Int. J. Electrochem. Sci., 12 **(2017)** 842 – 851, doi: 10.20964/2017.02.23

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Biosynthesis of Gold Nanoparticles Using *Pleurotus ostreatus* extract with Their Electrochemical Activity of Detection of Carbendazim in vegetable

Wei Wang[1], *Fangjie Yao*[1,*], *Daxiang Wang*[4], *Xudan Zhou*[1], *Xiaomei Wang*[2], *Chengai Zhao*[3], *Ming Fang*[1] *and Jing Cai*[1]

[1] College of Horticulture, Jilin Agricultural University, No.2888 Xincheng St, Changchun, Jilin, 130118, P.R. China
[2] College of Agricultural, Jilin Agricultural University, No.2888 Xincheng St, Changchun, Jilin, 130118, P.R. China
[3] Collage of Resources and Environment. Jilin Agricultural University, No.2888 Xincheng St, Changchun, Jilin, 130118, P.R. China
[4] Xinyang of Henan Province Hydroglogy and Water Resources Survey Bureau， Xinyang, Henan, P.R.China,
[*]E-mail: yaofangjie_228@qq.com

Received: 28 October 2016 / *Accepted:* 5 December 2016 / *Published:* 30 December 2016

Biosynthesis has attracted numerous attentions recently in the field of nanomaterial synthesis owing to its non-toxicity and environmental protection. Herein, AuNPs were successfully prepared by biosynthesis with *Pleurotus ostreatus* produced laccase as reducing agent. The formation of metallic Au was confirmed by both UV-vis spectroscopy, X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). As shown form the results of scanning electron microscopy, the mean size of the biosynthesized AuNPs were 47 nm. The biosynthesized AuNPs were then applied for the modification of screen printed electrode. The electrochemical sensor constructed with AuNPs/SPE electrode demonstrated remarkable performance towards the determination of carbendazim in vegetable.

Keywords: Biosensor; Carbendazim; Electrode modification; *Pleurotus ostreatus*; Au NPs

1. INTRODUCTION

Development of nanomaterials with controllable morphology and remarkable properties have gained wide attention from both academia and industries with regard to the applications in various fields such as electrodes, sensing and energy devices [1, 2]. The size of nanomaterials is of great importance to its prospect future in many fields, and the desired size ranges from 1 to 100 nm. As a kind of nanomaterials, metal nanoparticles have demonstrated promising potential applications such as

supercapacitor [3], photocatalysis [4], absorbent [5, 6], sensing [7], optoelectronics [8, 9] and thermal management [10, 11]. Until now, a large amount of metals including silver [12, 13], gold [14, 15], copper [16, 17], zinc [18, 19], titanium [20, 21], cadmium [22, 23], iron [24, 25], palladium [26, 27] and platinum [28, 29] can be synthesized successfully in nanometer size. Gold nanoparticles have a wide variety of uses such as catalysis, biosensor, surface enhanced Raman spectroscopy, immunosensing, vapor sensing and drug delivery. Recently, a lot of synthetic methods including chemical vapor [30] or electrochemical [31] deposition, green chemical synthesis method have been developed for the preparation of AuNPs.

As a newly developed research field in the preparation of metal nanomaterials, biosynthesis has numerous advantages including environmental protection, non-toxicity and excellent pharmaceutical compatibility, which makes it outperforms other physical or chemical synthesis method. A great deal of environment-friendly substances including bacterial, leaf extract, algae and fungi could be applied as reducing agent for the reduction of metal salt. As to Au nanoparticles, certain substances including bamboo (*Bambusa chungii*) leaf extracts, *Ficus racemosa* latex, ethanolic extract of *Brassica oleracea* L and fungus *Penicillium chrysogenum* as well have been verified to be effective as reducing agent.

In this study, a new biosynthesis method was proposed for the preparation of AuNPs with laccase as reducing agent which could be extracted from *Pleurotus ostreatus*. The morphology and properties of as-synthesized Au nanoparticle were investigated with a series of characterization methods. The biosynthesized AuNPs have demonstrated outstanding electrochemical performance towards the determination carbendazim (methyl 1H-benzimidazol-2-ylcarbamate) in vegetable. The study shows the possibility of the application of biosynthesized nanomaterial in the electrochemical field.

