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GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLES USING *PENICILLIUM AURANTIOGRISEUM, PENICILLIUM CITRINUM* AND *PENICILLIUM WAKSMANII*

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Biosynthesis of metal oxide nanoparticles using microorganisms is an important area of research in nanobiotechnology which is an emerging eco-friendly science of well-defined sizes, shapes and controlled monodispersity. The present study proposed a green process for the extracellular production of copper oxide nanoparticles (NPs). Copper NPs synthesized and stabilized using *Penicillium aurantiogriseum, Penicillium citrinum* and *Penicillium waksmanii* isolated from soil. The synthesized NPs were formed fairly uniform with spherical shape determined by Scanning Electron Microscope (SEM). Dynamic Light Scattering (DLS) was carried out by nano zetasizer to ascertain the size and polydispersity of NPs. The results were further supported by UV-vis and fluorescence Spectrum to show the presence of some secreted proteins from fungi through the culture which are capable of hydrolyzing metal precursors to form metal oxides extracellularly. In addition, we investigated the effect of several parameters on the particle size and the polydispersity index for the synthesis of nanoparticles in the ambient condition. By this approach, it is suggestive that this rapid synthesis of nanoparticles would be proper for developing a biological process for mass scale production.

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1. Introduction

Nanobiotechnology is an enabling technology that deals with nano-meter sized materials in different field of science such as biotechnology, nanotechnology, physics, chemistry and material science. Beside many physical and chemical methods which have been developed for preparing metallic nanoparticles, nanobiotechnology also serves as an important method in the development of clean, nontoxic, and environmentally friendly procedures for the synthesis and assembly of metallic nanoparticles. Biosynthesis of metallic nanoparticles using microorganisms is a fabulous and emerging eco-friendly science of well-defined sizes, shapes and controlled monodispersity. These nanoparticles have unique catalytic, electronic and optical properties different from the metallic particles [1-3].

Copper nanoparticles, due to their unique physical and chemical properties and the low cost of preparation, have been of great interest recently. Copper nanoparticles are exploited in wound dressings and socks to give them biocidal properties [4-5]. Furthermore, copper nanoparticles have potential industrial use such as gas sensors, catalytic processes,

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high temperature superconductors, solar cells and so on [6-8]. Copper nanoparticles can easily oxidize to form copper oxide. If the application requires the copper nanoparticles to be protected from oxidation, the copper NPs are usually encapsulated in organic or inorganic material such as carbon and silica [9-12].

In view of these bright application prospects of copper nanoparticles, many investigations have already been carried out to prepare them [13-15]. The green synthesis of metallic nanoparticles includes use of biological agents such as bacteria, fungi, actinomycetes, yeast and plants [16]. In green nanotechnology, different microorganisms produce inorganic materials, either intracellularly or extracellularly with properties similar to chemically synthesized materials [17]. Usha R. *et al* (2010) reported a green synthesis of copper oxide by *Streptomyces Sp.* for development of antimicrobial textiles which can be useful in hospitals to prevent or to minimize infection with pathogenic bacteria [13]. Singh V. *et al* (2010) reported biological synthesis of copper oxide nanoparticles using *Escherichia coli* with a variable size and shapes [14]. Meanwhile, the use of fungi is potentially exciting since they secrete large amounts of enzymes and are simpler to deal with in the laboratory [18].

However, the exact mechanism for the synthesis of nanoparticles using biological agents has not been devised yet but it suggested that different biomolecules are responsible for the synthesis of nanoparticles. But in extracellular biosynthesis of nanoparticles especially in the case of fungi numerous reducing and capping agents secrete that are possible to be involved and also the effect of these reducing agents on the shape and size of nanoparticles need to be clarified [19-20]. Application of fungi *Penicillium aurantiogriseum, Penicillium citrinum* and *Penicillium waksmanii* for extracellular production of copper nanoparticles, is the emphasis of the present paper.

2. Materials and methods

Yeast extract was purchased from Liofilchem, Italy, $CuSo_4$ and other chemical reagents were purchased from Merck Germany. The pure colonies of *Penicillium aurantiogriseum, Penicillium citrinum* and *Penicillium waksmanii* were approved by department of mycology and plant pathology of Sari Agriculture and Natural Resources University, Iran.

