Journal of Southeast University (English Edition) Vol. 31, No. 4, pp. 506 - 510 Dec. 2015 ISSN 1003—7985

Microbially induced deposition of barium phosphates and its ingredient, morphology and size under different pH values

Yu Xiaoniu Qian Chunxiang Wang Xin

(School of Materials Science and Engineering, Southeast University, Nanjing 211189, China)
(Research Institute of Green Construction Materials, Southeast University, Nanjing 211189, China)

Abstract: A phosphate-mineralization microbe was used to induce barium phosphates precipitation, and the precipitates with different types were obtained under different pH values. The average crystallite size of the barium phosphates was calculated by particle size distribution curves, and the size of the products was 33.40, 29.37, 24.13, 47.76 and 96.53 μm when the pH values of the mixed solution are 7, 8, 9, 10 and 11, respectively. The results of X-ray diffraction (XRD) show that the structures of the particles controlled by the mixed solution are mainly $BaHPO_4$ when pH < 10; the barium phosphates are synthesized by biological deposition which is the mixture of $BaHPO_4$ and $Ba_5(PO_4)_3OH$ when pH = 10; when pH = 11, the barium phosphates are also the mixtures, which are $Ba_5(PO_4)_3OH$ and $BaNaPO_4$. The above results indicate that the phosphate-mineralization microbe can produce a certain enzyme which constantly hydrolyzes phosphate monoester in the mixed solution, and then PO_4^{3-} ions are obtained.

Key words: phosphate-mineralization microbe; barium phosphates; morphology; X-ray diffraction; phosphate monoester

doi: 10.3969/j.issn.1003 - 7985.2015.04.013

Microbially induced precipitation of inorganic compounds are natural inorganic/organic hybrid materials formed through a cooperative interaction of inorganic materials with organic macromolecules, where the macromolecules control the nucleation, growth, morphology, structure, and crystal orientation of the inorganic components[1-2]. The morphology and size of inorganic crystals are usually controlled by adding organic matrices, such as $BaHPO_4$[3], $CaCO_3$[4-6], $LiFePO_4/C$[7], CdS[8], Ag_2S[9-10], etc. Therefore, inorganic materials via bio-inspired precipitation have achieved much attention from biologists, chemists, material scientists, and so on[11-15].

Barium phosphates have been extensively studied in the past for their applications in different fields, such as catalysts, bioceramics, ionic conductivity, ferroelectrics, luminescence, metal-doped, corrosion resistance, etc. These applied domains show that barium phosphates are important inorganic phosphate materials. Barium phosphates are usually prepared by the sol-gel method, hydrothermal synthesis, chemical precipitation, and so on. However, few investigations focused on the synthesis, ingredients, size and morphology of barium phosphates through microbiological technique. In this work, the influence of pH on the ingredient, morphology and size of barium phosphates precipitated in the mixed solution of bacteria and substrate is studied. Bacterial body and biomacromolecules can tailor the size and shape of synthesized particles, which have important influences on the size and morphology of the products. Therefore, barium phosphates precipitated in the mixture solution is taken as a more complicated process than other methods because the microbial cells of negative charge and functional groups of organic matrix can chelate metal ionsand regulate nucleation, growth, size, and orientation of the crystals[3, 16-18].

1 Materials and Methods

All reagents and solvents from commercial sources were used without further purification. Deionized water was self-made by our laboratory. The phosphate-mineralization microbe was selected to investigate microbially induced barium phosphates precipitation. Cultivation of the phosphate-mineralization microbe was conducted in a medium containing 3 g/L of beef extract, 5 g/L of peptone and 1 g/L of sodium chloride. The microbe with the OD_{600} value of 1.69 was used in this study. In general, the harvested microorganisms were kept in a refrigerator at (4 ± 0.2) ℃ for stock prior to use.

Microbially induced deposition of barium phosphates was prepared as follows: 20 mmol of phosphate monoester was completely dissolved in deionized water (40 mL), and the solution was poured into 200 mL of bacterial solution. The mixed solution of bacteria and substrate was allowed to stand under static conditions for 24 h at

Received 2015-05-09.

Biographies: Yu Xiaoniu (1988—), male, graduate; Qian Chunxiang (corresponding author), female, doctor, professor, cxqian@seu.edu.cn.

Foundation items: The National Natural Science Foundation of China (No. 51372038, No. 51178104), the Scientific Research Foundation of Graduate School of Southeast University (No. YBJJ1453), the 333 Project of Jiangsu Province.

