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MICRO-BIOLOGICAL MINERALIZATION: *BACILLUS* -INDUCED VIVIANITE $Fe_3(PO_4)_2 \cdot 8H_2O$ PRECIPITATION

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Nano-sized Vivianiteis prepared via biomineral processunder the assistance of*Bacillus*.The structural characterization by Fourier transform infrared spectroscopy (FTIR),energy dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD) and morphological observation via scanning electron microscopy (SEM) and transmission electron microscopy (TEM) show that the nanostructures of Vivianitehasbeen prepared. And thermal properties of Vivianitenanoparticles are investigated by thermogravimetric-differential scanning calorimetry(TG-DSC) analysis. SEM photographs display that synthesizedVivianite in the form of nanoparticleclusters and honeycomb-like in shape.TEM images show that Vivianitenanoparticles arequadrilateral, hexagonal and irregular in shapewith the size of20-200nm.Powder XRD patterns furnished evidence for the formation of Vivianitehaving average crystallite size 31.89nm.This workshows that nano-sized Vivianiteis easily achievable using*Bacillus*, which plays an important role in the process of crystal nucleation, growth and accumulation of Vivianitenanoparticles.

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1.Introduction

Biomineral process is one of common mostly phenomena in nature, and the process occurs in all living organisms,includinganimals, plants, and microorganisms.These organismscontain a large number of organic matrices, having a series of functional groups (N-H, O-H, COO-, C-O, C-C, C=C, C-H, etc), and them can adjust crystallizationprocess.Biominerals with controlled structure are generallyformed by self-organization and under mild solutionconditions.Nanomaterials are prepared by biomimetic process, which have been became an attractive alternativeoptionin compared with physical and chemical methods.As such, the process hasbeen achievingconsiderable attention from materialscientists,biologists, and chemists [1-4].For example,scientists in different fieldshave been used bacteria ororganic matrixto controlmorphologyor size of inorganic and metal materials, such asAu [5],Ag_2S [6, 7], $CaCO_3$[2, 8],CdS [9], $BaHPO_4$[1], $FePO_4$ [4], Fe_3O_4or Fe_3S_4 [10, 11], SiO_2 [3], and so on.

Vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$)is an importantinorganic phosphates, which can be used as raw materials of battery. However,Vivianitenanoparticlescan be synthesized by otherconventional-methodswhichare alwaysrelatively complex or indispensable harsh reaction condition [12].In this paper, we have used bacterial biomineralization process to synthesize Vivianitenanoparticles. And the morphology, structure, and thermal decomposition properties of theVivianitenanoparticles werecharacterized by Fourier transform infrared spectroscopy (FTIR),

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energy dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and thermogravimetric-differential scanning calorimetry (TG-DSC).

2. Experimental

All the reagents and solvents from commercial sourceswere used without further purification. *Bacillus* was purchased from China Center of Industrial Culture Collection, which culture with OD_{600} value of 1.0 and enzyme activity value of 0.7mmol/(L min) was used in this study.Cultivation of the *Bacillus* was conducted in a medium containing 3g/L beef extract, 5g/L typtone and 1g/L sodium chloride. Then, the harvested*Bacillus*was kept in a refrigerator at 4 °C for stock prior to use.

Microbiological precipitation of Vivianitenanoparticles: phosphatemonoesterwas completely dissolved in a beakerwith 30ml deionized waterwhosepH wasadjusted to 9.0using 18% HCl solution.The solution was injected into a 200ml solution of *Bacillus*, and then the mixture solution wasallowed to stand under static conditions for 12h at 30±2°C.As a result,Vivianitewas precipitated by adding $FeSO_4 \cdot 7H_2O$ (12mM) into the mixture solutionunder vigorousstirring. And after 2 min of stirring, the precipitated solution wasallowed to stand under static conditions for 72h at roomtemperature. Theproducts was filtrated and washed three times with deionized water and ethanol and then driedat 70 °C for 24 h. The samples was collected andcharacterized.