2.1 Biosynthesis of copper nanoparticles

Each fungus were cultured on fluid zapex dox broth including 21g sucrose and 3g yeast extract in 1000cc distilled water and incubated on a rotary shaker at 200 rpm for ten days at 28°C. The cultures were centrifuged at 10,000 rpm for 5 minutes. Then the supernatants were used for the synthesis of copper NPs in different situations to observe the effect of pH value and salt concentration on the Z-average size of NPs. For that, one set of 1, 3 and 5 mM solution of CuSo4 were prepared in double distilled water, separately and the supernatants were observed with pH adjusted in the range of 5, 6, 7, 8 and 9, using 0.25 N HCl or 0.25 N NaOH. Then 100 ml of each $CuSo_4$ solution added to 100 ml of supernatants for *P. aurantiogriseum, P. citrinum* and *P. waksmanii,* separately and incubated again on a rotary shaker at 120 rpm for 24 hours at 28°C. Then the evaluation of Size and polydispersity of particles were setting by a Zetasizer Nanoparticle Analyzer using Zetasizer 3600 at 25°C with a scattering angle of 90° (Malvern instruments, UK). The analysis of the surface and shape characteristics of Copper NPs were determined by Scanning electron microscope model 2360 (Leo Oxford England). The fluorescence measurements were carried out on a Perkin-Elmer LS 50B luminescence spectrophotometer.

3. Results and discussions

Addition of each fungus biomass to copper NPs solution led to changes from pale yellow to brownish color in solution which is suggested the formation of copper NPs in the solutions (Fig.1). Many metals can be treated as free-electron systems. Such metal called plasma contains

equal numbers of positive ions which are fixed in position and conduction electrons which are free and highly mobile. Under the irradiation of an electromagnetic wave, the free electrons are driven by the electric field to oscillate coherently. These collective oscillations of the free electrons are called plasmons. These plasmons can interact, under certain conditions, with visible light in a phenomenon called surface plasmon resonance (SPR) [21-22]. An example of this interaction between light and electrons of a metal particle is illustrated in Fig.1. The position, the shape and intensity of the surface plasmon resonance strongly depend on various factors including the size, shape and monodispersity of the NPs, as well as the composition of the surrounding media and interactions between stabilizing ligands and the NPs [23-24]. Therefore, we should observe the effect of different factors on the size, shape and monodispersity of the NPs to design a suitable formulation for production of NPs. Copper nanoparticles with different size and shape can be produced by biological method depending on concentration of the Cu^{2+} ion in solution, the enzymes released by fungal strains and pH of the solution.

Fig. 1. The color changed from pale yellow to brown arises due to excitation of surface plasmon resonance in the metal nanoparticles indicating the formation of copper NPs.

The UV-vis Spectrum reveals that low wavelength region recorded from the reaction medium exhibited an absorption band around 265 nm and it was attributed to aromatic amino acids of proteins (Fig.2). It is well known that the absorption band at 265 nm arises due to electronic excitation in tryptophan and tyrosine residue in the protein. This is in accordance with the results of other researchers [25,26]. The fluorescence spectrum showed a broad emission peak of copper nanoparticles at 448 nm when excited at 433 nm. The nature of the emission band indicates that the proteins bound to the nanoparticle surface and those present in the solution exist in the native form [25]. We believe that the presence of proteins in the fungal biomass plays an important role in nanoparticle synthesis and stabilization. Besides, Gericke M, and Pinches A (2006) sugested that some of these proteins are capable of hydrolyzing metal precursors to form metal oxides extracellularly [27].

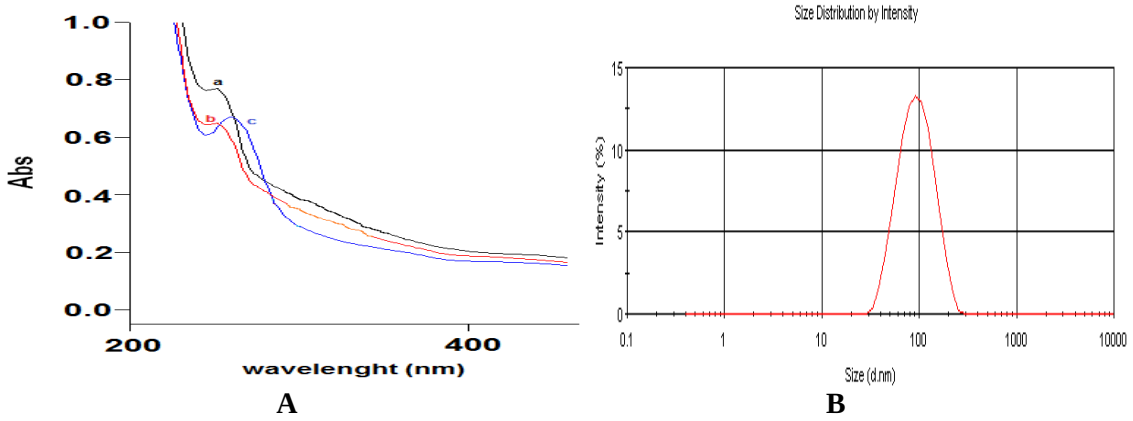

Fig. 2A. The UV-Vis spectra in low wavelength region recorded from the reaction medium exhibited an absorption band around 265 nm, where the reducing protein present in the culture medium for Penicillium citrinum (a), Penicillium waksmanii (b) and Penicillium aurantiogriseum (c). Fig. 2B. The fairly well-defined dimensions and good monodispersity of synthesized NPs.

