Digest Journal of Nanomaterials and Biostructures Vol. 11, No. 2, April – June 2016, p. 393 - 399

MICROBIAL-INDUCED SYNTHESIS OF NANOPARTICLES OF ZINC PHOSPHATE AND BASIC ZINC CARBONATE BASED ON THE DEGRADATION OF GLYPHOSATE

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Microbial-induced synthesis of nanoparticles of zinc phosphate and basic zinc carbonate based on the degradation of glyphosate, as a new green and environmental method**,** is being paid extensive attention to in that it has low cost, simple operation and rapid effects. This research, glyphosate was decomposed into phosphate ions, carbon dioxide and water under the enzymatic action of *Bacillus subtilis* and *Saccharomyces sp.*. Meanwhile, carbon dioxide was absorbed, transformed and produced carbonate ions under the enzymatic action of *Paenibacillus mucilaginosus*. Phosphate ions and carbonate ions which obtained by the degradation of glyphosate could mineralize zinc ions into phosphate and carbonate precipitations. The compositional and morphological properties of phosphate and carbonate precipitations were studied by means of X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and thermogravimetric analysis (TGA). The characterization data obtained showed that zinc phosphate and basic zinc carbonate were successfully synthesized by microbial method in this process. Zinc phosphate and basic zinc carbonate was shown to be nanometer-grade in size and sphere-like in shape. Furthermore, the phase composition, functional groups, and surface morphology of samples obtained by microbial method were mainly similar to that by chemical method.

(Received March 1, 2016; Accepted April 13, 2016)

Keywords: microbial-induced, degradation, mineralization, glyphosate, nanoparticles

1. Introduction

As a highly toxic pesticides, glyphosate are widely used in agricultural production and pest control. Due to the large amount of residue and degradation hardly, the long-term production and use of glyphosate has caused serious environmental pollution[1-4]. Heavy metals are another type of pollutants, and heavy metals pollution has become more serious than ever before[5]. The dual pollution of glyphosate and heavy metals has brought great challenges to the work of environmental protection.

Generally, three measures can be taken to control and remedy glyphosate and heavy metals pollution—physical method, chemical method and biological method[6-9]. Due to high energy consumption, large investment, complex operation and likely secondary pollution to the environment, physical and chemical methods are relatively difficult to be applied to the

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remediation of soil pollution in large areas[10-11]. Nevertheless, biological method has stable and reliable effects, no secondary pollution[12-17], which, therefore, has become the most promising method in degradation of glyphosate and remediation of heavy metals[18-20].

This research, *Bacillus subtilis*, *Saccharomyces sp.* and *Paenibacillus mucilaginosus* were selected based on the previous study. Glyphosate was decomposed into phosphate ions, carbon dioxide and water under the enzymatic action of *Bacillus subtilis* and *Saccharomyces sp.*. Meanwhile, carbon dioxide was absorbed, transformed and produced carbonate ions under the enzymatic action of *Paenibacillus mucilaginosus*. Phosphate ions and carbonate ions which obtained by the degradation of glyphosate could mineralize zinc ions into phosphate and carbonate precipitations. In addition, the particular composition, the morphology, and thermal decomposition properties of phosphate and carbonate precipitations were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared (FTIR), and differential scanning calorimetry-thermogravimetry (DSC-TG).

2. Materials and Methods

All the materials from commercial sources were used without further purification, and deionized water was selfmade. *Bacillus subtilis*, *Saccharomyces sp.* and *Paenibacillus mucilaginosus* were purchased from china center of industrial culture collection. Three kinds of microorganisms were cultured respectively in different conditions. Cultivation of the *Bacillus subtilis* was conducted in beef extract peptone culture (3 g of yeast extract, 5 g sodium chloride and 5 g of peptone were dissolved in deionized water to 1 L, and the pH value was adjusted to about 8.0) at 30 °C for 24 h. Cultivation of the *Saccharomyces sp.* was conducted in 1 L wort culture at 28 °C for 24 h. Cultivation of the *Paenibacillus mucilaginosus* was conducted in sucrose culture (10 g of sucrose and 3 g of sodium hydrogen phosphate were dissolved in deionized water to 1 L, and the pH value was adjusted to about 8.0) at 35 °C for 24 h. Then, the harvested microorganisms were kept in a refrigerator at 4 °C for stock prior to use respectively.

Biosynthetic method

500 mL of 100 mmol· L^{-1} $ZnSO_4$ and 500 mmol· L^{-1} glyphosate solution was prepared in advance. Next, 20 ml of *Bacillus subtilis* culture, 10 ml *Saccharomyces sp.* culture and 20 ml *Paenibacillus mucilaginosus* culture were added to above solution, respectively. The reaction solution was also allowed to stand under static conditions at room temperature for 24 hours. The precipitations were filtrated and washed three times with deionized water and ethanol, and then dried at 60°C in oven. Afterwards, the precipitates were collected and characterized.