2. EXPERIMENTS

Pleurotus ostreatus was supplied by local nursery. 5 mg of carbon source that contains 66% moisture was added to 8 mL of distilled water and then the fermentation was performed in Erlenmeyer flasks with the volume of 250 mL. After certain amount of inducers were added, the mixture was autoclaved at 121 °C for 20 min. 2 mL of spore suspension with the concentration of $\sim 8 \times 10^6$ spores/mL was used as medium. The fungus was mixed into the above-mentioned medium and then incubated at 29 °C statically in complete darkness.

After seven days, the fermentation broth in Erlenmeyer flask were soaked in 100 mL of citrate phosphate buffer with the concentration of 1 mM and pH of 5 for 2 h. And then the mixture was placed in a shaker (LAB-Line R Orbit Environ, U.S.A) with the rotation rate of 200 rpm. Finally, the mixture was extracted in tincture press and treated with cooling centrifugation (Hettich Universal 16 R, Germany) at 6 °C for 10 min in order to remove particles.

Spectrophotometrically (JASCO V/560 UV/Vis, Japan) with the wavelength of 525 nm was employed for the laccase activity measurement, and the mixture of 1 mM syringaldazine ($\epsilon = 65\ mM^{-1}\ cm^{-1}$), culture filtrate and 50 mM phosphate buffer with pH of 5 was used as reaction medium. The measured absorbance increase after 4 min can be applied for the calculation of degree of

syringaldazine oxidation. Enzyme activity was expressed in units (U) and one unit was defined as 1 μmol of syringaldazine oxidized per min.

Ammonium sulphate was mixed into the cell free filtrate which was obtained from *Pleurotus ostreatus*to that attains 80% saturation and then the mixture was kept at 4 °C for 48 h. The precipitate was obtained by centrifugation at 4 °C for 20 min with the centrifugal speed of 2500 g. The pellet was dissolved in 50 mL of citrate phosphate buffer with the concentration of 1 mM and pH of 5. In order to remove substances that will interfere with the enzyme activity as previously reported, the precipitate was desalted by dialysis bag. Bradford assay was applied for the quantification of protein concentration and bovine serum albumin was used as standard.

Laccase was incubated with activators or inhibitors, optimized buffer and syringaldazine, and the reaction mixture was kept at optimized temperature. In order to evaluate the effect of activators and inhibitors on the activity of enzyme, spectrophotometric assays were applied to measure the change of absorbance. Results were conveniently shown as percentage of non-treated laccase.

AuNPs were synthesized by mixing 0.1 mL of tetrachloroauric acid (10 mg/L) into 3 mL of laccase enzyme that contains 250 IU/mg under stirring. The colour of solution started to change from yellow to pink and then violet within 90 min. As affirmed by UV/Visible spectrophotometer, AuNPs were successfully prepared. Particle sizing system was used for the determination of average particle size and the corresponding size distribution. Spectrophotometer was applied for the measurement of UV/Visible spectra of AuNPs and the measuring range was 300-800 nm at a resolution of 1 nm. Spectrophotometer with KBr pellet was employed for the FT-IR measurements. Scanning electron microscope (SEM) was employed for the investigation of size and morphology of as-synthesized AuNPs.

Three electrode system with biosynthesized AuNPs modified screen printed electrode (SPE) as working electrode, Pt foil as counter electrode and saturated Ag/AgCl as reference electrode has been developed for the electrochemical detection of carbendazim. The modification of SPE surface by biosynthesized AuNPs was performed by dropping 0.1 mL of biosynthesized AuNPs with the concentration of 1 mg/mL onto the surface of SPE and then the SPE surface was dried at room temperature. The enhancement of electron transfer performance of electrode surface after modification was evaluated with electrochemical impedance spectroscopy (EIS). Specific experimental parameters are as follows: 5 mM $[Fe(CN)_6]^{3-/4-}$ as probe, 0.1 M KCl as supporting electrolyte, 10^1-10^5 Hz as frequency range and 5 mV as amplitude .

The modified SPE with biosynthesized AuNPs was applied for the analysis of carbendazim in vegetable sample with linear sweep voltammetry (LSV) method. Typically, 500μL of component solvent containing ethylene glycol and choline chloride was added into 1 g of vegetable sample in a Petri dish. After 15 min, the Au/SPE electrode was inset into the soil sample.

