Short Communication

Investigation of culture conditions for biosynthesis of silver nanoparticles using *Aspergillus fumigatus*

Zahra Ranjbar Navazi, Mohammad Pazouki*, Farah Sadat Halek

Environmental Group, Department of Energy, Materials and Energy Research Center, Karaj, I.R. Iran

Abstract
In this study, silver nanoparticles were synthesized using the fungus, *Aspergillus fumigatus*. The effects of three independent variables including glucose content of culture media, initial pH and initial spore concentration on biosynthesis of silver nanoparticles were investigated. These variables affect cell morphology, cell mass, size and morphology of silver nanoparticles and degree of silver ion reduction. The formation of silver nanoparticles was confirmed spectrophotomterically. Size and morphology of silver nanoparticles were investigated using transmission electron microscopy (TEM). The effects of culture conditions on cell mass concentration as well as the amount and size of synthesized silver nanoparticles were studied. As a result, the optimum culture condition for biosynthesis of silver nanoparticles consisted of a glucose concentration of 16 g/l, pH of 4.5 and spore concentration of 1.5×10^7 spore/l. TEM micrographs showed that the size of nanoparticles in the sample synthesized under optimized condition was in the range of 7-19 nm.
Keywords: Silver nanoparticles; Biosynthesis; *Aspergillus fumigatus*; Culture conditions

One of the most important criteria of nanotechnology is that of the development of clean, nontoxic and environmentally acceptable ''green chemistry'' procedures, involving organisms ranging from bacteria to fungi and even plants (Bhattacharya *et al*., 2005; Sastry *et al*., 2004). The interactions between microorganisms and metals have been well documented and the ability of microorganisms to extract and/or accumulate metals is already employed in biotechnological processes such as bioleaching and bioremediation (Gericke *et al*., 2006).

Synthesis of nanoparticles employing microorganisms has attracted great interest due to their unusual optical (Krolikowska *et al*., 2003), chemical (Kumar *et al*., 2003), photoelectrochemical (Chandrasekharan *et al*., 2000) and electronic properties (Peto *et al*., 2002), which enable the synthesis of nanoparticles of different chemical compositions, well-defined sizes and distinct morphologies (Bhattacharya *et al*., 2005). It is well known that many organisms can synthesize inorganic materials either intra- or extracellularly (Dickson, 1999; Kroger *et al*., 1999; Mandal *et al*., 2006; Mann, 1996; Oliver *et al*., 1995; Mann, 1993; Simkiss *et al*., 1989; Lovley *et al*., 1987).

The use of fungi in the synthesis of nanoparticles is a relatively recent addition to the list of microorganisms possessing nanoparticle biosynthesis "ability". Application of fungi to produce nanoparticles is potentially exciting because of their ability to secrete large amounts of enzymes. Some of the microorganisms, which have been widely used for synthesizing of silver nanoparticles include microorganisms such as, *Verticillium* sp., *Fusarium oxysporum*, *Pseudomonas stutzeri* AG259 (Kuber *et al*., 2006; Sastry *et al*., 2003) and *Aspergillus fumigatus* (Bhainsa and D'Souza, 2006). Except for *F. oxysporum* and *A. fumigatus*, biosynthesis of silver nanoparticles by the abovementioned microorganisms is intracellular. It is possible to

Correspondence to:* **Mohammad Pazouki, Ph.D.
Tel: +98 261 62800 36; Fax: +98 261 62800 30
E-mail: mpazouki@merc.ac.ir, mpaz6@yahoo.com

release intracellular silver and gold nanoparticles via ultrasound treatment of the biomass-nanoparticles composite or via reaction with suitable detergents. Nonetheless, it would be far more practical if the metal ions exposed to the fungus could be reduced outside the fungal biomass, leading to the formation of metal nanoparticles in solution. (Mandal *et al*., 2006) Therefore in this study *A. fumigatus* was used to reduce silver ions extracellularly. According to the preliminary study of Bhainsa and D'Souza (2006), this fungus is a good candidate for rapid biosynthesis of silver nanoparticles. In this research, the effects of glucose concentration, pH and number of spores on the size and morphology of silver nanoprticles were investigated using UV- visible spectrophotometry and transmission electron microscopy (TEM).