Analysis of precipitations

The crystal structure of the precipitates was examined by X-ray diffraction (XRD) with Bruker D8-Discover diffractometer using graphite-monochromatized high-intensity Cu Kα radiation (λ = 1.5406 Å). The scanning angle range was from 10° to 80° 2θ with the step at 0.2 s·$step^{-1}$. SEM (FEI Company, Netherlands) with a GENESIS 60S energy dispersive X-ray spectroscope (EDS) spectroscopy system with magnification from 10,000 to 200,000 was used to observe the morphology and to measure the elemental compositions of the precipitations. The accelerating voltage and spot size of the secondary electron detector were 20 kV and 4.0, respectively.

The FTIR spectrum of the precipitations was recorded using a Nicolet 5700 spectrometer by KBr pellet technique with the resolution of 4 cm^{-1} and scanning the product for 20 times in the range of 4,000~400 cm^{-1}.

Thermogravimetric-differential scanning calorimetry (TG-DSC) analysis was carried out on STA449 F3 thermogravimetric analyzer (Netzsch, Germany). The analyses were carried out simultaneously in a nitrogen atmosphere at a heating rate of 10 $°C\cdot min^{-1}$ between room temperature and 1000 °C.

3 Results and Discussion

3.1 XRD analysis

The XRD pattern of precipitations obtained by microbial method is shown in Fig. 1. It was to be noted that the peaks of XRD patterns are well consistent with JCPDS card number 30-1489 and 19-1458, and the precipitations are characterized as mixture of zinc phosphate and basic zinc carbonate. Meanwhile, the precipitations were well crystallized, and almost no peaks attributable to impurities were observed. However, compared with the diffraction peak intensity of the zinc phosphate obtained by chemical method[21], it was slower relatively by microbial method. The diffraction peak intensity of the basic zinc carbonate obtained by microbial method was also slower in contrast with that by chemical method[22]. It was well known that microbial activity and microorganism had an effect on crystal growth and lead to poor crystallinity.

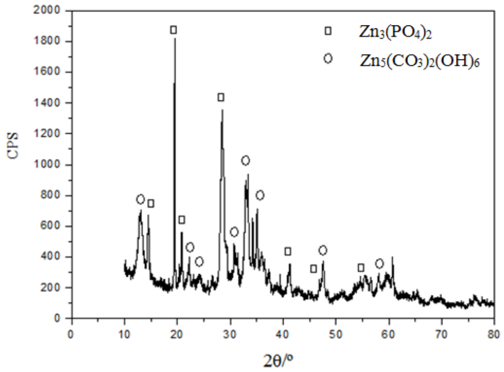
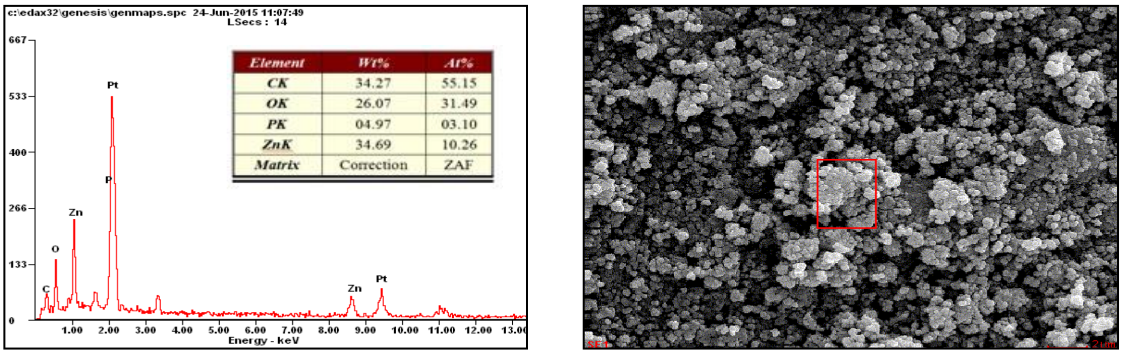


Fig. 1 The XRD pattern of precipitations obtained by microbial method

3.2 SEM analysis

EDS spectrum of precipitations obtained microbial method is shown in Fig. 2. An elemental analysis of precipitations composition was performed by EDS which certify the presence of elemental C, O, P and Zn in the sampled particles. It was in keeping with the XRD results and proved that biosynthesis of zinc phosphate and zinc basic carbonate based on microbial-induced degradation of glyphosate was feasible.



