Chin.J.Geochem.(2012)31:390–397
DOI: 10.1007/s11631-012-0589-8

Weathering of phosphorus-bearing mineral powder and calcium phosphate by *Aspergillus niger*

QIU Shuangshuang[1,2] and LIAN Bin[1]

[1] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China*

[2] *Graduate University of Chinese Academy of Sciences, Beijing 100049, China*

* *Corresponding author, E-mail: bin2368@vip.163.com*

Received July 8, 2011; accepted August 10, 2011

Abstract The weathering of phosphorus-bearing mineral powder (PBMP) and calcium phosphate (CP) by *Aspergillus niger* was compared, and the mechanism involved was analyzed for the comprehensive utilization of PBMP. The concentration of water-soluble phosphorus (WSP), Mg^{2+}, and Ca^{2+} at different culture times, microstructures of particles, and mineral compositions was examined by ultraviolet-visible spectrometer (UV), atomic absorption spectrometry (AAS), scanning electron microscopy (SEM), and X-ray diffraction (XRD). Results showed that the change in the concentrations of WSP, Mg^{2+}, and Ca^{2+} were related to the growth of *A. niger* and the different mineral compositions. Compared with CP, PBMP was weathered harder by fungi. Traces of the weathered mineral were found through SEM. CP not only showed traces of erosion damage but also appeared as a rhombohedron-like substance. The XRD test indicated that the weathering minerals can form water calcium oxalate. Further analysis revealed that the mechanism of PBMP and CP weathering by *A. niger* was the collaborative result of mycelium biomechanical effects and the acid-soluble role of acidic metabolites. The phosphorus dissolution rate of PBMP after 20 d was 46.83%, whereas that of CP after 12 d was 91.01%. The findings of this study are significant to the effective use of waste PBMP and to the exploitation of low-grade phosphate rock resources.

Key words *Aspergillus niger*; phosphate rock; mineral weathering; calcium phosphate

1 Introduction

Phosphorus is an important component of organisms and is one of the essential macronutrients of plants. However, the deficiency in agricultural soil phosphorus worldwide, especially in China, is serious as it affects crop yield and quality (Sheng Xuebin, 1995; Shen Shanmin, 1998). The application of phosphate fertilizer in soil is necessary to promote the growth of crops. The production of phosphate fertilizer requires high-grade phosphate rock as raw material. However, although phosphate resources are available, most of these are low-grade phosphate rocks (Liu Daijun et al., 2005; Huang Zhiliang et al., 2008). Because the development and use of phosphate resources for the existing levels of industrial technology are difficult, most low-grade phosphate rocks are abandoned. Hence, the search for a cheap, environmentally friendly method that takes full advantage of low-grade phosphate rocks is necessary (Xiao Wengding and Xiao Taiyang, 2007; Rosling et al., 2007; Chen Shu et al., 2009).

From the beginning of the 20th century, people began to study the relationship between microbial activities and phosphorus transformation. In 1908, for instance, Sackett (1908) found that 36 strains of bacteria had phosphorus-dissolving characteristics when bone meal and phosphate rock were dissolved by 50 different strains of bacteria. Such study initiated the use of microorganisms to dissolve insoluble phosphates. Thereafter, many scholars have studied and reported about the separation and the use of soil mi-

croorganisms, as well as the transformation of insoluble phosphates. Nishanth and Biswas (2008) examined the release of phosphorus in phosphate rocks by *Aspergillus awamori* and the effects of the release on the yield and the nutrient uptake of wheat. The results showed sharp increases in the release of water-soluble phosphorus (WSP) and a high yield, uptake, nutrient recovery, and fertility of P and K in soils. Hameeda et al. (2008) conducted glasshouse and field experiments using two efficient strains: *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35. Under glasshouse conditions, plant biomass increased by 99% with EB 67 and by 94% with CDB 35. Under field conditions, plant biomass increased by 66% with EB 67 and by 51% with CDB 35. Seed treatment with EB 67 and CDB 35 increased the grain yield of field-grown maize by 85% and 64%, respectively, compared with the uninoculated control group. Mittal et al. (2008) investigated the effect of *A. awamori* and *Penicillium citrinum* on the growth and the seed production of chickpea plants. The results indicated that *A. awamori* was slightly stronger than *P. citrinum*, but no stimulatory effect was observed when a consortium of the two strains was used. In their study on the solubilization of Morocco phosphorite by *Aspergillus niger*, Bojinova et al. (2008) found that fungi growth caused greater dissolution of phosphate rocks when WSP compounds were not added to the medium. Hamdali et al. (2008) used eight strains of actinomycetes from phosphate mines to dissolve phosphate rocks. They found that the mechanisms involved in these weathering processes were siderophores that were produced by fungals and not by organic acids. In the early 1950s in China, multiple strains of *Bacillus megaterium* that were capable of dissolving organic phosphorus were separated from northeast black and gray soils (Jin Shuchao, 2006). Lin Qimei et al. (2002) and Zhao Xiaorong et al. (2003) used *Arthrobacter* sp., *Pseudomonas* sp., and *Aspergillus* sp. to dissolve rock phosphate and found that phosphate solubilization was related to the type and dosage of the phosphate rock. Sun Dongmei et al. (2008) measured the phosphorus solubilizing ability of *Bacillus* that was isolated from soil and found that the soluble phosphorus content increased and then decreased. Li Mingxiao et al. (2008) examined the phosphate rock solubility of five phosphate-solubilizing bacteria under high temperature. They proposed that the microbial biomass of phosphorus was an important aspect of the analysis on the solubilization of phosphate- solubilizing bacteria. Chen Shu et al. (2008) studied the effect of phosphorite weathering by *Bacillus mucilaginosus* and analyzed bacterial proteins, by which they proposed that some proteins were expressed and activated in the process of weathering.