In order to prepare the required biomass for the biosynthesis of silver nanoparticles, spores of *A. fumigatus* $U_{B2}60^{o}$ which had originally been isolated from the Bidestan Distillery and Food Products, Qazvin, Iran for biodecolorization of distillery spent wash (Pazouki *et al*., 2006; Pazouki *et al*., 2005) were maintained on potato dextrose agar (PDA) slants for 5 days. Twenty experiments were designed using the central composite design (CCD) at five levels (Montgomery, 2005) to investigate the effects of culture media conditions on the biosynthesis of silver nanoparticles. This design has the advantage of using a minimum number of trials. The parameters studied, were glucose concentration, initial pH and the number of spores in liquid culture media. These parameters were selected due to their influence on morphology and metabolite production (Pazouki and Panda, 2000). Hence 20 liquid media containing (g/l) KH_2PO_4 7.0, K_2HPO_4 2.0, $MgSO_4{\cdot}7H_2O$ 0.1, $(NH_4)_2SO_4$ 1.0, yeast extract 0.6, different glucose concentrations and pH were prepared using CCD (Table 1). Then, the respective numbers of spores were inoculated and the fungus was grown aerobically. The flasks were incubated at 25ºC on an orbital shaker (Sm-920, Borhan Pouya Co., Iran) with agitation at 160 rpm. After 72 h of cultivation, the resulting biomasses in the flasks were harvested using a sterilized sieve and washed extensively with distilled water to remove medium components. The obtained biomass in each run of the experiment as it was produced, was added to 100 ml of Milli-Q deionized water and incubated at 30°C for 72 h on an orbital shaker, with shaking at 130 rpm. After the incubation period, for the purpose of the synthesis of silver nanoparticles, $AgNO_3$ (1 mM) was mixed with the biomass filtrate that was obtained by passing it through Whatman filter paper no. 42, in a 250 ml Erlenmeyer flask . The resulting mixture was incubated at 25ºC in the dark, with agitation at 130 rpm (Bhainsa and D'Souza, 2006).

In order to observe the effect of biomass on synthesized silver nanoparticles, silver nitrate was added to the samples under the same conditions but, containing biomass. Whereas the rapid synthesis of silver nanoparticles is possible using *A. fumigatus*, the UV spectra of samples were obtained using UV-visible spectrophotometer (CECIL 30E 9500, England) after 4 h in order to compare the intensities of peaks related to silver nanoparticles. The sample of biomass filtrate which had the desirable UV spectrum with regard to

Table 1. Culture conditions of *A. fumigatus* for biosynthesis of silver nanoparticles using central composite design CCD.

Sample	Glucose (g/l)	pH	Spore concentration ($\times 10^6$/l)
1	16	4.5	8
2	16	4.5	8
3	7	6	3.7
4	7	6	12.3
5	25	3	12.3
6	16	4.5	8
7	25	6	12.3
8	16	4.5	8
9	25	3	3.7
10	25	6	3.7
11	7	3	12.3
12	7	3	3.7
13	0.86	4.5	8
14	16	4.5	8
15	16	4.5	7.5
16	16	4.5	8
17	31.14	4.5	8
18	16	7.02	8
19	16	4.5	15.2
20	16	1.98	8

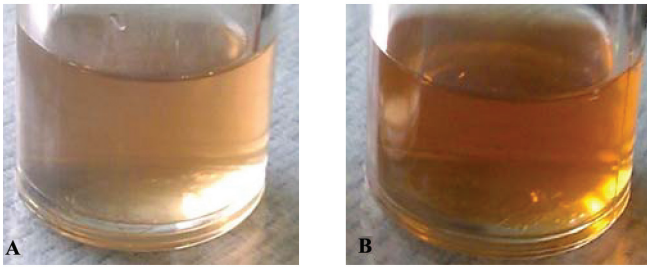

Figure 1. Color change of cell filtrate A: after adding silver nitrate (1mM) at the start of the reaction and B: after 72 h.

the silver nanoparticles was used for electron transmission microscopy (TEM). A drop of the sample was placed on the carbon coated copper grid of the microscope (CEM902A, Zeiss, Germany).