3. RESULTS AND DISCUSSION

The biosynthesis reaction began immediately at the moment that *Pleurotus ostreatus* extract was mixed into the $HAuCl_3$ solution. The nucleation of AuNPs started immediately as can be seen from the color of solution changing from initial light yellow to purplish yellow. For the newly formed

AuNPs dispersion, surface plasmon resonances of AuNPs was observed owing to the scattering and absorbing effect of metallic nanoparticles for light at a particular wavelength. Numerous factors such as morphology, size and solvent will affect the optical property of AuNPs. As can be seen from the UV-vis spectrum of biosynthesized AuNPs (Fig. 1A), a distinct absorption peak at 485 nm that could be attributed to the surface plasmon resonance of AuNPs was observed, indicating the successful preparation of metallic Au. In addition, the average size of formed AuNPs could be indirectly identified from the peak position of surface plasmon resonance. The mean size of the biosynthesized AuNPs was concluded to range from 25 to 70 nm.

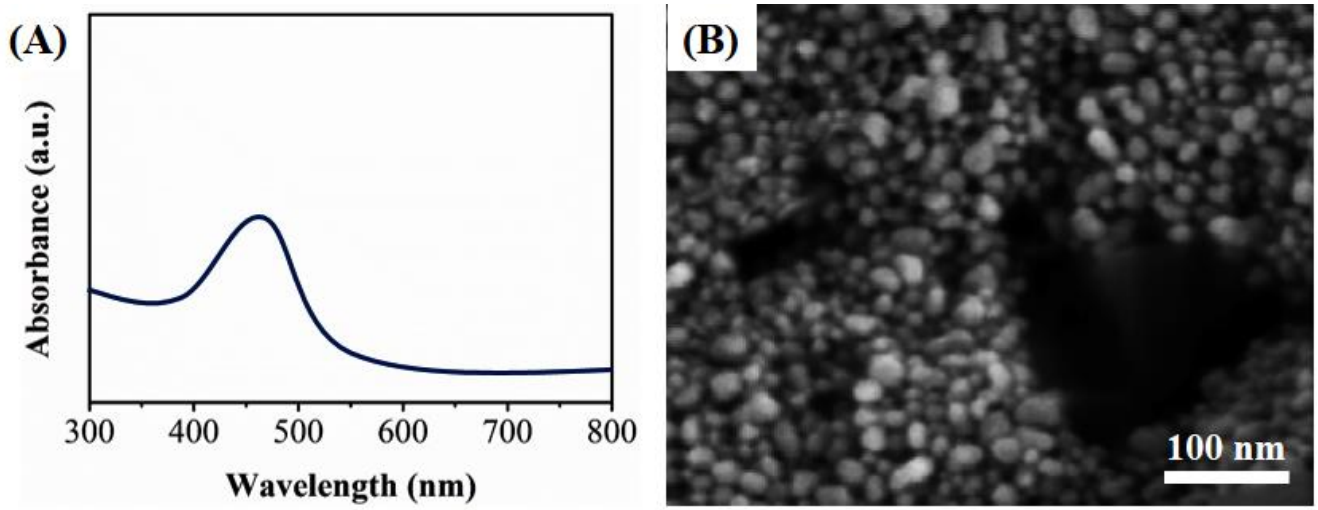

Figure 1. (A) UV-vis spectrum and (B) SEM image of biosynthesized AuNPs.

X-ray photoelectron spectroscopy (XPS) was employed for the investigation of formed AuNPs as well. As shown in the Au_{4f} high resolution XPS (Fig. 2A), the observed peaks at 83.4 eV and 87.3 eV could be attributed to $Au_{4f\ 7/2}$ and $Au_{4f\ 5/2}$ peaks, respectively [32, 33]. Owing to the surface attachment of *Pleurotus ostreatus* extract which was used as reducing agent in the preparation of AuNPs, the observed Au_{4f} peaks exhibited a shift from the theoretical peak position of Au^0 that is at 84.1 and 87.6 eV, respectively.

As shown form the XRD pattern of biosynthesized AuNPs (Fig. 2B), the observed diffraction peaks at 39.3°, 45.9°, 67.7° and 81.9° can be ascribed to (111), (200), (220) and (311) planes of Au in face-centered-cubic (fcc) structure (JCPDS 4-0783), respectively. Therefore, metallic Au was successfully prepared as confirmed by XRD result. The broadening of Bragg's peaks indicates the formation of NPs. Nearly monodispersed Au NPs with controllable size and uniform shape can be easily obtained in the simple aqueous reduction method. The mean size of Au NPs was calculated using the Debye–Scherrer's equation by determining the width of the (111) Bragg's reflection [34].