Dynamic Light Scattering (DLS) is a noninvasive technique to measure the size and size distribution of nanoparticles dispersed in a liquid. DLS was carried out by nano zeta sizer (model of 3000, produced by Malvern company) to ascertain average metal oxide NPs size. Table.1 depicts the effect of pH value and salt concentration on the particle size of the nanoparticles, only the pH value and salt concentration were changed in the experiments and all other parameters were kept constant. In this study, rate of shaker was considered at 120 rpm and the result registered after 24 hours incubation. It is interesting to note that the variety in the Z-average size of copper NPs formed at different pH levels indicates that the changes in this parameter could play an important role during optimization of the process controlling nanoparticle morphology. We investigated the effect of these different parameters on the particle size and the polydispersity index, where the polydispersity index measures the second moment of the size distribution of the nanoparticle population. The PDI ranges are from a value of 0.01 for monodispersed particles and up to values of 0.5–0.7. Samples with very broad size distribution have polydispersity index values > 0.7 [28]. The polydispersity indexes registered in different situation in the present study defined nanoparticles (Tablet.1).

In addition, the diagram of DLS analysis demonstrated that the copper NPs formed with fairly well-defined dimensions and good monodispersity (Fig.2B).

The smallest nanoparticles were prepared in lower salt concentration in 1 mM. Increasing the salt concentration to 3 and 5 mM increased the particle size. This might be explained that increased concentration of salt levels allowed particle growth at a faster rate. Moreover, Particles in higher salt concentration maybe aggregate and produce bigger particles. From the practical point of view, it would be better if the metal ions could be prepared in lower concentration, leading to the formation of smaller nanometer-sized particles in the solution. Furthermore, the pH value of the solution was found to strongly influence the Z-average size of these NPs. The size of the nanoparticles was smaller at the acidic pH of 5.0 as compared to that at the pH of 6.0–9.0. A possible explanation of this observation is that at pH 5.0 nanoparticles are highly positively charged. The electrostatic repulsion prevents nanoparticles from uncontrolled agglomeration.

Because, when the nanoparticles are formed, their surface has a sufficient zeta potential to prevent further agglomeration of the particles.

Table 1. Influence of the pH value and salt concentration on nanoparticle size for Penicillium citrinum, Penicillium waksmanii and Penicillium aurantiogriseum.

Trial	pH	[a]Conce (mol/lit)	T (℃)	Rate (rpm)	*Penicillium citrinum*		*Penicillium waksmanii*		*Penicillium aurantiogriseum*	
					[b]PDI	Z-average size (nm)	[b]PDI	Z-average size (nm)	[b]PDI	Z-average size (nm)
1	5	1	28	120	0.473	91	0.330	80	0.323	91
2	5	3	28	120	0.379	106	0.252	92	0.304	94
3	5	5	28	120	0.383	116	0.305	87	0.403	96
4	6	1	28	120	0.286	114	0.374	88	0.345	91
5	6	3	28	120	0.270	160	0.355	91	0.222	184
6	6	5	28	120	0.408	233	0.167	179	0.367	250
7	7	1	28	120	0.519	85	0.432	89	0.347	95
8	7	3	28	120	0.240	149	0.287	106	0.297	119
9	7	5	28	120	0.407	279	0.204	128	0.388	218
10	8	1	28	120	0.245	102	0.394	83	0.284	109
11	8	3	28	120	0.298	140	0.393	121	0.261	139
12	8	5	28	120	0.425	295	0.189	171	0.241	202
13	9	1	28	120	0.294	133	0.354	88	0.306	89
14	9	3	28	120	0.244	151	0.239	79	0.252	97
15	9	5	28	120	0.475	225	0.288	179	0.326	102

[a]Concentration of salt
[b] Poly Dispersity Index

The SEM image could provide us two main types of data, one is the topographic structure of the surface and the second one is the distinction of different phases in the sample. Through this technique, the source of electron beam is mainly a tungsten filament which radiates on the sample by an objective lens. The beam scans the surface of the nanoparticles. Different kinds of detectors are used to collect signals, and using secondary and backscattered electrons the image is formed. Secondary electrons have relatively low energy and are a surface-sensitive signal. This signal causes high-resolution topographic information and SEM imaging. In this study, the SEM micrographs of nanoparticle obtained in the filtrate showed that the copper NPs produced by *Penicillium citrinum, Penicillium waksmanii* and *Penicillium aurantiogriseum* are spherical shaped and well distributed in solution (Fig. 4). The atomic force microscope (AFM) is becoming an important bio-physical technique for studying the morphology of nanoparticles and biomolecules [29].