Reduction of silver ions was reflected in the changing color of the cell filtrate, from pale yellow to a brown shade as shown in Figure 1. Due to the completion of the reaction, the intensity of the brownish color increased. In addition, the presence and production of silver nanoparticles were confirmed by UV-visible spectrophotometry, whereby light absorption at approximately 420 nm was attributed to to the surface plasmon resonance of the silver nanoparticles .

Desirable UV spectra were obtained by samples with high absorption intensity due to high levels of reduced silver ions or smooth curves due to better size distributions (Khosravi *et al*., 2007). As denoted in Figure 2, the sample from run 19 had the highest intensity of light absorption at 420 nm but the shape of the UV spectrum of the sample from run 15 was much smoother.

Figure 3 shows the UV spectrum of the samples from runs 15 and 19 containing biomass. The UV spectrum of samples containing biomass did not show admissible absorption intensity at 420 nm as compared to the samples containing cell filtrate, indicating low concentration of silver nanoparticles in the media. The color of media did not change significantly either, instead the color of the biomass surfaces changed to a brownish red after adding silver nitrate. It shows that

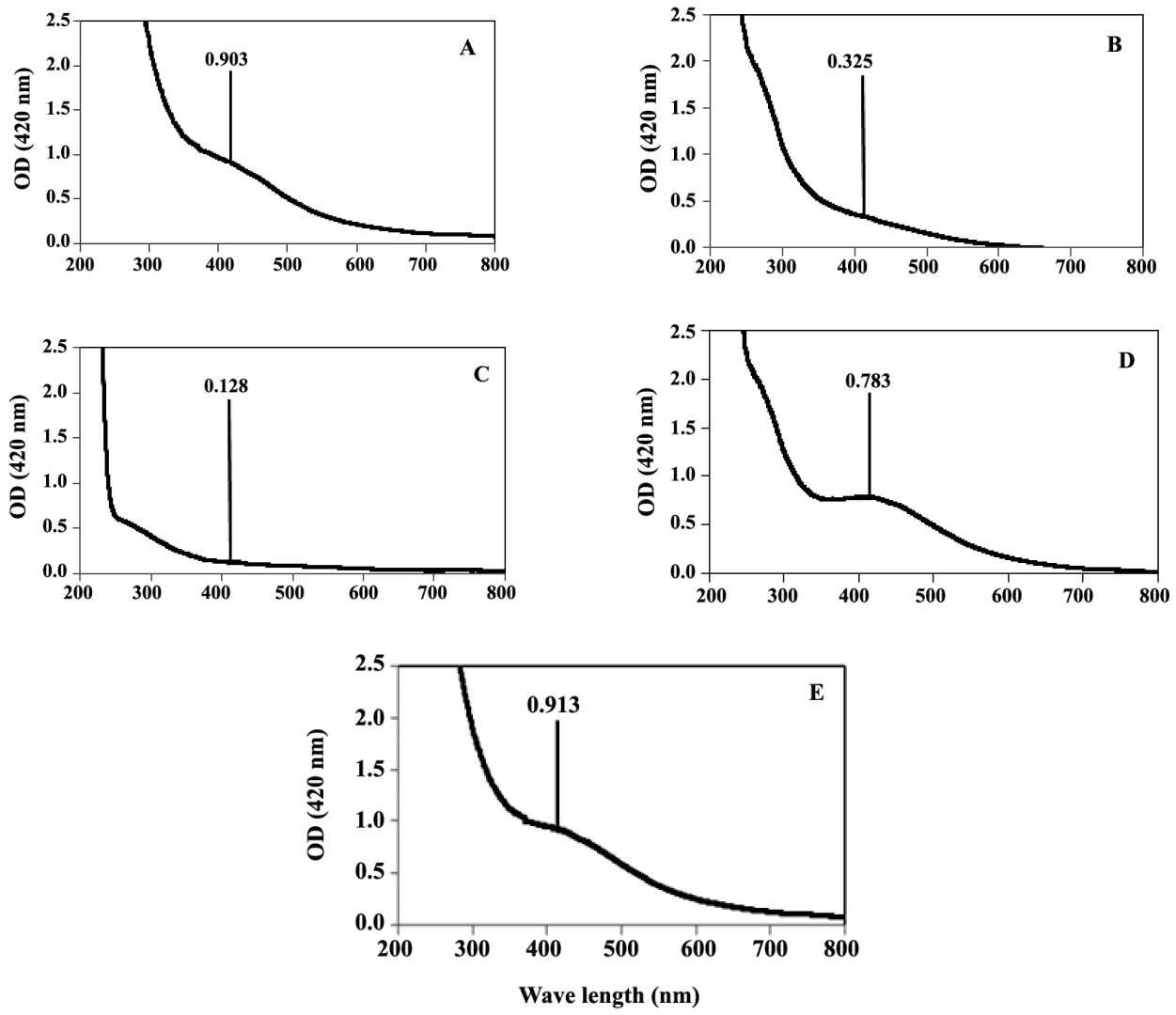

Figure 2. Ultraviolet spectra of selected samples from the central composite design (CCD) experiment where,: A: 1, B: 4, C: 13, D: 15, E: 19.

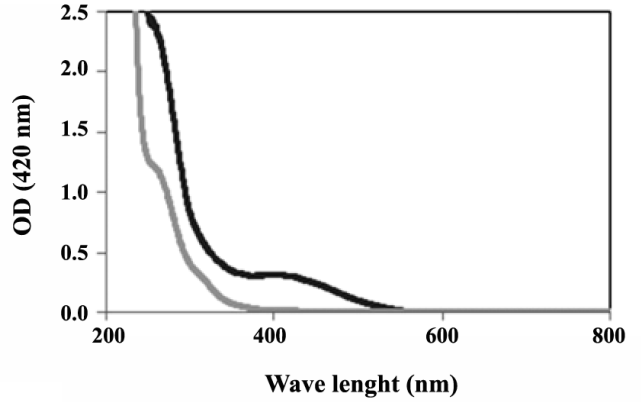

Figure 3. Ultraviolet spectrum of samples 15 (Black) and 19 (Grey) containing biomass.

the synthesized silver nanoparticles had accumulated on the cell wall. Hence, the benefits of external biosynthesis of silver nanoparticles could not be appreciated because the detachment of nanoparticles would be a difficult and extra process.