These studies show the wide range of phosphorus-dissolving microbes, including bacteria, fungi, actinomycetes, and so on. Among these microorganisms, *A. niger* had been found to have good phosphorus-dissolving ability. In his study of phosphorus-dissolving fungi, Johnston (1952) found that *A. niger* was the strongest among the separated multiple strains of fungi. In their study on the roles of *Bacillus subtilis*, *Pseudomonas*, and *Aspergillus* on the decomposition of low-grade phosphate rocks, Chi Ruan et al. (2005) confirmed that the phosphate-solubilizing ability of *Aspergillus* was far stronger than that of *B. subtilis* and *Pseudomonas*. In their research on phosphate rock weathering by *A. niger*, Chen Shu et al. (2009) indicated that the main factors causing phosphate rock weathering were the biophysical damage caused by the growth of *A. niger* and the biochemical degradation of extracellular secretion. These studies show that most microbial phosphate solubilization reports have focused on the isolation of microbes from soil, the microbial dissolution of insoluble phosphates, and the role of microbes in promoting the growth of plants. Because microbial phosphate solubilization is slow and inefficient, strengthening research in this area is necessary, especially in the microbial weathering of apatite and the formation of secondary minerals. This paper examined the variations in mineral composition and analyzed the mechanism of microbial weathering using the weathering of PBMP and CP by *A. niger*. The aim is to provide basic information on the microbial weathering of phosphate rocks (PRs) and on the development and use of PR resources.

2 Materials and methods

2.1 Fungal culture

A. niger (preserved in the Biotechnology Research Center of the Institute of Geochemistry, Chinese Academy of Sciences) was grown on Czapek plates (a 1000 mL medium consisting of 30 g sucrose, 3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4·7H_2O$, 0.01 g $FeSO_4·7H_2O$, and 20 g agar in secondary deionized water) at 30℃ for two days and harvested by an inoculating pin. It was placed in 100 mL of sterile liquid medium (Czapek less agar) and incubated in a rotary shaker at 110 rpm and 30℃ for one day, until the mycelial pellets were about 1 mm in diameter.

2.2 Mineral phases and chemical compositions of PBMP

PBMP (collected from Fuquan City, Guizhou Province, China) was crushed and sieved to obtain grains of ＜200 mesh. It was ultrasonically washed with secondary deionized water and dried at 60℃ to constant weight. The mineral compositions and the

relative contents were identified by X-ray diffractometry (XRD, Rigaku D/Max-2200, CuKαat 40 kV, and 30 mA at 3º/min scan rate). The chemical compositions were determined through X-ray fluorescence spectrometry (XRF, Panalytical Axios PW4400). The PBMP XRF and XRD analyses are listed in Table 1.

Table 1 Mineralogical and chemical compositions of the PBMP

Mineralogical composition (%) by XRD					
Dolomite		Fluorapatite		Quartz	
75.74		20.52		3.74	
Chemical composition (%) by XRF					
CaO	MgO	Na_2O	K_2O	P_2O_5	LOI
32.11	12.73	0.25	0.23	11.15	38.34

2.3 Experimental settings for PBMP and CP weathering by *A. niger*

A batch of experiments was performed to explore the different aspects of fungal-mineral interactions over a period of 20 d. Two treatment groups were created: 1000 mg grains of PBMP and 250 mg grains of CP, each of which was added to 250 mL flasks (containing 100 mL of the culture medium). The removal of K_2HPO_4 and $MgSO_4{\cdot}7H_2O$ from the medium is necessary for the consumption of PMBP by *A. niger* to ensure that the soluble P and Mg^{2+} of the supernatant are released in the weathering of PBMP by *A. niger*. For the same reason, K_2HPO_4 must be removed from the medium in the CP treatment group. The two treatment groups were sterilized at 121℃ for 20 min, followed by inoculation of 10 mycelial pellets, and incubation in a rotary shaker at 110 rpm and 30℃. Each group was incubated for 0, 1, 2, 3, 4, 5, 6, 7, 10, 12, 16, and 20 d. Three control experiments were performed parallel to the treatment runs: Control I was identical to the treatment group but had autoclaved mycelial pellets; Control II used the liquid medium only without mycelial pellets or autoclaved mycelial pellets; and Control III used secondary deionized water instead of liquid medium without mycelial pellets or autoclaved mycelial pellets. The initial pH values of the treatments and the control experiments were adjusted to 7.5 to 8.5 to eliminate the effect of the different experimental designs.