Citation: Yu Xiaoniu, Qian Chunxiang, Wang Xin. Microbially induced deposition of barium phosphates and its ingredient, morphology and size under different pH values[J]. Journal of Southeast University (English Edition), 2015, 31(4): 506 - 510. [doi: 10.3969/j.issn.1003 - 7985.2015.04.013]

(30 ± 2)℃. The pH values of the mixed solution were adjusted to 7, 8, 9, 10 and 11 by using 18% HCl and 5% NaOH solution. Next, 20 mmol of $BaCl_2 \cdot 2H_2O$ were added to the above solution. After stirring, the precipitated solution was allowed to stand under static conditions for 24 h at ambient temperature. Finally, all precipitates were collected and characterized.

The phase purity of products was examined by the powder X-ray diffraction (XRD) with Bruker D8-Discover diffractometer using graphite-monochromatized high-intensity Cu Kα radiation ($\lambda = 0.154\,06$ nm). The scanning electron microscope (SEM, FEI Company, Netherlands, operating voltage 20 kV) with a Genesis 60S energy dispersive X-ray spectroscopy (EDS) spectroscopy system was used to conduct morphological studies and to measure the elemental compositions of the samples. The particle size was determined using a Microtrac S3500 particle size analyzer (Advanced Research Tools Corporation, Downers Grove, Illinois, USA) for dispersions of barium phosphates particles in pure water.

2 Results and Discussion

The XRD of materials analysis confirms that the barium phosphates at pH < 10, patterns can be readily indexed to the reported structures of $BaHPO_4$ (JCPDS No. 17-0929, 17-0929 and 72-1370, respectively), and no peaks attributable to impurities are observed (see Fig. 1). The precipitates are mainly mixtures when the pH values of the mixed solution are 10 and 11. The standard XRD patterns of the reported structures of the mixture of $BaHPO_4$ and $Ba_5(PO_4)_3OH$ used are JCPDS No. 72-1370 and 78-1141 when pH = 10 (see Fig. 1). When pH = 11, the standard XRD patterns of the reported structures by the mixture of $Ba_5(PO_4)_3OH$ and $NaBaPO_4$ used are JCPDS No. 06-0272 and 33-1210 (see Fig. 1). The barium phosphates are precipitated in the mixed solution under different pH values, which can be explained by the following steps:

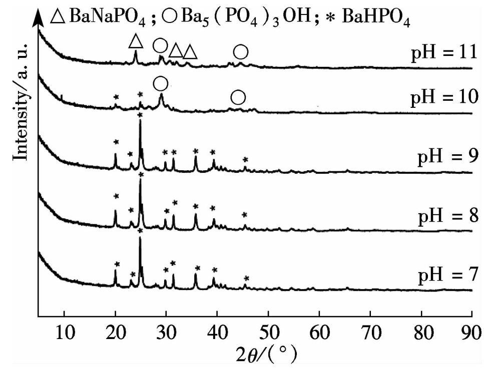

Fig. 1 XRD patterns of barium phosphates at different pH values

$$\text{Phosphate monoester} + H_2O \xrightarrow{\text{Phosphate-mineralization microbe}} HPO_4^{2-}/PO_4^{3-}$$

$$HPO_4^{2-}/PO_4^{3-} \xrightarrow{pH=7,8,9} HPO_4^{2-}$$

$$HPO_4^{2-} + BA^{2+} \xrightarrow{pH=7,8,9} BaHPO_4$$

$$HPO_4^{2-}/PO_4^{3-} \xrightarrow{pH=10} PO_4^{3-} + HPO_4^{2-} + OH^-$$

$$PO_4^{3-} + HPO_4^{2-} + OH^- + Ba^{2+} \xrightarrow{pH=10} Ba_5(PO_4)_3OH + BaHPO_4$$

$$HPO_4^{2-}/PO_4^{3-} \xrightarrow{pH=11} PO_4^{3-} + OH^-$$

$$PO_4^{3-} + OH^- + Ba^{2+} + Na^+ \xrightarrow{pH=11} Ba_5(PO_4)_3OH + BaNaPO_4$$