Fourier transform infrared spectroscopy (FTIR)spectra of the samples were recorded using a Nicolet 5700 spectrometer by KBr pellet technique in the range of 500-4000 cm^{-1}.The phase purity of products was examined by powder X-ray diffraction (XRD) with Bruker D8-Discoverdiffractometer using graphite-monochromatized high-intensity Cu Kα radiation (λ=1.5406Å). Scanning electron microscopy (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.Transmission electron microscopy (TEM) images were obtained on a FEI, G2 20 equipment. TEM grids were prepared using a few drops of nanoparticles followed by drying. Thermogravimetric-differential scanning calorimetry (TG-DSC) of Vivianitewere measured byNETZSCH STA449 F3 devicein the temperature range of room temperature to800°Cat the heating rate of 10°C •min^{-1}under an N_2 flow of 20 mL•min^{-1}.

3. Results and discussion

Vivianiteprecipitate induced by*Bacillus* is taken as a more complicated process than the one induced in other solution[12]. *Bacillus* can produce alkaline phosphatase (EC 3.1.3.1) which constantly hydrolyzedphosphatemonoester in bacterial solution, and obtain PO_4^{3-} and various alcohols [13, 14]. The formation of Vivianitenanoparticlesdepositing on*Bacillus* can be explained by the following steps:

$$\text{Phosphate monoester}+H_2O \xrightarrow{Alkaline\ phosphatase} PO_4^{3-} \quad (1)$$

$$Fe^{2+} + PO_4^{3-} + H_2O \xrightarrow{Bacillus} Fe_3(PO_4)_2 \cdot 8H_2O \quad (2)$$

The FTIR spectrum is effectively used to identify the functional groups of the samples.The

FTIR spectrum of Vivianite is displayed in Fig. 1.The broad peaks at 3000-3500 cm^{-1} and the strong peak at 1644.90 cm^{-1} are attributed to the O-H stretching vibration of water. The nanoparticles shows strong transmission bands for PO_4 stretching at1056.82cm^{-1} andbending at553.89 cm^{-1}in good agreementwith the investigations on Vivianite byWalpersdorfandFrost[15, 16].

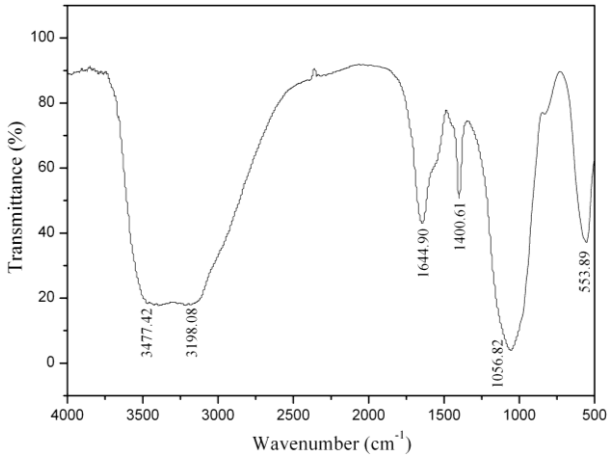
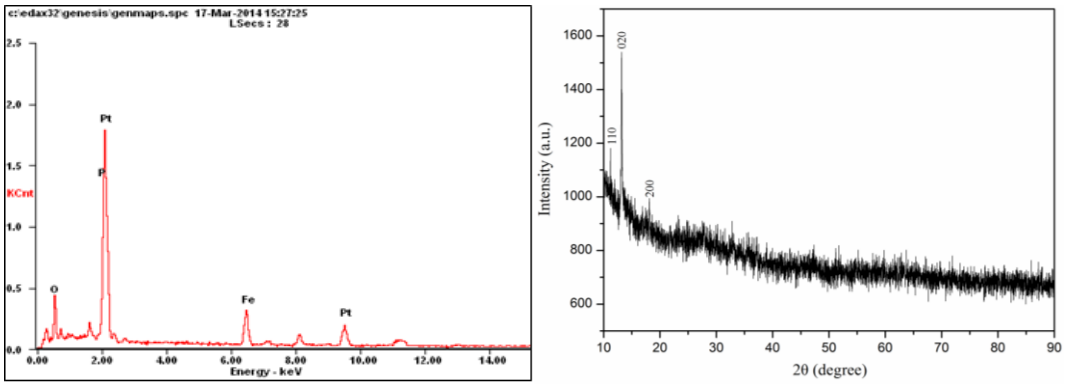