The tapping mode AFM images were developed especially for studying biofunctionalized samples. Fig. 4d shows a typical medium scale AFM image (10.1μm×10.1μm) of the bio-functionalized organic layer which consists of organic moieties at the surface. From the topographical view, it is evident that the nanoparticles are spherical in shape. The results noted in SEM images are quite agreeable to AFM observations.

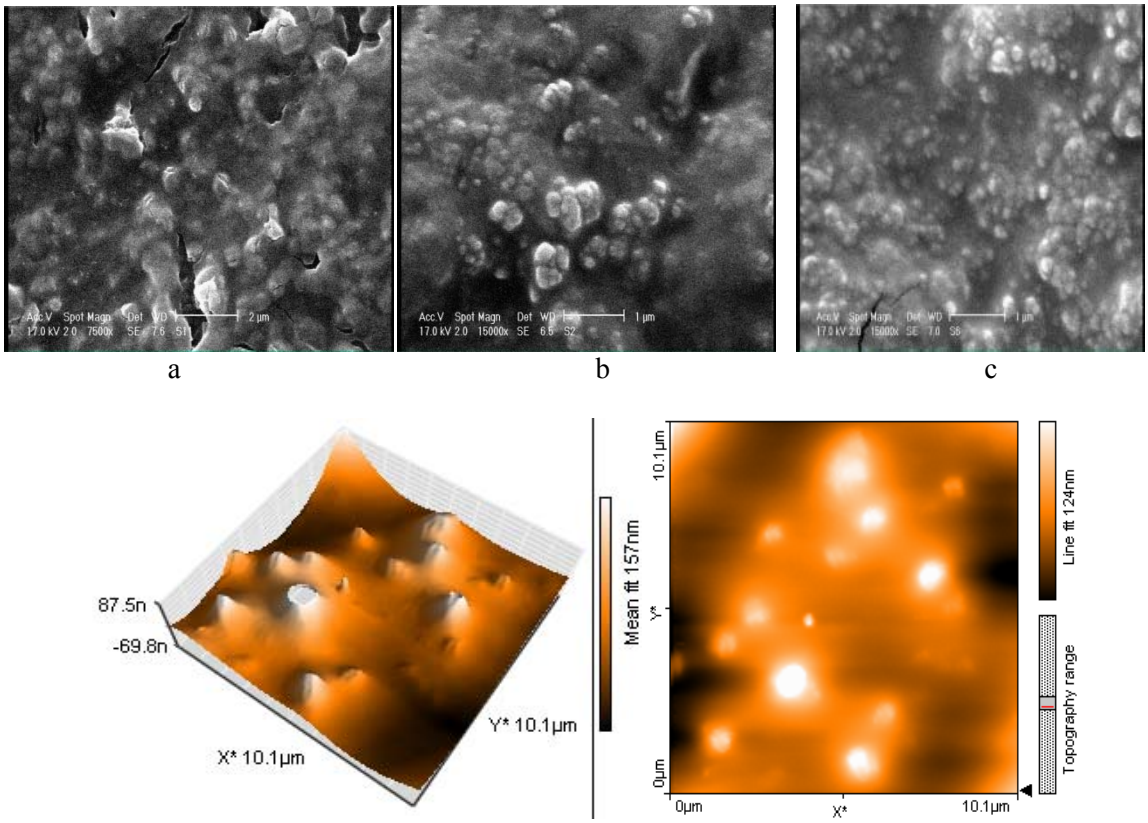

Fig. 4. SEM image of copper NPs produced by Penicillium citrinum(a), Penicillium waksmanii (b) and Penicillium aurantiogriseum(c). AFM image (10.1µm×10.1µm) of copper NPs produced by Penicillium citrinum(d).

4. Conclusion

Developments in nanotechnology and detection of chemical and biological warfare agents for the manipulation and control of metallic nano-scales production have revolutionized disease diagnosis and treatment. In this study, we have reported the biological process for the formation of copper NPs using *P. aurantiogriseum, P. citrinum* and *P. waksmanii*. It was concluded that there is a direct correlation existing between pH value, concentration of salt, polydispersity index and particle size. This technology will be smaller, cheaper, lighter yet more functional effect and require less energy and less raw materials to manufacture. In spite of the successes achieved in biological synthesis of nanoparticles, there is still a need to improve the rate of synthesis and monodispersity of nanoparticles. Also, microbial cultivation and downstream processing techniques must be improved, and more efficient methods should be developed.