The distribution coefficients of ions δ can be used here to explain the above results of XRD and the forms of phosphate ions with different values. The distribution coefficient of phosphate ions in the solution depends on the concentration of the hydrogen ion, and the distribution coefficient can be calculated by the distribution coefficient equations as

$$\delta_0 = \frac{[H_3PO_4]}{C} = \frac{[H^+]^3}{[H^+]^3 + Ka_1[H^+]^2 + Ka_1Ka_2[H^+] + Ka_1Ka_2Ka_3} \quad (1)$$

$$\delta_1 = \frac{[H_2PO_4^-]}{C} = \frac{Ka_1[H^+]^2}{[H^+]^3 + Ka_1[H^+]^2 + Ka_1Ka_2[H^+] + Ka_1Ka_2Ka_3} \quad (2)$$

$$\delta_2 = \frac{[HPO_4^{2-}]}{C} = \frac{Ka_1Ka_2[H^+]}{[H^+]^3 + Ka_1[H^+]^2 + Ka_1Ka_2[H^+] + Ka_1Ka_2Ka_3} \quad (3)$$

$$\delta_3 = \frac{[PO_4^{3-}]}{C} = \frac{Ka_1Ka_2Ka_3}{[H^+]^3 + Ka_1[H^+]^2 + Ka_1Ka_2[H^+] + Ka_1Ka_2Ka_3} \quad (4)$$

where $Ka_1 = 7.25 \times 10^{-3}$, $Ka_2 = 6.31 \times 10^{-8}$, $Ka_3 = 4.80 \times 10^{-13}$.

The distribution coefficient of $[HPO_4^{2-}]$ (δ_2) is large under pH = 7, 8 and 9, so $BaHPO_4$ is the main precipitate when pH < 10. The distribution coefficient of $[PO_4^{3-}]$ (δ_3) is smaller than $[HPO_4^{2-}]$ (δ_2) in theory. But δ_3 is not ignored by the X-ray diffraction peaks analysis when pH = 10 and 11 (see Tab. 1). The difference in product types is mainly ascribed to the pH values of the mixed solution and the property of $BaCl_2 \cdot 2H_2O$. For instance, all PO_4^{3-} ions are changed into HPO_4^{2-} ions since the aqueous solution of $BaCl_2 \cdot 2H_2O$ is weakly acidic when pH < 10. A certain percentage of PO_4^{3-} ions is changed into HPO_4^{2-} ions when pH = 10, and no PO_4^{3-} ions is changed into HPO_4^{2-} ions when pH = 11. It is easy to conclude that phosphate monoester can be constantly hydrolyzed by a specific enzyme, which is secreted by the phosphate-mineralization microbe, and then the PO_4^{3-} ions are obtained.

Tab. 1 Distribution coefficients of ions under different pH values calculated according to Eqs. (1) to (4)

pH	δ_1	δ_2	δ_3
7.0	0.613	0.410	2.39×10^{-6}
8.0	0.137	0.863	5.41×10^{-5}
9.0	0.016 1	0.984	6.23×10^{-4}
10.0	1.57×10^{-3}	0.991	6.19×10^{-3}
11.0	1.52×10^{-4}	0.941	5.94×10^{-2}

The SEM images and particle size distribution curves of barium phosphates obtained in the mixed solution of bacteria and substrate under different pH values are shown in Figs. 2 and 3, respectively. When the pH of the mixed solution is 7, the morphology of precipitates is an irregular particle, and the length and width of the particle is less than 124.40 μm (see Fig. 2(a) and Fig. 3(a)). When pH = 8, the spheres are accompanied by some irregular sphere appearing, as shown in Fig. 2(b). The size of particles is nonuniform and the diameter ranges from 4.62 to 87.99 μm (see Fig. 3(b)). Fig. 2(c) and Fig. 3(c) show that the shape of the products is dumbbell-shaped and the size of particles is 5.50 to 62.22 μm when the pH of the mixed solution is 9. When the pH of the mixed solution is increased up to 10, the shape of the mixture of $BaHPO_4$ and $Ba_5(PO_4)_3OH$ displays irregularly with the nonuniform size, as shown in Fig. 2(d). The irregular particles' diameters ranges from 3.89 to 176.00 μm (see Fig. 3(d)). When the pH of the mixed solution is increased to 11, the shape of the mixture of $Ba_5(PO_4)_3OH$ and $NaBaPO_4$ is also irregular with the nonuniform size, as shown in Fig. 2(e). The irregular particles size is 4.62 to 418.60 μm (see Fig. 3(e)). The average crystallite size of barium phosphates is 33.40, 29.37, 24.13, 47.76, and 96.53 μm when the pH values of the mixed solution are 7, 8, 9, 10 and 11, respectively (see Fig. 3).