2.4 Experimental sample analysis

At specified incubation times, 10 mL aliquots were collected from the flasks and centrifuged at 5000 rpm for 10 min to collect the supernatant. Each 10 mL aliquot was immediately processed as follows, 2 mL was filtered for pH value determination, 3 mL was filtered for Ca^{2+} and Mg^{2+} concentration measurement through atom absorption spectrometry (Perkin-Elmer PE-5100), and the remaining 5 mL was filtered for WSP concentration measurement through UV (ultraviolet-visible spectrometer, Purkinje General-T6).

The mineral grains collected at the end of the experiments were processed as follows. Mycelial pellets were picked open, air-dried, and analyzed by scanning electron microscopy (Shimadzu-SS550, 25 kV, 0.25 nA) and energy disperse spectroscopy (EDS, Genesis EDAX, 25 kV, 0.45 nA, and 0.1 μm beam spot diameter). The remaining mycelial pellets in the bottles were washed with secondary deionized water, picked open with tweezers, and washed again. The washed grains were collected and dried at 60℃ for testing by XRD.

3 Experimental results and discussion

3.1 pH variation of supernatants during PBMP and CP weathering by *A. niger*

On days 0, 1, 2, 3, 4, 5, 6, 7, 10, 12, 16, and 20, supernatants were collected to determine the pH values of both the treatment and the control groups. The results are shown in Figure 1.

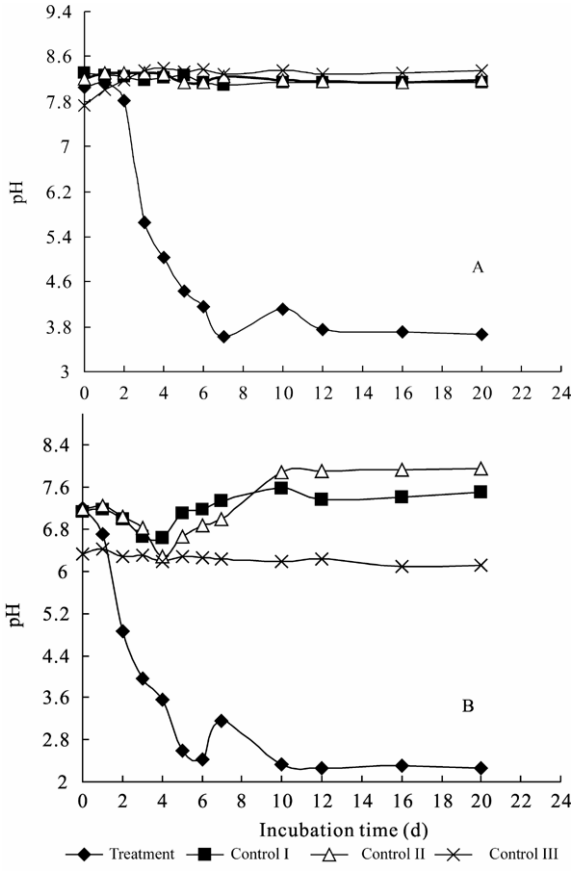

Fig. 1. pH values in different treatment media at different culture times: A. with PBMP, B. with CP.

The results show that the pH values of the treatment groups were significantly lower than those of the control groups. The values became acidic from weak alkaline, and this result means that *A. niger* produced acidic metabolites that helped weather PBMP and CP in the process of PBMP and CP weathering. These acids can be inorganic or organic (e.g. acetic acid, oxalic acid, citric acid, etc.) (Bennett et al., 2001; Routh et al., 2001; Welch et al., 2002).

The pH value curve of PBMP and CP weathering by *A. niger* shows that the pH change was fastest within a two- to five-day period. This result suggests that fungi had the most exuberant vitality at this period, in which PBMP and CP weathering was most obvious. In Figure 1 B, the pH values of the control Ⅱ and control Ⅲ groups slightly decreased and then increased. The slightly elevated pH values could be attributed to the part of atmospheric CO_2 that is dissolved in the culture medium.

3.2 Analysis and concentration of WSP in the supernatants

The characteristics of WSP concentration as it varied with time are shown in Figure 2.

The figure indicates that the WSP concentration of both the treatment and the control groups increased with the culture time. The increase was particularly evident in the treatment group, from 0 mg/L on 0 d to 773.26 mg/L on 20 d, at a phosphorus dissolution rate of 46.83%. The increase in WSP concentration in the control groups was not as obvious as that in the treatment group. *A. niger* had a strong weathering ability toward PBMP at the designed experimental conditions compared with the control groups. This capability may be attributed to the large amounts of organic acid that were secreted during the growth of fungi. On one hand, organic acid can reduce the pH of the medium, and on the other hand, it can bind with Ca^{2+} to dissolve PBMP and to release WSP.