Fig. 3 showed FTIR spectra of laccase before and after the formation of AuNPs. The intensity of the peaks of functional groups displayed certain change after the formation of AuNPs. The peaks at 3016 cm^{-1} and 1631 cm^{-1} correspond to OH/NH functional groups and carbonyl group, respectively. The changes of peak intensity for both above-mentioned two peaks could be attributed to secondary

amide structure. Thus FTIR analysis clearly shows that capping and reducing of NPs by biomolecules present in seed aqueous extract of *Pleurotus ostreatus* could be responsible for prolonged stability.

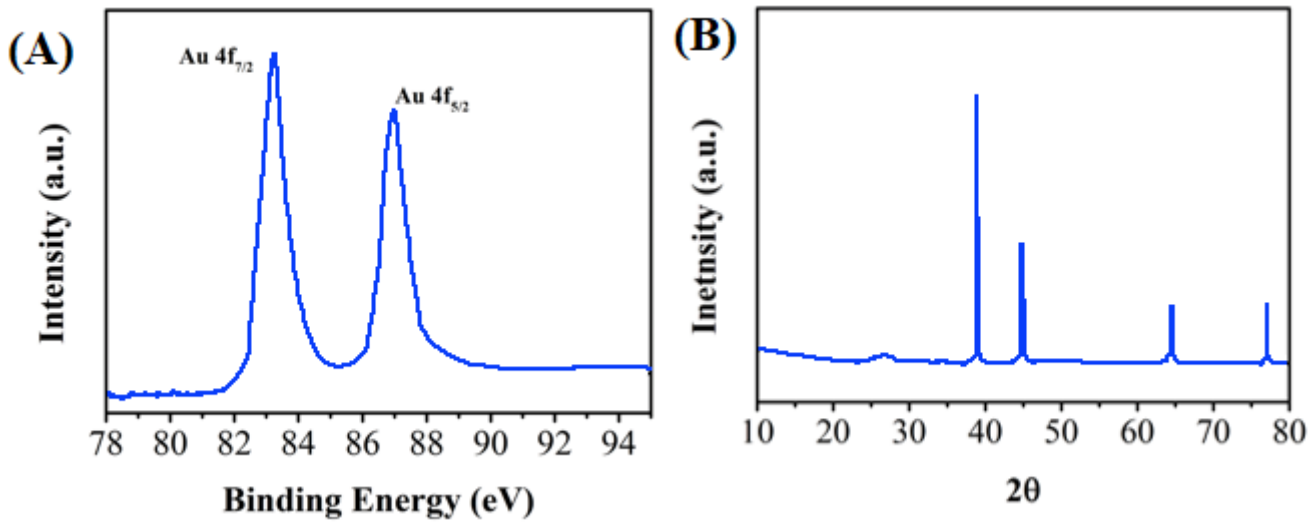

Figure 2. (A) High resolution Au 4f scan and (B) XRD pattern of biosynthesized AuNPs.

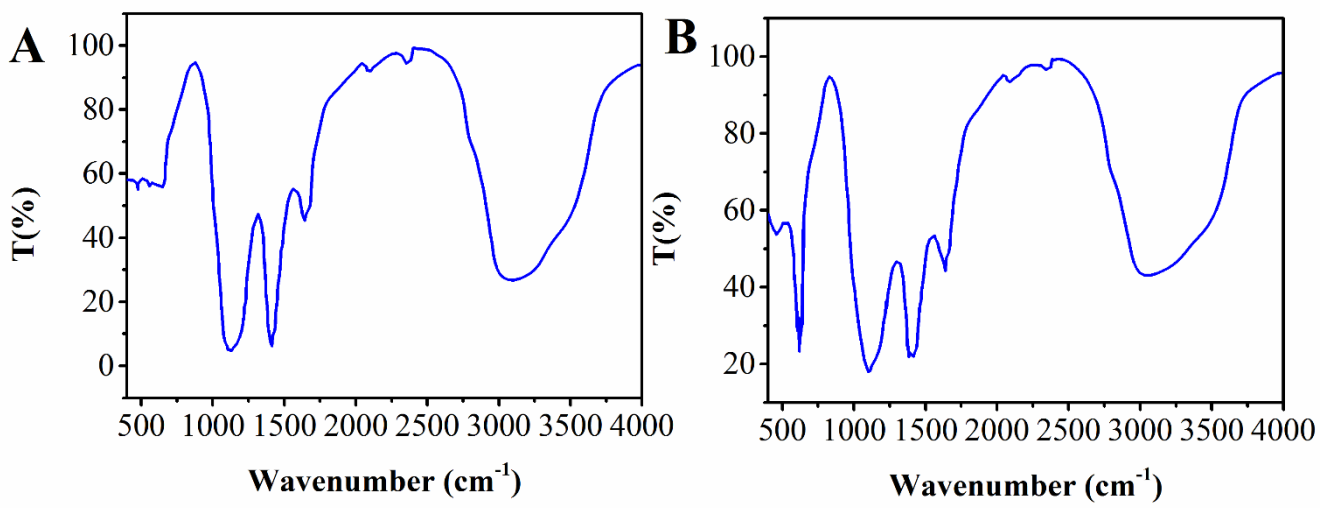

Figure 3. FTIR spectra of laccase (A) before and (B) after the formation of AuNPs.