Figure 4 shows the TEM micrograph of silver nanoparticles synthesized according to the culture conditions of runs 15 and 19. As seen in this Figure, the morphology of nanoparticles is almost spherical. The sizes the nanoparticles in the sample of run 19 ranged from 7- to 35 nm and the sizes of nanoparticles in the sample from run 15 was in the range of 16-50 nm. Moreover, there was some aggregation between the silver nanoparticles synthesized in the sample from run 15.

Table 2 includes the cell dry mass produced during each run, indicating the effects of culture conditions on the amount of cells produced. In addition, it was observed that these parameters affected the morphology and even the color of the fungal cells. Therefore, the chosen culture variables including glucose concentration, initial pH and number of spores were required to be considered in order to obtain desirable nanoparticles. According to the obtained results, the optimum culture conditions of *A. fumigatus* for biosynthesis of silver nanoparticles included a glucose concentration of 16 g/l, pH of 4.5 and spore concentration of 1.5×10^7/l.

Application of biological systems for synthesis of silver nanoparticles has already been reported (Sastry *et al*., 2003; Klaus-Jeorger *et al*., 2001). However, the exact mechanism leading to the formation of silver nanoparticles by all these organisms is yet to be elucidated. Ahmad *et al*. (2003) have reported that certain NADH dependent reductases of *F. oxysporum* were involved in the reduction of silver ions. Also, Duran *et al*. (2005) have studied several strains of the fungus *F. oxysporum* and have reported that the reduction of the metal ions occurs by a nitrate-dependent reductase and a shuttle quinone extracellular process. One reason for aggregation of sample 15 could be the use of a fewer number of spores as compared with that of sample 19. Therefore, the spore inoculum concentration affects the synthesized nanoparticles.