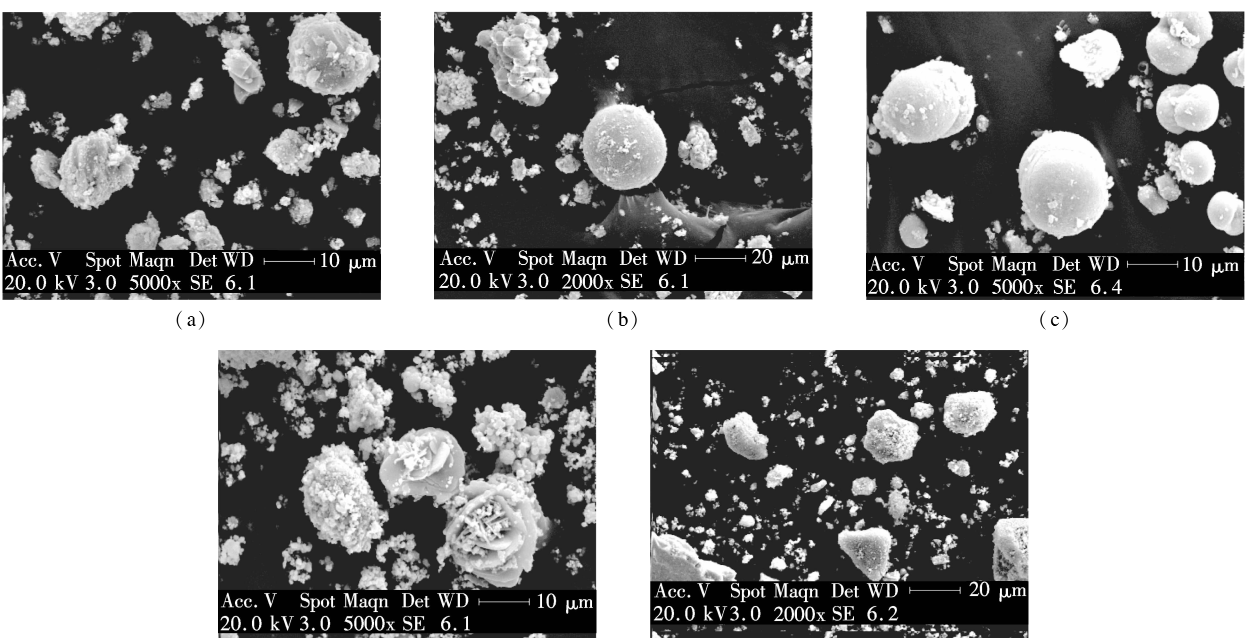

Fig. 2 SEM images of barium phosphates obtained in the mixed solution of bacteria and substrate under different pH values. (a) pH = 7; (b) pH = 8; (c) pH = 9; (d) pH = 10; (e) pH = 11

3 Conclusion

The diverse shapes and size of the barium phosphates can be obtained in the presence of the mixed solution of bacteria and substrate under different pH values. The shapes of barium hydrogen phosphate are the irregular particle, irregular sphere and dumbbell-shaped with a wide diameter distribution when pH = 7, 8 and 9, respectively. However, the mixtures of $BaHPO_4$ and $Ba_5(PO_4)_3OH$, and $Ba_5(PO_4)_3OH$ and $BaNaPO_4$ are prepared when the pH values of the mixed solution are 10 and 11, respectively. It is easy to conclude that $Ba_5(PO_4)_3OH$ can be easily synthesized when a large number of PO_4^{3-} ions are contained in the mixed solution. The