The surface active area of biosynthesized AuNPs modified SPE was compared with that of bare SPE by CV scans. As shown from the CV scans obtained on bare SPE and Au/SPE electrode in 0.5 M H_2SO_4 (Fig. 4A), the background obtained with Au/SPE electrode was obviously higher than that obtained with bare SPE, suggesting that the electrode surface area was largely enhanced after the modification of SPE electrode with biosynthesized AuNPs. Electrochemical impedance spectroscopy (EIS) was employed for investigating the interface properties of both electrodes as well. As can be seen from the Nyquist plots obtained with both electrodes (Fig. 4B), the diameter of semicircle in the high frequency region obtained with Au/SPE electrode was much smaller than that obtained with bare

SPE electrode, demonstrating that the electron transfer resistance decreased after the modification of SPE electrode with biosynthesized AuNPs. The calculated electron transfer resistance with Randle equivalent circuit mode was 180 and 111 Ω for bare SPE and Au/SPE electrodes, respectively. In addition, the content of electroactive substance on Au/SPE was calculated to be 4.55×10^{-5} μM/cm with Laviron equations [35].

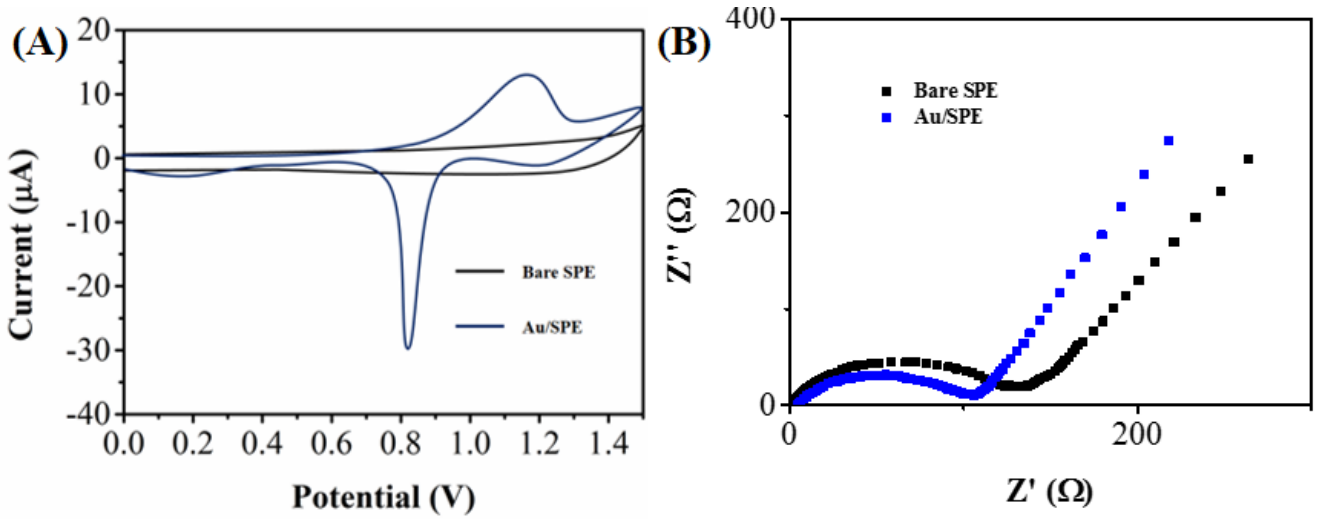

Figure 4. (A) CVs obtained with bare SPE and Au/SPE electrodes in 0.5 M H_2SO_4 at scan rate of 50 mV/s. (B) Nyquist plots obtained with bare SPE and Au/SPE electrodes in the presence of 5 mM $Fe(CN)_6 3^{-/4-}$ and 0.1 M KCl.