Figure 2B shows that the WSP concentration in CP across all groups increased with the culture time. The variation in characteristics was similar to PBMP weathering, with slight differences. In the treatment group, the WSP concentration increased from 24.70 mg/L on day 0 to 1102.22 mg/L on day 20, with a phosphorus dissolution rate of 91.01%. Instead of increasing, however, the concentration began to decrease. After day 20, the concentration decreased to 563.95 mg/L possibly because of the lesser acid production in the subsequent growth stages of the fungi. This decrease in concentration resulted in less fluorapatite dissolution. Part of the WSP that rebonded with Ca^{2+}, as well as the WSP concentration, was also observed decreased.

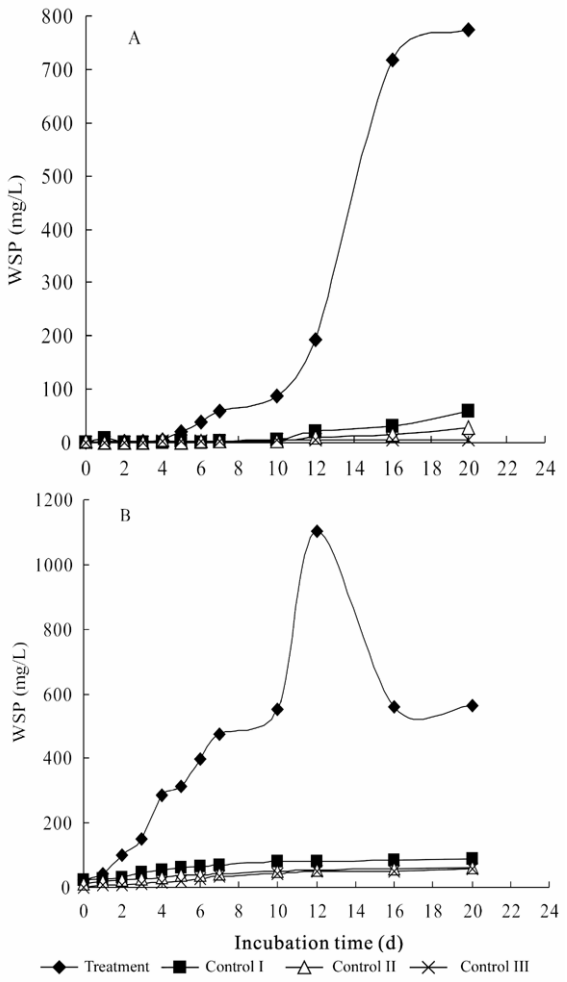

Fig. 2. WSP concentration in different treatment media at different culture times: A. with PBMP, B. with CP.

3.3 Analysis and concentration of Ca^{2+} in the supernatants

The Ca^{2+} concentration in the supernatants of PBMP and CP weathering by *A. niger* was measured, and the results are shown in Figure 3.

The results in Figure 3A show that the Ca^{2+} concentration in the treatment group during PBMP weathering rapidly increased from day 0 to day 3. On day 3, the Ca^{2+} concentration reached the maximum value of 63.53 mg/L. Thereafter, it began to decline until the value reached 3.87 mg/L on day 7, and then a slight rebound occurred. On day 20, the Ca^{2+} concentration was 8.85 mg/L. The control groups had a slight and insignificant increase. Notably, the experimental use of PBMP containing 75.74% dolomite resulted in carbonate minerals that were easier to weather than phosphate minerals, as well as in a WSP concentration of PBMP on the first four days that was almost 0 mg/L (Figure 2A). Therefore, we can assume that the previous four days were dominated mainly by dolomite

weathering accompanied with PBMP weathering. After day 4, because the fungi grew well and secreted large amounts of organic acids, minerals were decomposed, and Ca^{2+} was released. Part of the Ca^{2+} that was released was absorbed by the mycelium, and the rest combined with organic acids in the form of precipitate. The combination decreased the Ca^{2+} concentration in the supernatant. After day 8, a small fraction of Ca^{2+} that was absorbed by the mycelium was released into the supernatant, and the Ca^{2+} concentration slightly increased due to bacterial autolysis.