Element	*Wt%*	*At%*
CK	34.27	55.15
OK	26.07	31.49
PK	04.97	03.10
ZnK	34.69	10.26
Matrix	Correction	ZAF

Fig. 2 EDS spectrum of precipitations obtained microbial method

SEM photographs of precipitations obtained by microbial method are shown in Fig. 3. As Fig. 3 showed, the overall evaluation of the morphology of the precipitations indicated size-different and sphere-like of nanoparticles. By comparison, particle size of larger particles was 200~300nm, while that of smaller particles was 50~100nm. It was found that particle size of zinc phosphate particles was 20~80nm[21]. As a result, particle size of larger particles might be zinc basic carbonate, and that of smaller particles might be zinc phosphate.

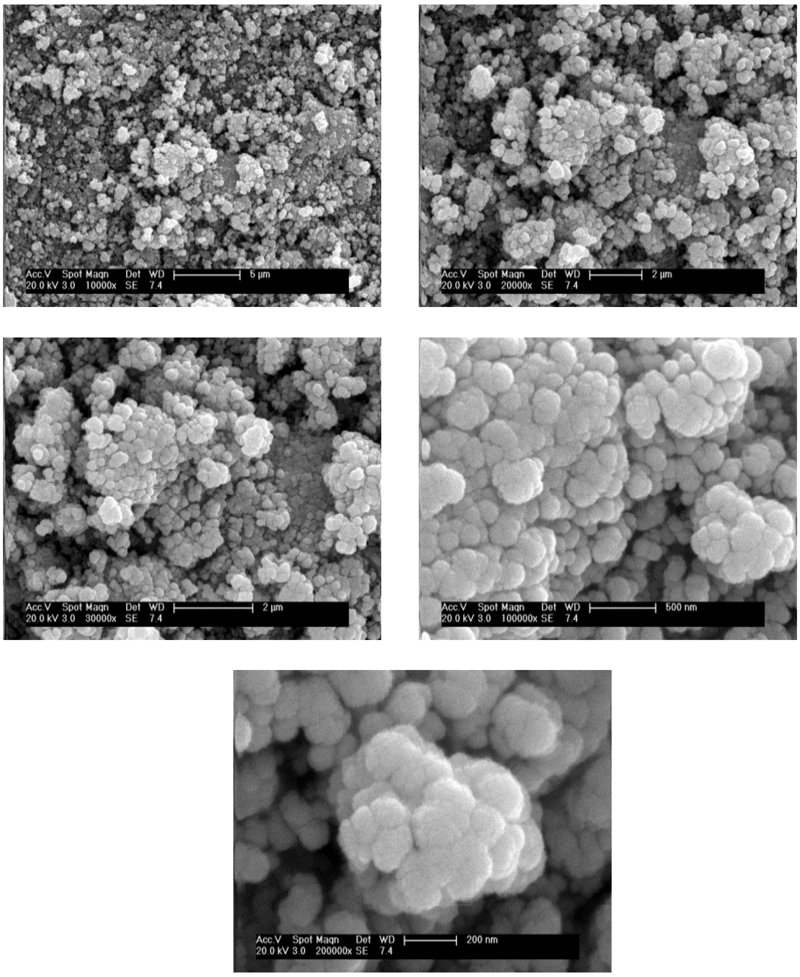


Fig. 3 Different magnification of SEM images of precipitations obtained by microbial method

3.3 FTIR analysis

In order to achieve complementary information about the precipitations, FTIR analysis was also conducted. The FTIR spectrum of precipitations is shown in Figure 4. Fig. 4 demonstrated FTIR results in the spectral region from 4,000 to 400 cm^{-1} .

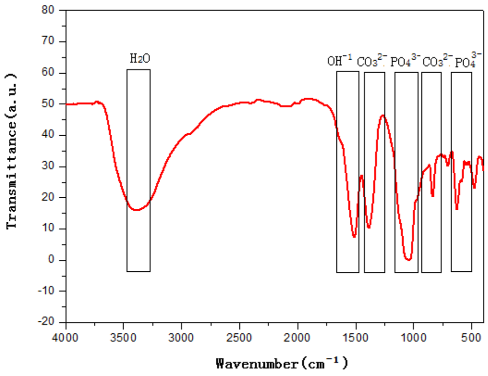


Fig. 4 FTIR spectra of precipitates obtained by microbial method

The vibrational absorptions appeared at 793 and 1389 cm^{-1} are corresponding to CO_3^{2-}, while the vibrational absorptions appeared at 671 and 1123 cm^{-1} were corresponding to PO_4^{3-}. The wide band of crystal H_2O at around 3,397 cm^{-1} could be observed in the FTIR analysis of the samples. Strong transmission band for OH^- was to be reflected in 1561 cm^{-1}. Above results demonstrated that the functional groups of precipitations obtained by microbial method were CO_3^{2-} , PO_4^{3-} and OH^- , and the precipitations are a mixture of zinc phosphate and zinc basic carbonate.