There are a few reports on the effects of culture conditions on the biosynthesis of metal nanoparticles. Mohammadian *et al*. (2007) have investigated the effects of growth conditions on the biosynthesis of silver nanoparticles by *F. oxysporum*. They have reported that consistency in the fungus growth conditions

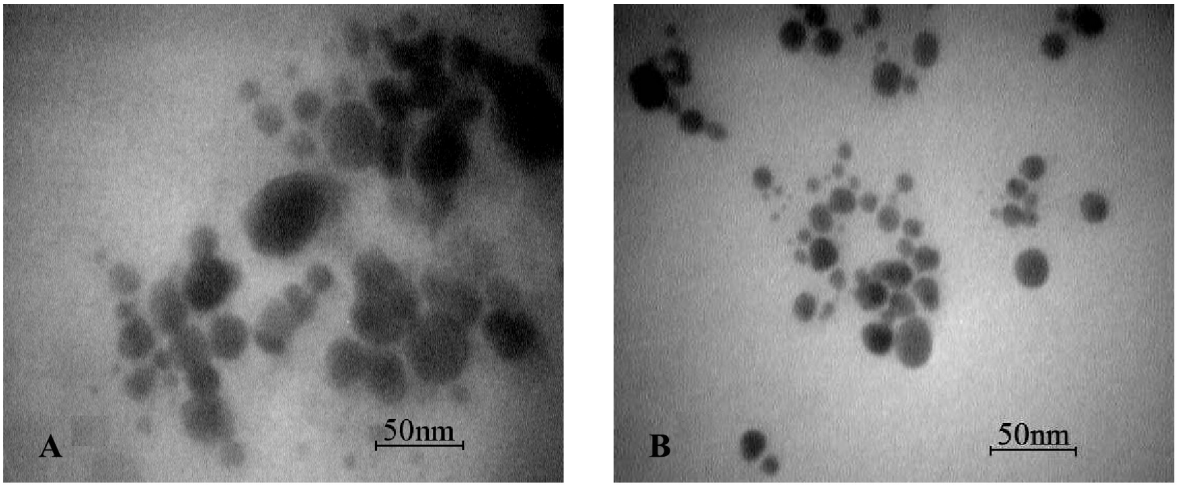

Figure 4. Transmission Electron Microscopy (TEM) micrograph of silver nanoparticles synthesized by *A. fumigatus* under culture conditions of A: sample 15 (scale bar: 50 nm) B: sample 19 (scale bar: 50 nm).

Table 2. Cell mass obtained under different culture conditions obtained by Central Composite Design CCD.

Sample	Cell mass (g)	Sample	Cell mass (g)
1	0.336	11	0.191
2	0.327	12	0.150
3	0.186	13	0.337
4	0.159	14	0.650
5	0.438	15	0.637
6	0.264	16	0.714
7	0.498	17	0.738
8	0.296	18	0.760
9	0.457	19	0.665
10	0.474	20	0

including cultivation temperature, carbon source concentration and shaking frequency is not a determining factor affecting the rate of nanoparticles biosynthesis. However, there is no report on the effects of the numbers of spores on biosynthesis of metal nanoparticles. In this study, some of the fungus culture variables including initial pH, glucose concentration and spore number have been found to affect the morphology and amount of fungal biomass produced and also the size and morphology of synthesized silver nanoparticles.

Acknowledgments

This study was performed at the Materials and Energy Research Center and financially supported by the Graduate Studies Office, Karaj, Tehran.

References

Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M (2003). Extracellular biosynthesis of monodispersed gold nanoparticles by a novel extermophillic actinomycete, *Thermonospora* sp. *Langmuir* 19: 3550-3553.

Bhainsa KC, D'Souza SF (2006). Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. *Coll Surf B Bioint*. 47: 160-164.

Bhattacharya D, Rajinder G (2005). Nanotechnology and potential of microorganisms. *Crit Rev Biotechnol*. 25: 199-204.