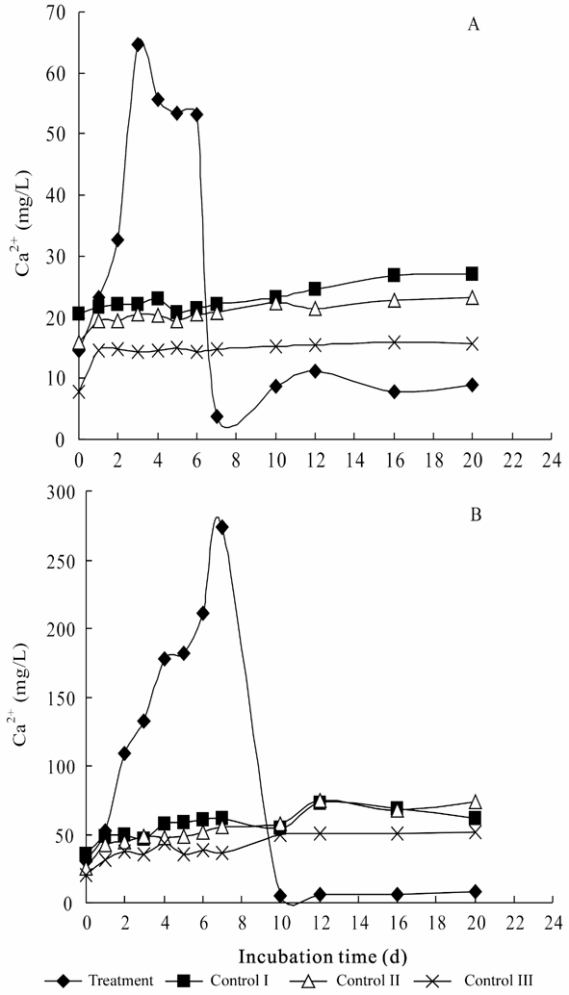

Fig. 3. Ca^{2+} concentration in different treatment media at different culture times: A. with PBMP, B. with CP.

Figure 3B had a similar mechanism as Figure 3A, but it differed in that the Ca^{2+} concentration of the CP treatment group increased on day 0 to day 7 and then decreased in the following days. The difference in concentrations of PBMP and CP is explained by the nature of the solid. In PBMP, all minerals existed in the form of mineral crystals, and *A. niger* needed to use more energy to produce Ca^{2+} and grow well. Because the crystal structure of CP was not as stable as that of PBMP, CP weathered easily, and the Ca^{2+} concentration was high.

CP was taken as an example to observe the SEM morphology and to analyze the EDS feature of CP that was weathered by fungi and by the medium for over 20 d. The purpose was to verify that the released Ca^{2+} did combine with the organic acids secreted by the fungi and did form a precipitate in the process of PBMP and CP weathering. The results are shown in Figure 4.

Figure 4B shows that the elemental composition of CP in the micro region was mainly O, Ca, and P under the action of the medium alone and that a small amount of C was produced from the medium. Figure 4c shows that a large number of hyphae existed in the sample after weathering by *A. niger*. CP was wrapped, interspersed by, or attached to the mycelium. The particles existed in the form of diamond arranged in a short columnar structure, and the aggregates were Chrysanthemum. The corresponding spectroscopy (Fig. 4D) evidently shows the changes in the elements. The elements contained not only O, Ca, and P but also C without P. The reason is that in the process of CP weathering, the metabolites combined with Ca^{2+} and formed a new mineral. This mineral had a similar morphology as calcite, but the exact conclusion needs further validation.

3.4 Analysis and concentration of Mg^{2+} in the supernatants

Because PBMP contained some dolomite, its weathering process was accompanied by dolomite weathering and Mg^{2+} release. Thus, the Mg^{2+} concentration in the supernatants was also measured, and the results are shown in Figure 5. In the CP weathering group, the Mg^{2+} in the supernatants, which was added in the form of $MgSO_4 \cdot 7H_2O$, was not released by CP. Thus, the Mg^{2+} concentration of the CP weathering group was not measured.

Figure 5 shows that the Mg^{2+} concentration in both the treatment and the control groups increased with the culture time. However, the increase was particularly obvious in the treatment group, from 8.77 mg/L on day 0 to 479.25 mg/L on day 20. The increase in Mg^{2+} concentration in the control groups was not as obvious as that in the treatment group. Compared with those in the control groups, the Mg^{2+} concentrations in the treatment group were 14.9, 9.5, and 50.6 times more than those in the control Ⅰ group, the control Ⅱ group, and the control Ⅲ group, respectively, after day 20. The results of the analysis of Mg^{2+} concentration further show that *A. niger* had strong weathering ability toward PBMP at the specified experimental conditions. After day 3, the Ca^{2+} concentration was 63.53 mg/L, and the Mg^{2+} concentration was 65.12 mg/L in the supernatants. Evidently,