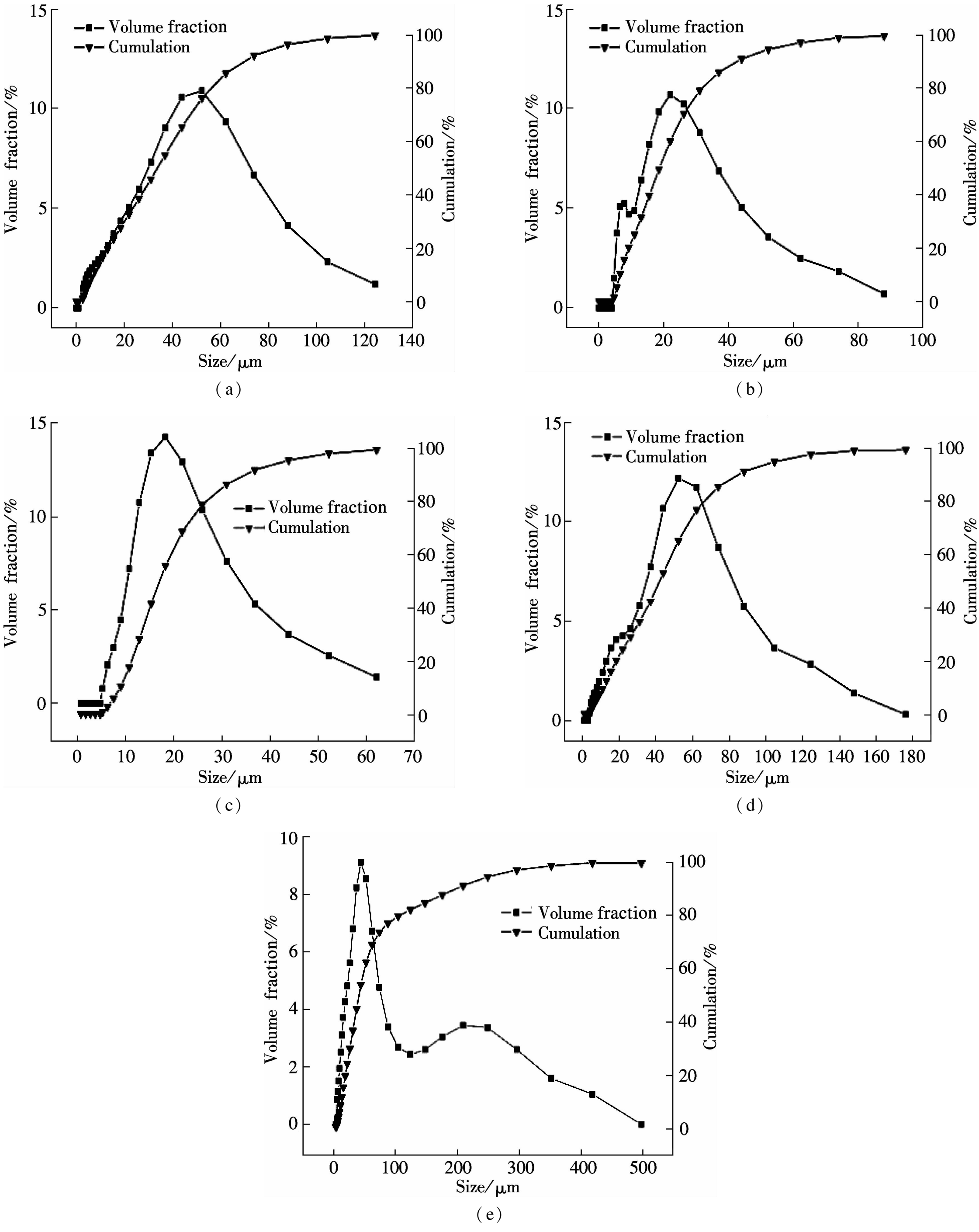

Fig. 3 Particle size distribution of barium phosphates with different pH values. (a) pH = 7; (b) pH = 8; (c) pH = 9; (d) pH = 10; (e) pH = 11

above results show that bacteria and biomacromolecules play an important role in the process of crystal nucleation, growth and the accumulation of barium phosphates.

References

[1] Xu X R, Han J T, Cho K. Formation of amorphous calcium carbonate thin films and their role in biomineralization [J]. *Chemistry of Materials*, 2004, **16**(9):1740 - 1746.

[2] Mann S. Molecular recognition in biomineralization [J]. *Nature*, 1988, **332**(6160):119 - 124.

[3] Wang F, Xu G Y, Zhang Z Q. The effect of pH on morphological control of barium hydrogen phosphate crystal by a double-hydrophilic copolymer [J]. *Materials Letters*, 2005, **59**(7):808 - 812.

[4] Ma Y F, Qiao L, Feng Q L. In-vitro study on calcium carbonate crystal growth mediated by organic matrix extracted from fresh water pearls [J]. *Materials Science & Engineering C*, 2012, **32**(7): 1963 - 1970.

[5] Mann S, Heywood B R, Rajam S, et al. Controlled crystallization of $CaCO_3$ under stearic acid monolayers [J]. *Nature*, 1988, **334**(6184): 692 - 695.

