Mann S. (2001) *Biomineralization: Principles and Concepts in Bioinorganic Material Chemistry*. Oxford University Press, New York.

Mel'nikova E. O., Avakyan Z. A. and Karavaiko G. I. (1990) Microbiological destruction of silicate minerals containing beryllium. *Mikrobiologiya* **59**, 63–69.

Mehta A. P., Torma A. E. and Murr L. E. (1979) Effect of environmental parameters on the efficiency of biodegradation of basalt rock by fungi. *Biotechnol. Bioeng.* **21**, 875–885.

Monib M., Zahra M. K., Abdel El-Al. and Heggo A. (1984) Role of silicate bacteria in releasing K and Si from biolite and orthoclase. *Soil Bioconserv. Biosphere.* **2**, 733–743.

Nagy K. L. (1995) Dissolution and precipitation kinetics of sheet silicates. *Rev. Mineral* **31**, 173–233.

Nahas E. (1996) Factors determining rock phosphate solubilization by microorganisms. *World J. Microbiol. Biotechnol.* **112**, 567–572.

Narsian V. and Patel H. H. (2000) *Aspergillus aculeatus* as a rock phosphate solubilizer. *Soil Biol. Biochem.* **32**, 559–565.

Qi Z. T. (1997) *Chinese Fungi, v.5: Aspergillus sp. and Its Reproduction*. Science Press, Beijing, pp. 28–30.

Panasenko V. T. (1976) Ecology of microfungi. *Bot. Rev.* **33**, 189–215.

Reyes I., Bernier L. and Simard R. R. (1999) Effect of nitrogen sources on the solubilization of different inorganic phosphates by an isolate *Penicillium rugulosum* and two UV-induced mutants. *FEMS Microbiol. Ecol.* **28**, 281–290.

Reyes I. (1999) Characteristics of phosphate solubilization by an isolate of a tropical *Penicillium rugulosum* and two UV-induced mutants. *FEMS Microbiol. Ecol.* **28**, 291–295.

Rossi G. (1979) Potassium recovery through leucite bioleaching: possibilities and limitations. In *Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena* (eds. L. E. Murr, A. E. Torma and J. A. Brierley). Academic Press, New York, pp. 279–319.

Rozanova E. P. (1986) Leaching of glass and during microbiological oxidation of oil. *Mikrobiologiya* **55**, 787–791.

Staley J. T., Palmer F. and Adams J. B. (1981) Microcolonial fungi: common inhabitants of desert rocks? *Science* **215**, 1093–1095.

Sterflinger K. (2000) Fungi as geologic agents. *Geomicrob. J.* **17**, 97–124.

Tansey M. R. and Jack M. A. (1976) Thermophilic fungi in sun-heated soils. *Mycologia* **68**, 1061–1075.

Verrecchia E. P. (1990) Litho-diagenetic implications of the calcium-oxalate carbonate biogeochemical cycle in semiarid calcretes, Nathareth Israel. *Geomicrob. J.* **8**, 87–99.

Verrecchia E. P. and Dumont J. L. (1996) A biogeochemical model for chalk alteration by fungi in semiarid environments. *Biogeochemistry* **35**, 447–470.

Wallander H. and Tonie W. (1999) Biotite and microcline as potassium sources in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. *Mycorrhiza* **9**, 25–32.

Weiner S. and Dove P. M. (2003) An overview of biomineralization processes and the problem of the vital effect. *Rev. Mineral Geochem.* **54**, 1–29.

Welch S. A., Barker W. W. and Barfield J. F. (1999) Microbial extracellular polysaccharides and plagioclase dissolution. *Geochim. Cosmochim. Acta* **63**, 1405–1419.

Whitdaw M. A. (1999) Phosphate solubillzation in solution culture by the soil *Penicillium radicum*. *Soil Biol. Biochem.* **31**, 655–665.

Wollenzien U., de Hoog G. S., Krumbein W. E. and Urzi C. (1995) On the isolation of microcolonial fungi occurring on and in marble and other calcareous rocks. *Sci. Total Environ.* **167**, 287–294.

Yuan L., Fang D. H., Wang Z. H., Shun H. and Huang J. G. (2000) Bio-mobilization of potassium from clay minerals: I. By ectomycorrhizas. *Pedosphere* **10**, 339–346.

Yuan L., Huang J. G., Li X. L. and Christie P. (2004) Biological mobilization of potassium from clay minerals by ectomycorrhizal fungi and eucalypt seedling roots. *Plant Soil* **262**, 351–361.

Associate editor: Johnson R. Haas