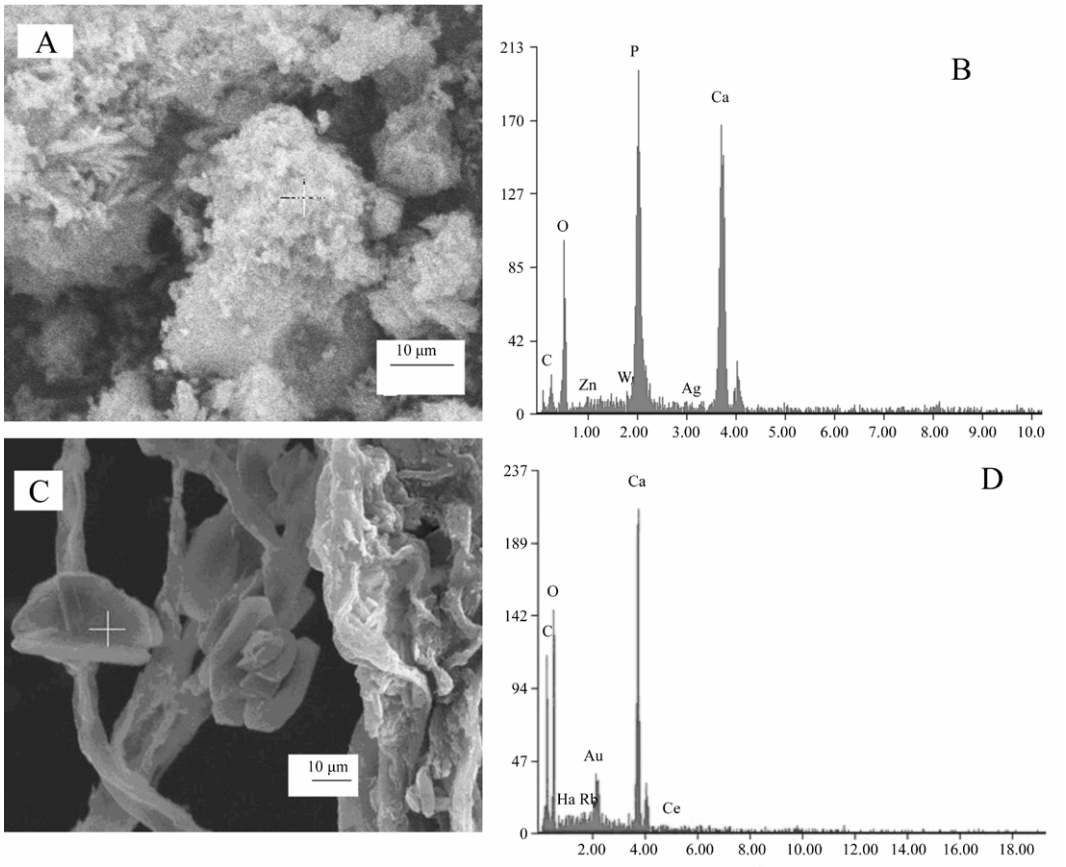

Fig. 4. SEM and EDS analyses of CP weathered by medium (A, B) and by fungi (C, D).

Ca^{2+} and Mg^{2+} were released in an approximate ratio of 1:1, and this release was determined by the crystal structure of dolomite. Because of their difference in solubility, Mg^{2+} compared with Ca^{2+} was not susceptible to combine with organic acids in the form of precipitate. According to Chen Shu et al. (2009) and Li Luli et al. (2010), *A. niger* produced large amounts of oxalic acid, and this oxalic acid combined with Ca^{2+} to form calcium oxalate in the process of *A. niger* weathering of phosphate rocks. The difference in the solubilities of calcium oxalate and magnesium oxalate was 155.2 times: the solubility of calcium oxalate was 6.7×10^{-4}, whereas that of magnesium oxalate was 0.104. Thus, the Mg^{2+} concentration in the supernatant did not decrease but rather increased.

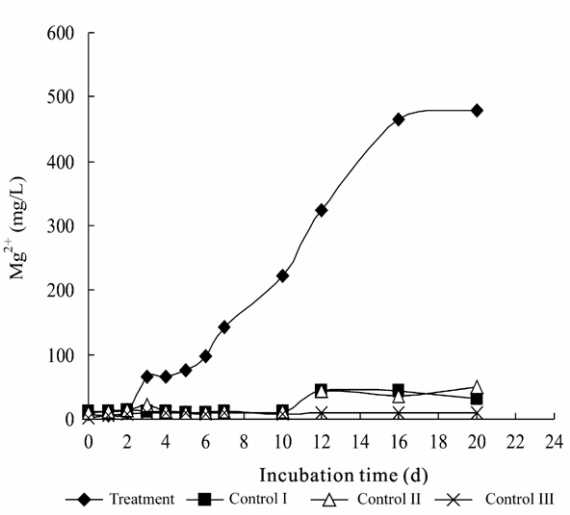

Fig. 5. Mg^{2+} concentration in different treatment media at different culture times.

3.5 Change in mineral composition of PBMP with *A. niger*

After day 20, the mycelial pellets in the CP treatment group produced an insufficient amount of mineral powders for the collection of sediments for XRD analysis. Thus, only the PBMP that was weathered by *A. niger* and by the medium groups after day 20 were included in XRD analysis.

The results of the XRD analysis show that the mineral powders that were weathered by *A. niger* generated new substances, like water calcium oxalate ($CaC_2O_4\cdot H_2O$) (Figure 6). The residual powders consisted of 74.72% water calcium oxalate, 16.26% fluorapatite, and 9.02% quartz. The original dolomite in the PBMP was completely converted into water

calcium oxalate. The XRD results of the mineral powders that were weathered by the medium indicate that no new substance was generated. The residual powders consisted of 26.63% fluorapatite, 68.15% dolomite, and 5.22% quartz. Relative to the original mineral compositions, the fluorapatite and quartz contents were slightly increased, whereas the dolomite content was decreased.