[6] Fu G, Valiyaveettil S, Wopenka B, et al. $CaCO_3$ biomineralization: acidic 8-kDa proteins isolated from ara-

gonitic abalone shell nacre can specifically modify calcite crystal morphology [J]. *Biomacromolecules*, 2005, **6**(3):1289 - 1298.

[7] Zhang X D, He W, Yue Y Z, et al. Bio-synthesis participated mechanism of mesoporous $LiFePO_4$/C nanocomposite microspheres for lithium ion battery [J]. *Journal of Materials Chemistry*, 2012, **22**(37):19948 - 19956.

[8] Lin J, Cates E, Bianconi P A. A synthetic analogue of the biomineralization process: Controlled crystallization of an inorganic phase by a polymer matrix [J]. *Journal of the American Chemical Society*, 1994, **116**(11):4738 - 4745.

[9] Chen J, Kong Y F, Ji J J, et al. Protein-induced structural evolution of silver sulfide at the nanoscale: from hollow particles to solid spheres [J]. *Nanoscale*, 2012, **4**(15):4455 - 4458.

[10] Qin D Z, Zhang L, He G X, et al. Synthesis of Ag_2S nanorods by biomimetic method in the lysozyme matrix [J]. *Materials Research Bulletin*, 2013, **48**(9):3644 - 3647.

[11] Zhu W K, Luo X G, Zhang C, et al. Microbiological precipitation of barium carbonate [J]. *Chinese Journal of Inorganic Chemistry*, 2011, **27**(10):2053 - 2060.

[12] Cheng L, Cord-Ruwisch R, Shahin M A. Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation [J]. *Canadian Geotechnical Journal*, 2013, **50**(1):81 - 90.

[13] DeJong J T, Fritzges M B, Nusslein K. Microbially induced cementation to control sand response to undrained shear [J]. *Journal of Geotechnical and Geoenvironmental Engineering*, 2006, **132**(11): 1381 - 1392.

[14] Schüler D, Baeuerlein E. Dynamics of iron uptake and Fe_3O_4 biomineralization during aerobic and microaerobic growth of magnetospirillum gryphiswaldense [J]. *Journal of Bacteriology*, 1998, **180**(1): 159 - 162.

[15] Rong H, Qian C, Li L. Influence of molding process on mechanical properties of sandstone cemented by microbe cement [J]. *Construction and Building Materials*, 2012, **28**(1): 238 - 243.

[16] Höppe H A, Daub M, Oeckler O. Synthesis, crystal structure, infrared spectrum and thermal behavior of α-$BaHPO_4$[J]. *Solid State Sciences*, 2009, **11**(8):1484 - 1488.

[17] Chaabane T B, Smiri L, Bulou A. Vibrational study and crystal structure refinement of $BaHPO_4$ [J]. *Solid State Sciences*, 2004, **6**(2):197 - 204.

[18] Nallamuthu D, Selvarajan P, Freeda T H. Studies on various properties of pure and Li-doped barium hydrogen phosphate (BHP) single crystals [J]. *Physica B: Condensed Matter*, 2010, **405**(24): 4908 - 4913.

微生物诱导磷酸钡盐沉积及其不同 pH 值条件下的成份、形貌和尺寸

於孝牛　钱春香　王　欣

(东南大学材料科学与工程学院, 南京 211189)
(东南大学绿色建材技术研究所,南京 211189)

摘要:利用磷酸盐矿化菌诱导磷酸钡盐沉淀,并在不同 pH 值条件下赋予不同的形貌. 通过粒径分布曲线计算磷酸钡盐的平均结晶尺寸,当细菌与混合溶液的 pH 为 7, 8, 9, 10 和 11 时,对应产物的尺寸分别为 33. 40, 29. 37, 24. 13, 47. 76 和 96. 53 μm. X-射线衍射的结果表明:当 $pH < 10$ 时,混合溶液控制颗粒的主要成分为 $BaHPO_4$. 当 $pH = 10$ 时,生物沉积的磷酸钡盐主要成份为 $BaHPO_4$和 $Ba_5(PO_4)_3OH$ 的混合物. 当 $pH = 11$ 时,主要为 $Ba_5(PO_4)_3OH$ 和 $BaNaPO_4$ 混合物. 上述结果显示磷酸盐矿化菌可以产生特定的酶不断水解磷酸单酯,然后获得 PO_4^{3-} 离子.

关键词:磷酸盐矿化菌;磷酸钡盐;形貌;X-射线衍射;磷酸单酯

中图分类号:O782