Fig. 1. The FTIR spectrum of Vivianitenanoparticles

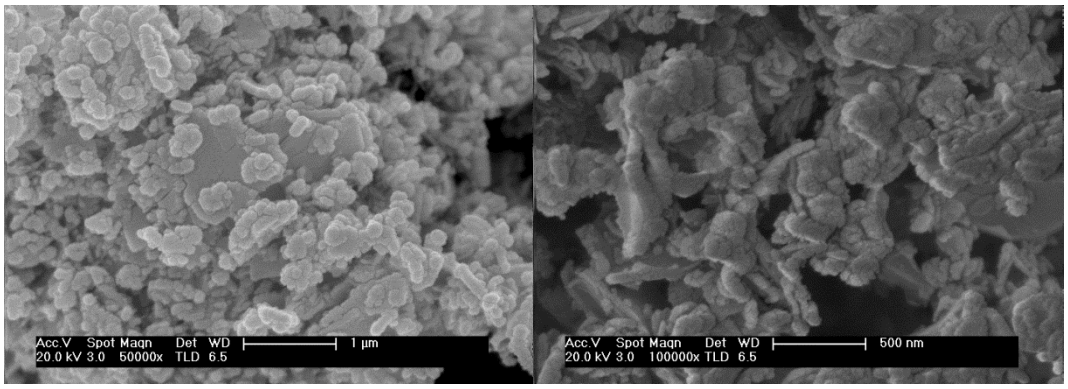
An elemental analysis of samplescomposition is performed using EDSto confirm the presence of elements O, Pand Fein the Vivianitesample(Fig. 2 a). The XRD analysis of materials further confirms that Vivianitepatterns can be readily indexed to the reported structures of $Fe_3(PO_4)_2 \cdot 8H_2O$ (JCPDS Card No.03-0070)(Fig. 2 b), and no peaks attributable to impurities are observed.



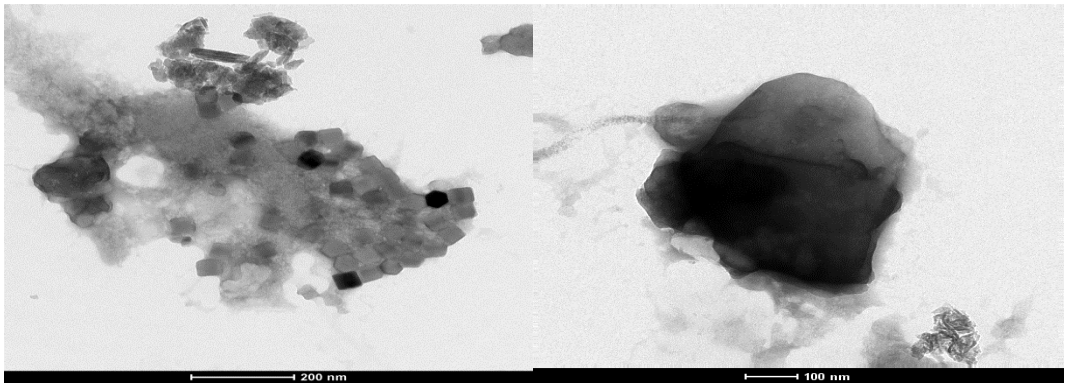
(a) (b)

Fig. 2. EDS spectrum and XRD patterns of Vivianitenanoparticles

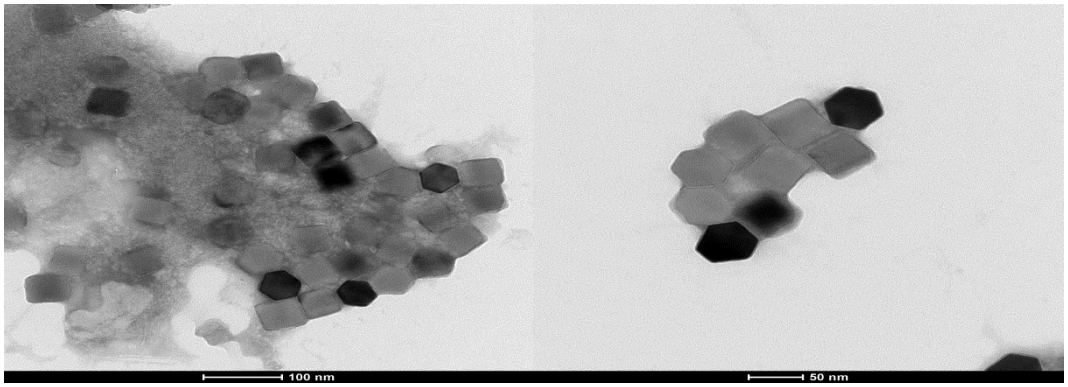
Fig. 3 showsSEM, TEMand ED images of Vivianitenanoparticlesto obtainunder the assistance of *Bacillus*. The SEM image showsthat synthesizedVivianitenanoparticlesin the form of aggregates and honeycomb-like in shape(Fig. 3 a, b).The TEM image shows that synthesizedVivianitenanoparticles arequadrilateral, hexagonaland irregular in shape witha size in the range of 20-200nm(Fig. 3 c, d, e, f). The average crystallite size is 31.89nm, whichcalculate by the Scherrer formula. The electron diffraction (ED)pattern indicatesthat the synthesized Vivianiteis well crystallized(Fig. 3 g).