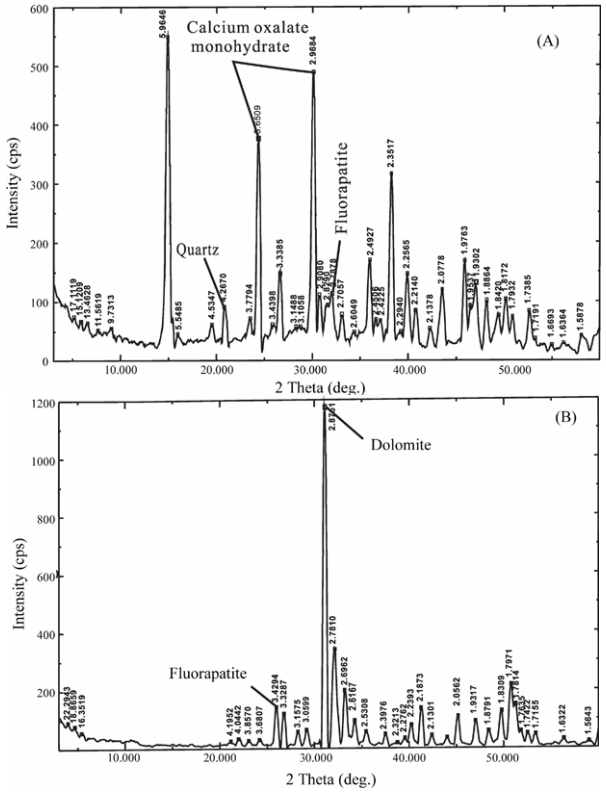

Fig. 6. PBMP X-ray diffraction of weathering by medium (A) and weathering by fungi (B).

From the information on pH, WSP concentration, and Ca^{2+} and Mg^{2+}concentration, CP and PBMP weathering by *A. niger* had similar experimental results. Further, from the SEM and EDS results (Fig. 4C and D) of CP weathering by *A. niger*, we can assume that the residues on day 20 contained large quantities of water calcium oxalate.

4 Conclusions

(1) In CP and PBMP weathering, *A. niger* produced a large number of organic acids to cause mineral dissolution. The mineral particles were wrapped in spherical fungi-mineral aggregates. This wrapping reinforced the biological mechanical damage effects of mycelium on the mineral particles.

(2) In PBMP weathering by *A. niger*, the 0 d to 4 d period was mainly dominated by dolomite weathering, with rare apatite dissolution. After 20 d of weathering by *A. niger*, the phosphate solubilization rate of PBMP was 46.83%, whereas that of CP was 91.01%. This result indicates that *A. niger* is a highly effective phosphate solubilization bacteria.

(3) *A. niger* produced large amounts of organic acids, which combined with Ca^{2+} to form calcium oxalate in *A. niger* weathering of PBMP and CP.

Acknowledgements The authors are grateful to the National Natural Science Foundation of China for Creative Research Groups (Grant No. 41021062) and the "Hundred Talents" Program of the Chinese Academy of Sciences for their financial support. Great thanks are also due to Prof. Wan Guojiang of the Institute of Geochemistry, Chinese Academy of Sciences for his helpful comments.

References

Bennett P.C., Rogers J.R., Choi W.J., and Hiebert F.K. (2001) Silicates, silicate weathering, and microbial ecology [J]. *Geomicrobiology Journal*. **18**, 3−19.

Bojinova D., Velkova R., and Ivanova R. (2008) Solubilizaton of Morocco phosphorite by Aspergillus niger [J]. *Bioresource Technology*. **99**, 7348−7353.

Chen Shu, Lian Bin, and Liu Congqiang (2008) Effect of *Bacillus mucilaginosus* on weathering of phosphorite and a preliminary analysis of bacterial proteins [J]. *Chinese Journal of Geochemistry*. **27**, 209−216.

Chen Shu, Liu Congqiang, and Lian Bin (2009) Two-dimenttional Gel Electrophoresis analysis of bacterial proteins during Bacillus mucilaginosus on weathering of phosphorite [J]. *Microbiology*. **36**, 334−338 (in Chinese with English abstract).

Chen Shu, Liu Congqiang, and Lian Bin (2009) Effect of a strain of *Aspergillus Niger* on weathering of phosphorite [J]. *Acta Mineralogica Sinica*. **29**, 103−108 (in Chinese with English abstract).

Chi Ruan, Xiao Chunqiao, Gao Hong, Wu Yuanxin, Li Shirong, and Wang Cunwen (2005) Biodecomposition of low-grade rock phosphate with some bacteria and fungi [J]. *The Chinese Journal of Process Engineering*. **5**, 636−639 (in Chinese with English abstract).