CV profiles of carbendazim oxidation in PBS (1 μM) was obtained on bare SPE and biosynthesized AuNPs modified SPE and the results were shown in Fig. 5A. The obtained current with bare SPE at potential of 0.96 V was quite small, indicating that the carbendazim oxidation at bare SPE electrode was very weak. In contrast, the current response obtained with Au/SPE was much higher. It was found that no peak response could be observed without the addition of carbendazim, suggesting that the peak at potential of 0.73 V indeed belongs to the carbendazim oxidation process. The potential of oxidation peak was observed to shift, indicating that the over potential of carbendazim oxidation was lower owing to the electrocatalytic activity of the biosynthesized AuNPs.

The amperometric response of Au/SPE electrode in function of successive addition of carbendazim was shown in Fig. 5B. The response was able to achieve steady-state in 4 s after the addition of carbendazim, indicating the extremely fast detection process of Au/SPE electrode. As shown form Fig. 5B, the current responses were related linearly with carbendazim concentrations ranging from 0.05 μM to 50 μM. The detection limit was 10 nM according to the ratio of signal to noise being 3. The performance of Au/SPE based carbendazim electrochemical sensor was compared with that of other reported sensors and the results were shown in Table 1. It was found that the performance of biosensor based on AuNPs/SPE electrode for carbendazim determination was comparable with other sensors.

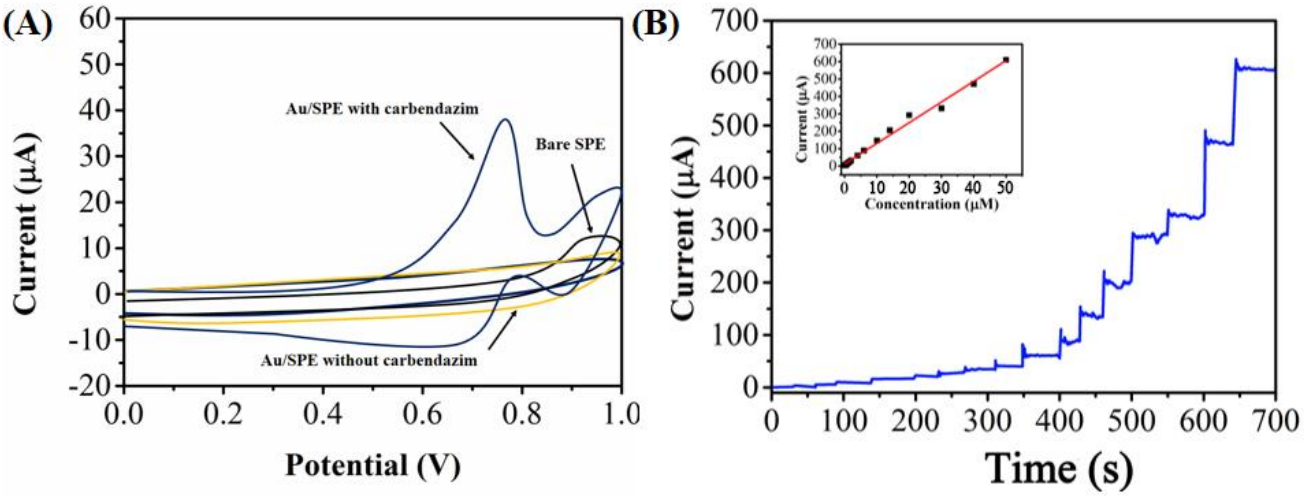

Figure 5. (A) CVs of carbendazim oxidation obtained on bare SPE and Au/SPE electrodes with scan rate of 50 mV/s. (B) Amperometric response obtained on Au/SPE electrode with the potential of 0.73 V in function of successive addition of carbendazim into PBS. Inset shows the magnification of current responses in the concentration range from 0.05 to 25 μM.

Table 1. Comparison of constructed carbendazim electrochemical sensor based on AuNPs/SPE with other previous reported sensors.

Electrode	LDR (μM)	LOD (μM)	Reference
Cyclodextrin–graphene	0.005-0.45	0.002	[36]
Multiwalled carbon nanotubes-polymeric methyl red film	0.2-10	0.009	[37]
GO–MWNTs	0.01-4	0.005	[38]
Carboxylic group functionalized poly(3,4-ethylenedioxythiophene)	0.012–0.35	0.0035	[39]
Carbon fiber	0.5-7	0.03	[40]
Multiwalled carbon nanotube	0.05-10	0.007	[41]
Diamond electrode	0.5-20	0.03	[42]
Tricresyl phosphate	0.05-10	0.01	[43]
Biosynthesized Au/SPE	0.05-50	0.01	This work

CV responses of 50 μM carbendazim at AuNPs/SPE were measured after 4 days, 1 week, and 2 weeks, and the percentages of current lost were found to be 3.5 % (after 4 days), 7.7 % (1 week), and 12.5 % (2 weeks), respectively. The possible reason for this drop might be due to loss of the composite from the electrode during storage due to either physical or chemical processes [44].