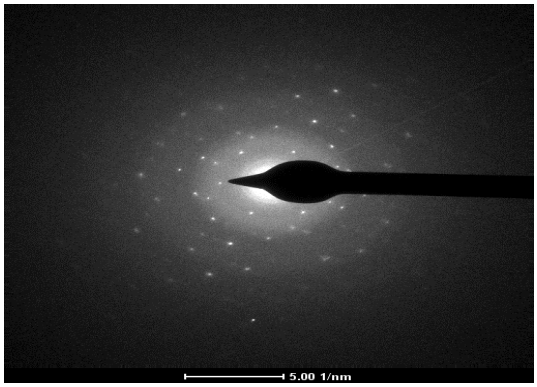
(a) (b)



(c) (d)



(e) (f)



(g)

Fig. 3. SEM images (a, b), TEM images (c, d, e, f)and ED pattern (g) of Vivianitenanoparticles

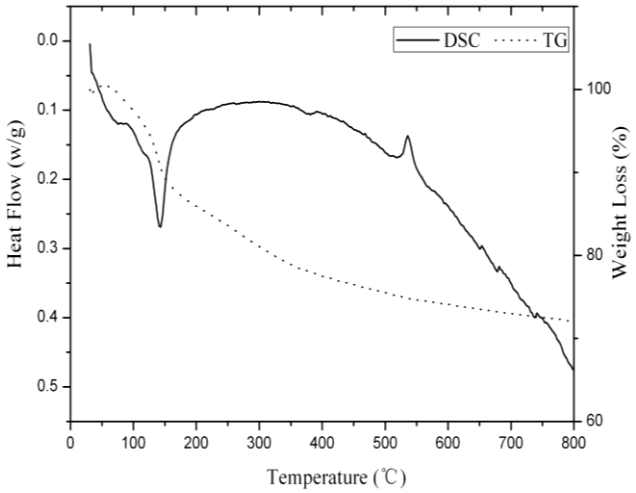


Fig. 4. TG-DSC curvesof Vivianitenanoparticles

Fig.4 shows the TG-DSC curves of Vivianitenanoparticles from room temperature to800°C.Over the temperature ranges, the TG curve shows continuous weight-loss about 28.38%,which consistswith the theoretical value (28.69%). The TG curves of nanoparticlesshow that maximum weight loss occurs in the temperature range61-250°C which is due the elimination of crystal water of Vivianitenanoparticles.From the results, it is observed that DSC curves show endothermic peaks at 142.1°C and 536.1°C forVivianitenanoparticles.The peak at 142.1°C can be considered as the decomposition points of the materials.The small peak around 536.1°C can correspond to crystallization of$Fe_3(PO_4)_2$.

4. Conclusions

This work showed that diverse sizes of the Vivianitenanoparticles can be obtained in the presenceof *Bacillus*.FTIR spectra, EDSand XRD diffraction graph confirmed the structure of Vivianite, SEM morphology analysis displays that theVivianite powder has a honeycomb-like shape, and TEM morphology further analysis shows that the shapeof Vivianite nanoparticlesappearsquadrilateral, hexagonal and irregular,and withaveragesize is31.89nm.TG curveexhibits that total mass loss of Vivianite is 28.38%, which consists with the theoretical value.Results of this research provide a new route to synthesize nano-sizedVivianite.

Acknowledgements

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