Chandrasekharan N, Kamat PV (2000). Improving the photoelectrochemical performance of nanostructured TiO_2 films by adsorption of gold nanoparticles. *J Phys Chem B*. 104: 10851-10857.

Dickson DPE (1999). Nanostructured magnetism in living systems. *J Magn Mater*. 203: 46-49.

Gericke M, Pinches A (2006). Biological synthesis of metal nanoparticles. *Hydrometallurgy* 83: 132-140.

Duran N, Marcato PD, Alves OL, D'Souza IH-G, Esposito E (2005). Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. *J Nanobiotech*. 3: 8-12.

Khosravi A, Shojaosadati SA (2007). Biosynthesis of Silver nanoparticles and product evaluation. Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science (M.S.) in Nanotechnology, Tarbiat Modares University. 57-58.

Klaus-Jeorger T, Jeorger R, Olsson E, Granqvist C (2001). Bacteria as workers in the living factory: metal accumulating bacteria and their potential for material science. *Tran Biotech*. 19: 15-20.

Kroger N, Deutzmann R, Sumper M (1999). Polycationic peptides from diatom biosilica that direct silica nanosphere formation. Science 286: 1129-113.

Krolikowska A, Kudelski A, Michota A, Bukowska J (2003). SERS studies on the structure of thioglycolic acid monolayers on silver and gold. *Surf Sci*. 532: 227-232.

Kumar A, Mandal S, Selvakannan PR, Parischa R, Mandale AB, Sastry M (2003). Investigation into the interaction between surface-bound alkylamines and gold nanoparticles. *Langmuir* 19: 6277-6282.

Lovley DR, Stolz JF, Nord GL, Philips EJP (1987). Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. *Nature* 330: 252-254.

Mandal D, Bolander ME, Mukhopadhyay, Sarkar G, Mukherjee P (2006). The use of microorganisms for the formation of metal nanoparticles and their application. *J Appl Microbiol Biotechnol*. 69: 485-492.

Mann S (1993). Molecular tectonics in biomineralization and biomimetic materials chemistry. *Nature* 365: 499-505.

Mann S (1996). Biomineralization and Biomimetic Chemistry. In: *Biomimetic materials Chemistry*. VCH, New York. PP. 1-40.

Mohammadian A, Shojaosadati SA, Habibi Rezaee M (2007). *Fusarium oxysporum* mediates photogeneration of silver nanoparticles. *J Sci Iran*. 14: 323-326.

Montgomery DC (2005). Response surface methods and designs, In: *design and analysis of experiments*. 6th Edition, John Wiley & Sons. PP. 405-463.

Oliver S, Kupermann A, Coombs N, Lough A, Ozin GA (1995).

Lamellar aluminophosphates with surface patterns that mimic diatom and radiolarian microskeletons. *Nature* 378: 47-50.

Pazouki M, Hussainnia A, Moztarzadeh F, Banifathemi M (2006). Application of response surface methodology for optimization of important parameters in decolorizing treated distillery wastewater using *Aspergillus fumigatus* $U_{B2}60$. *Int Biodet Biodeg J*. 57: 195-199.

Pazouki M, Hussainnia A, Shayegan J, Banifathemi M (2005). Decolorization of distillery wastewater by UV irradiated spores of *Aspergillus fumigatus. Iran J Chem Eng*. 2: 49-55.

Pazouki M, Panda T (2000). Understanding the morphology of fungi. *Biopr Eng*. 22: 127-143.

Peto G, Molnar GL, Paszti Z, Geszti O, Beck A, Guczi L (2002). Electronic structure of gold nanoparticles deposited on SiOx/Si. *Mater Sci Eng C*. 19: 95-99.

Sastry M, Ahmad A, Khan MI, Kumar R (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. *Curr Sci*. 85: 162-170.

Sastry M, Ahmad A, Khan MI, Kumar R (2004). Microbial nanoparticle production. In: *Nanobiotechnology*, Niemeyer CM, Mirkin CA (eds). Wiley-VCH, Weinheim, Germany. PP. 126-135.

Simkiss K, Wilbur KM (1989). *Biomineralization*. Academic press, New York.