The biosensor constructed with biosynthesized AuNPs modified SPE electrode was applied for analyzing traces of carbendazim in vegetable sample as well. Two vegetable samples were purchased from local markets as real samples. As can be seen from the detected concentration of carbendazim in the two real samples (Table 2), the electrochemical sensor based on biosynthesized AuNPs modified SPE demonstrated excellent performance for the detection performances of carbendazim in vegetable samples. Consequently, the proposed carbendazim electrochemical sensor constructed with

AuNPs/SPE demonstrated promising potential applications in the real determination of carbendazim in various food samples.

Table 2. Detection of carbendazim in vegetable sample with electrochemical sensor constructed with biosynthesized AuNPs modified SPE.

Sample	Added (μM)	Found (μM)	Recovery (%)
Vegetable sample 1	0	0	—
	1	1.054	105.4
	2	1.977	98.9
	5	5.120	102.4
Vegetable sample 1	0	0	—
	0.5	0.522	104.4
	10	10.21	102.1
	30	28.44	94.8

4. CONCLUSIONS

In conclusion, a biosynthesis method with *Pleurotus ostreatus* produced laccase as reducing agent was developed for the preparation of spherical Au NPs. The average diameter of as-prepared AuNPs was 47 nm. The electrochemical sensor constructed with biosynthesized AuNPs modified SPE demonstrated excellent performance in the determination of carbendazim. The linear detection range and detection limit were found to be 0.05-50 μM and 10 nM, respectively. Moreover, the proposed electrochemical sensor could be employed for the determination of carbendazim in real vegetable samples.

ACKNOWLEDGMENTS
The study was supported by The Molecular Identification of Mating Type System and Construction of Analysis Platform of Mating Type Factors for Wood Ear (31272219) and The Foundation of Model Establishment and Model Reduction of Northeast Cold Facilities Vegetables Cost Pesticide Technology (2016YFD0201004)

References

1. S. Guo and E. Wang, *Nano Today*, 6 (2011) 240
2. P.C. Ray, *Chemical reviews*, 110 (2010) 5332
3. X. Peng, L. Peng, C. Wu and Y. Xie, *Chemical Society Reviews*, 43 (2014) 3303
4. Y. Ma, X. Wang, Y. Jia, X. Chen, H. Han and C. Li, *Chemical reviews*, 114 (2014) 9987
5. Z. Xiao, Z. Yang, L. Wang, H. Nie, M.e. Zhong, Q. Lai, X. Xu, L. Zhang and S. Huang, *Adv. Mater.*, 27 (2015) 2891
6. Z. Xiao, Z. Yang, L. Wang, H. Nie, M.e. Zhong, Q. Lai, X. Xu, L. Zhang and S. Huang, *Adv. Mater.*, 27 (2015) 2890
7. V. Biju, *Chemical Society Reviews*, 43 (2014) 744