3.4 TG-DSC analysis

The thermal stability of the precipitations were determined by thermogravimetric analysis in nitrogen atmosphere as shown in Fig.5. Fig.5 showed that there were two obvious weight loss behaviors, which correspond to respective thermal changes of DSC curves. The first DSC peak occurs at around 86 °C, and the corresponding weight loss was 1.26 %, which might result from the crystal water. The second peak at around 256 °C corresponded to the crystalline of basic zinc carbonate, which decomposed from 241 °C to 278 °C and brought about 13.63 % of weight losses. Ma et al. [23]illustrated that the strong endothermic peak of basic zinc carbonate appeared at 243°C, which decomposed from 221 °C to 257 °C, and it was in line with the result by microbial method. There was no significant weight loss over 450 °C, and it indicates that the thermodynamic properties of zinc phosphate was stable in addition to water loss condition. The results of this research was in accord with the literature reports[24].

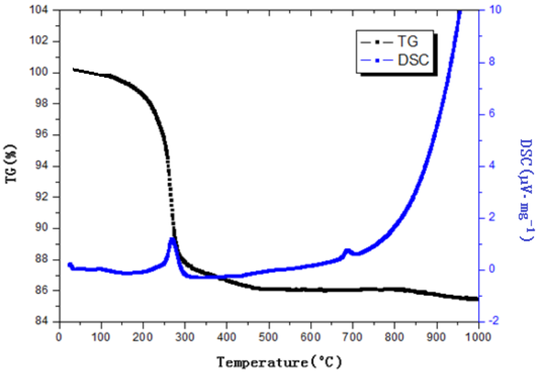


Fig. 5 Thermal analysis curves of precipitations obtained by microbial method

3.5 Mechanism

Microbial-induced synthesis of nanoparticles of zinc phosphate and basic zinc carbonate based on the degradation of glyphosate was a relatively complex physical and chemical process, and microbes played an important role in this process. Firstly, glyphosate was decomposed into phosphate ions, carbon dioxide and water under the enzymatic action of *Bacillus subtilis* and *Saccharomyces sp.*. Secondly, carbon dioxide was absorbed, transformed and produced bicarbonate ions under the enzymatic action of *Paenibacillus mucilaginosus*, and bicarbonate ions were transformed into carbonate ions under under the condition of alkaline environment. Finally, heavy metal ions in the solution were attracted to the bacteria cell wall due to the negative charge of the latter and upon addition of substrate to the bacteria. Meanwhile, heavy metal ions were mineralized and precipitated to phosphate and carbonate particles at the cell surface serving as the nucleation site. The mechanism of remediation of heavy metals pollution based on the degradation of glyphosate could be explained from the following equations:

$$\text{Glyphosate} \longleftrightarrow PO_4^{3-} + CO_2 + H_2O \tag{1}$$

$$CO_2 + H_2O \longleftrightarrow HCO_3^- + H^+ \tag{2}$$

$$HCO_3^- + OH^- \longleftrightarrow CO_3^{2-} + H_2O \tag{3}$$

$$Zn^{2+} + \text{Cell} \longleftrightarrow \text{Cell-}Zn^{2+} \tag{4}$$

$$\text{Cell-}Zn^{2+} + CO_3^{2-} + PO_4^{3-} \longleftrightarrow \text{Cell-}Zn_5(CO_3)_2(OH)_6\text{-}Zn_3(PO_4)_2 \tag{5}$$

4. Conclusions

This research, we conducted a fundamental laboratory experiment on the synthesis of nanoparticles of zinc phosphate and basic zinc carbonate by microbial-induced degradation of glyphosate and mineralization of zinc ions. In this process, *Bacillus subtilis*, *Saccharomyces sp.* and *Paenibacillus mucilaginosus* were selected based on the previous study. Glyphosate was decomposed into phosphate ions, carbon dioxide and water under the enzymatic action of *Bacillus subtilis* and *Saccharomyces sp.*. Meanwhile, carbon dioxide was absorbed, transformed and produced carbonate ions under the enzymatic action of *Paenibacillus mucilaginosus*. Phosphate ions and carbonate ions which obtained by the degradation of glyphosate could mineralize zinc ions into phosphate and carbonate precipitations. Compared with zinc phosphate and basic zinc carbonate prepared by conventional chemical method, it shows a similar property of production. XRD diffraction graph, EDS spectrum and FTIR spectra confirmed the structure of the mixture of zinc phosphate and basic zinc carbonate, and SEM morphology analysis shows that the mixture has a sphere-like shape and nano-sized particles.

Acknowledgements

This work was supported financially by the National Nature Science Foundation of China (Grant No. 51372038), Key Research and Development Program of Jiangsu Science and Technology Department (Grant No. BE2015678), Science and Technology Research Program of Jiangsu Transport Department (Grant No. 2015T32) and Scientific Research Foundation of Graduate School of Southeast University (YBJJ1566).

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