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References

[1**]** D. Mubarak Ali, M. Sasikala, M. Gunasekaran, N. Thajuddin. Dig. J. Nanomater. Bios. **6**, 385 (2011).

[2] J. Huang, C. Chen, N. He, J. Hong, Y. Lu, L. Qingbiao, W. Shao, D. Sun, X. H. Wang, Y. Wang, X. Yiang, Nanotechnology **18**, 105 (2007).

[3] M. Rai, A. Yadav, A. Gade, Crit. Rev. Biotechnol. **28**, 277 (2008).

[4] G. Borkow, Wound Repair Regen **18**, 266 (2010).

[5] G. Borkow, R. C. Zatcoff, J. Gabbay, Med. Hypotheses. **73**, 883 (2009).

[6] Y. Li, J. Liang, Z. Tao, J. Chen, Mater. Res. Bull. **43**, 2380 (2007).

[7] L. C. Carnes, K. J. Klabunde, J. Mol. Catal. A: Chem. **194**, 227 (2003).

[8] Z. Guo, X. Liang, T. Pereira, R. Scaffaro, H. T. Hahn, Compos. Sci. Tech. **67**, 2036 (2007).

[9] N. Cioffi, N. Ditaranto, L. Torsi, R.A. Picca, E. D. Giglio, P. G. Zambonin, Anal. Bioanal. Chem. **382**, 1912 (2005).

[10] Q. Xu, Y. Zhao, J. Z. Xu, J. J. Zhu, Sens. Actuators, B 114, 379 (2006).

[11] E. K. Athanassiou, R. N. Grass, W. J. Stark, Nanotechnology **17**, 1668 (2006).

[12] J. S. Moya, C. Pecharroman, A. Cubillo, I. Montero, J. Am. Ceram. Soc. **89**, 3043 (2006).

[13] R. Usha, E. Prabu, M. Palaniswamy, C. K. Venil, k. R. Rajendran, GJBB **5**, 153 (2010).

[14] V. Singh Ajay, R. Patil, A. Anand, P. Milani, W. N. Gade, Curr Nanosci **6**, 365 (2010).

[15] N. Tran, A. Mir, D. Mallik, A. Sinha, S. Nayar, T. J. Webster, Int J Nanomed **5**, 277 (2010).

[16] K.N. Thakkar, S. S. Mhatre, R. Y. Parikh, Nanomedicine **6**, 257 (2010).

[17] E. Bäuerlein, Angew. Chem. Int. Ed. **42**, 614 (2003).

[18] D. Mandal, M. E. Bolander, D. Mukhopadhyay, G. Sarkar, P. Mukherjee, Microbiol. Biotechnol. **69**, 485 (2006).

[19] P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. I. Khan, R. Ramani, R. Parischa, P. A. V. Kumar, M. Alam, M. Sastry, R. Kumar, Angew. Chem. Int. Ed. **40**, 3585 (2001).

[20] P. Mohanpuria, N. K. Rana, S. K. Yadav, J. Nanopart. Res. **10**, 507 (2008).

[21] A. Moores, F. Goettmann, New J. Chem. **30**, 1121 (2006).

[22] J. M. Pitark, V. M. Silkin, E. V. Chulkov, P. M. Echenique, J. Opt. A-Pure. Appl. Op. **7**, 73 (2005).

[23] I. Lisiecki, F. Billoudet, P. Pileni, J. Phys. Chem. 100, 4160 (1996).

[24] L. K. Kelly, E. Coronado, L. Zhao, G. C. Schatz, J. Phys. Chem. B **107**, 668 (2003).

[25] D. Nelson, D. M. Priscyla, L. A. Oswaldo, I. S. Gabriel, E. Elisa, J. Nanobiotechnology **3**, 3155 (2005).

[26] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M. I. Khan, R. Kumar, M. Sastry, Colloids Surf., B **28**, 313 (2003).

[27] M. Gericke, A. Pinches, Hydrometallurgy **83**, 132 (2006).

[28] M. Nidhin, R. Indumathy, K. J. Sreeram, U. Balachandran, Bull. Mater. Sci. **31**, 93 (2008).

[29] R. B. Best, D. J. Brockwell, J. L. Toca-Herrera, A. W. Blake, D. A. Smith, S. E. Radford, J. Clarke, Anal. Chim. Acta **87**, 479 (2003).