8. D. Jariwala, V.K. Sangwan, L.J. Lauhon, T.J. Marks and M.C. Hersam, *Chemical Society Reviews*, 42 (2013) 2824
9. H. Chang and H. Wu, *Adv Funct Mater*, 23 (2013) 1984
10. M.J. Meziani, W.L. Song, P. Wang, F. Lu, Z. Hou, A. Anderson, H. Maimaiti and Y.P. Sun, *Chemphyschem : a European journal of chemical physics and physical chemistry*, 16 (2015) 1339
11. V. Tyagi, A. Pandey, D. Buddhi and R. Kothari, *Energy and Buildings*, 117 (2016) 44
12. H. Lu, D. Zhang, J. Cheng, J. Liu, J. Mao and W.C. Choy, *Adv Funct Mater*, 25 (2015) 4211
13. M.S. Abdel-Aziz, K.S. Abou-El-Sherbini, E.M. Hamzawy, M.H. Amr and S. El-Dafrawy, *Applied biochemistry and biotechnology*, 176 (2015) 2225
14. D. Pan, Y. Gu, H. Lan, Y. Sun and H. Gao, *Anal. Chim. Acta.*, 853 (2015) 297
15. M. Kodiha, Y.M. Wang, E. Hutter, D. Maysinger and U. Stochaj, *Theranostics*, 5 (2015) 357
16. D. Chattopadhyay and B. Patel, *Universal Journal of Chemistry (UJC)*, 1 (2016) 24
17. D. Lopez-Espiricueta, E. Fearon, S. Edwardson and G. Dearden, *Physics Procedia*, 83 (2016) 194
18. X. Xiang, F. Xia, L. Zhan and B. Xie, *Sep. Purif. Technol.*, 142 (2015) 227
19. J. Joseph, R. Tangsali, V.M. Pillai, R. Choudhary, D. Phase and V. Ganeshan, *Mater. Res. Bull.*, 61 (2015) 475
20. H. Kangarlou, M.M. Aghgonbad and A. Abdollahi, *Mat. Sci. Semicon. Proc.*, 30 (2015) 1
21. R. Garci-Contreras, R. J. Scougall-Vilchis, R. Contreras-Bulnes, H. Sakagami, R.A. Morales-Luckie and H. Nakajima, *Journal of Applied Oral Science*, 23 (2015) 321
22. S.M. Goodman, H. Noh, V. Singh, J.N. Cha and P. Nagpal, *Applied Physics Letters*, 106 (2015) 083109
23. Y. Su, A.S. Adeleye, Y. Huang, X. Zhou, A.A. Keller and Y. Zhang, *Nature Publishing Group. Scientific Reports*, 6 (2016) 26918
24. M. Masnadi, N. Yao, N. Braidy and A. Moores, *Langmuir*, 31 (2015) 789
25. K. Nielsen, Y. Kalmykova, A.-M. Strömvall, A. Baun and E. Eriksson, *Science of the Total Environment*, 532 (2015) 103
26. S.J. McKeown and L.L. Goddard, Hydrogen Detection Using a Single Palladium Nano-Aperture on a Fiber Tip, Lab-on-Fiber Technology, Springer 2015, pp. 181.
27. M.R. Awual, M.M. Hasan and H. Znad, *Chem. Eng. J.*, 259 (2015) 611
28. M. Habibi, M. Fanaei and Z. Emami, *Microelectronic Engineering*, 131 (2015) 29
29. L. Peeters, A.J. Keller, V. Umansky, D. Mahalu and D. Goldhaber-Gordon, *Journal of Vacuum Science & Technology B*, 33 (2015) 051803
30. M. Motaghedifard, M. Behpour, S.M. Ghoreishi and E. Honarmand, *Russian Journal of Electrochemistry*, 52 (2016) 477
31. K.W. Kim, W. Song, M.W. Jung, M.-A. Kang, S.Y. Kwon, S. Myung, J. Lim, S.S. Lee and K.-S. An, *Carbon*, 82 (2015) 96
32. R. Leppelt, B. Schumacher, V. Plzak, M. Kinne and R. Behm, *J. Catal.*, 244 (2006) 137
33. J. Li, C.-y. Liu and Y. Liu, *Journal of Materials Chemistry*, 22 (2012) 8426
34. H. Borchert, E.V. Shevchenko, A. Robert, I. Mekis, A. Kornowski, G. Grübel and H. Weller, *Langmuir*, 21 (2005) 1931
35. E. Laviron, *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, 52 (1974) 355
36. Y. Guo, S. Guo, J. Li, E. Wang and S. Dong, *Talanta*, 84 (2011) 60
37. J. Li and Y. Chi, *Pesticide Biochemistry and Physiology*, 93 (2009) 101
38. S. Luo, Y. Wu and H. Gou, *Ionics*, 19 (2013) 673
39. Y. Yao, Y. Wen, L. Zhang, Z. Wang, H. Zhang and J. Xu, *Anal. Chim. Acta.*, 831 (2014) 38
40. M.G. de la Huebra, P. Hernández, O. Nieto, Y. Ballesteros and L. Hernández, *Fresenius J Anal Chem*, 367 (2000) 474
41. P.A. Sundari, S. Palaniappan and P. Manisankar, *Analytical letters*, 43 (2010) 1457
42. R.F. França, H.P.M. de Oliveira, V.A. Pedrosa and L. Codognoto, *Diamond and Related Materials*,

27–28 (2012) 54
43. A.M. Ashrafi, J. Đorđević, V. Guzsvány, I. Švancara, T. Trtić-Petrović, M. Purenović and K. Vytřas, *Int. J. Electrochem. Sci*, 7 (2012) 9717
44. T.S.H. Pham, L. Fu, P. Mahon, G. Lai and A. Yu, *Electrocatalysis*, 7 (2016) 411