Hamdali H., Bouizgame B., Hafidi M.，Lebrihi A., Virolle M., and Ouhdouch Y. (2008) Screening for rock phosphate solubilizing Actinomyceters from Moroccan phosphate mines [J]. *Applied Soil Ecology*. **38**, 12−19.

Hameeda B., Harini G.，Rupela O.P., Wani S.P., and Reddy G. (2008) Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna [J]. *Microbiological Research*. **163**, 234−242.

Huang Zhiliang, Liu Yu, Xue Huanyan, Shi Hebin, and Luo Huihua (2008) *Apatite Mineral Materials* [M]. pp. 63−65. Chemical Industry Press, Beijing (in Chinese with English abstract).

Jin Shuchao, Du Chunmei, Ping Wengxiang, Guan Hongyu, and Xu Baoxing (2006) Advance in phosphorus-dissolving microbes [J]. *Journal of Microbiology*. **26**, 73−78 (in Chinese with English abstract).

Johnston H.W. (1952) The solubilization of phosphate: The action of various organic compounds on dicalcium and tricalcium phosphates [J]. *New Zealand J. Sci. Tech. B.* **33**, 436−446.

Li Luli, Qiu Shuyi, Wang Yijuan, and He Guixiang (2010) Research on the transforming capacity of low grade phosphate tailings with *Aspergillus niger* [J]. *Chinese Agricultural Science Bulletin*. **26**, 335−338 (in Chinese with English abstract).

Li Mingxiao, Xi Beidou, Wei Zimin, Qi Ruijiang, Chen Jian, Yang Tianxue, and He Xiaosong (2008) Screening and characterization of phosphate-solubilizing microorganisms at high temperature [J]. *Research of Environmental Sciences*. **21**, 165−169 (in Chinese with English abstract).

Lin Qimei, Zhao Haiying, and Zhao Xiaorong (2002) Rock phosphates solubilization of some microorganisms [J]. *Scientia Agricultura Sinica*. **35**, 1232−1235 (in Chinese with English abstract).

Liu Daijun, Jiang Shaozhi, Luo Hongbo, and Zhang Yunxiang (2005) The tendency of phosphate rock deficiency and dilution and its countermeasure [J]. *Phosphate & Compound Fertilizer*. **20**, 6−9 (in Chinese with English abstract).

Mittal V., Singh O., Nayyar H., Kaur J., and Tewari R. (2008) Stimulatory effect of phosphate-solubilizing fungal strains (Aspergillus awamori and Penicillium) on the yield of chickpea (Cicer arietinum L. cv. GPF2) [J]. *Soil Biology & Biochemistry*. **40**, 718−727.

Nishanth D. and Biswas D.R. (2008) Kinetics of phosphorus and potassium release from rock phosphate and waste mica enriched compost and their effect on yield and nutrient uptake by wheat (Triticum aestivum) [J]. *Bioresource Technology*. **99**, 3342−3353.

Rosling A., Suttle K.B., Johansson E., Van Hees P.A.W., and Banfield J.F. (2007) Phosphorous availability influences the dissolution of apatite by soil fungi [J]. *Geobiology*. **5**, 265−280.

Routh J., Grossman E.L., Ulrich G. A., and Suflita J.M. (2001) Volatile organic acids and microbial processes in the Yegua formation, east-central Texas [J]. *Applied Geochemistry*. **16**, 183−195.

Sackett W.G., Pattern A.G., and Brown C.W. (1908) The solvent action of soil bacteria upon the in soluble phosphates of raw bone meal and natural rock phosphate [J]. *Central Bacteria*. **20**, 688−703.

Shen Shanmin (1998) *Chinese Soil Fertility* [M]. pp.10−12. China Agriculture Press, Beijing (in Chinese with English abstract).

Sheng Xuebin (1995) Studies on soil phosphorus status and trends [J]. *Advances in Environmental Science*. **3**, 11−21 (in Chinese with English abstract).

Sun Dongmei, Zhang Shuquan, Chi Li, Wang Yuting, and Zhao Min (2008) Two phosphorus dissolving microorganisms isolated and their characteristics [J]. *Chinese Agricultural Science Bulletin*. **24**, 239−242 (in Chinese with English abstract).

Welch S.A., Taunton A.E., and Banfield J.F. (2002) Effect of microorganisms and microbial metabolites on apatite dissolution [J]. *Geomicrobiology Journal*. **19**, 343−367.

Xiao Wengding and Xiao Taiyang (2007) Integration study of biological ore separation and chemical metallurgy for lower grade phosphate ore [J]. *Minerals and Geology*. **21**, 362−365 (in Chinese with English abstract).

Zhao Xiaorong, Lin Qimei, Zhao Zijuan, and Li Baoguo (2003) Dynamics of rock phosphate solubilization by *Aspergillus* 2TCiF2 [J]. *Journal of China Agricultural University*. **8**, 43−46 (in Chinese with English abstract).