Silver nanoparticles biosynthesized using *Opuntia ficus* aqueous extract

Silva-de-Hoyos L. E., Sánchez-Mendieta V., Rico-Moctezuma A., Vilchis-Nestor A. R.
Centro de Investigación en Química Sustentable, Facultad de Química, Universidad Autónoma del Estado de México. Carretera Toluca-Atlacomulco Km 14.5, San Cayetano, Piedras Blancas, Toluca, Estado de México, México. CP 50120.

Camacho-López M. A. *
Laboratorio de Fotomedicina, Biofotonica y Espectroscopia Láser de Pulsos Ultracortos, Facultad de Medicina, Universidad Autónoma del Estado de México, Paseo Tollocan s/n esq. Jesús Carranza, Toluca Edo. de México 50120, México

Avalos-Borja M.
Centro de Nanociencias y Nanotecnología, Universidad Nacional Autónoma de México, Km. 107 Carretera Tijuana-Ensenada, Ensenada, B.C. México. AP. 2681, CP 22800.
(Recibido: 28 de julio de 2011; Aceptado: 4 de enero de 2012)

Silver nanoparticles were synthesized using a green chemistry method. Stable silver nanoparticles in a colloidal aqueous solution were prepared successfully by the chemical reaction of silver nitrate ($AgNO_3$) and *Opuntia ficus indica* aqueous extract, used as both, reducing and stabilizing agent. Nanoparticle size, morphology and optical properties were analyzed by using transmission electron microscopy (TEM) and UV-Vis spectroscopy, respectively. TEM images revealed a nanoparticle average size of 23 nm. An absorption band centered around 398 nm was observed, this absorption corresponds to the surface plasmon resonance (SPR) of the silver nanoparticles.

Keywords: Silver nanoparticles; bioreduction; green nanochemistry; Opuntia ficus indica, biogenic synthesis, optical properties

A partir de una nueva ruta de síntesis empleando la química verde, se obtuvieron nanopartículas de plata. Una solución coloidal de nanopartículas de plata se obtuvo a través de la reacción química entre el nitrato de plata ($AgNO_3$) y una solución acuosa de extracto de nopal (*Opuntia ficus indica*), el cual juega el papel de agente reductor y estabilizador. El tamaño, morfología y propiedades ópticas de las nanopartículas fueron analizadas mediante microscopia electrónica de transmisión (TEM) y espectroscopia de absorción óptica UV-Vis, respectivamente. Imágenes de TEM mostraron que el tamaño promedio de las nanopartículas obtenidas fue de 23 nm. En los espectros absorción se observó una banda centrada en 398 nm, la cual corresponde a la resonancia del plasmón superficial de las nanopartículas de plata.

Palabras clave: Nanopartículas de plata; Biorreducción; Nanoquimica verde; Opuntia ficus indica; Síntesis biogénica; Propiedades ópticas

1. Introduction

The physicochemical and optoelectronic properties of metallic nanoparticles are strongly dependent on the size and size-distribution, but also nanoparticles shape contributes significantly to the control of their properties. Wide varieties of physical and chemical procedures have been developed in order to synthesize nanoparticles of different compositions, sizes, shapes and controlled polydispersity. Nevertheless, the routinely physicochemical techniques for nanoparticle production such as photochemical reduction [1], laser ablation [2], electrochemistry [3], lithography [4] or high energy irradiation [5], either remain expensive or employ hazardous substances, such as organic solvents, and toxic reducing agents like sodium borohydride and N,N-dimethylformamide. In addition, due to the high surface energy of the nanoparticles, these tend to form aggregates; therefore, surface passivating and capping reagents are frequently added to the reaction systems to avoid coalescence.

The development of reliable, eco-friendly processes for the synthesis of nanomaterials is an important aspect of nanotechnology. Nanotechnology also requires the synthesis of nanomaterials of different chemical compositions, sizes and morphology with an excellent control over these characteristics.

With the growing need to minimize or eliminate the use of environmental-risk substances, as the green chemistry principles describe [6], the synthesis of nanoparticles using biological entities has received increasing attention in the last decade [7]. The biosynthetic procedures involve either living organisms such as bacteria [8], fungi [9] and plants [10] or biomass, like plant extracts [11-14]. Biological synthetic processes have emerged as a simple and viable alternative to more complex physicochemical approaches to obtain nanomaterials with adequate control of size and shape [15].

The use of the highly structured physical and biosynthetic activities of microbial cells for the synthesis of nanosized materials has recently emerged as a novel approach for the synthesis of metal nanoparticles. The interactions between

*macamachol@uaemex.mx

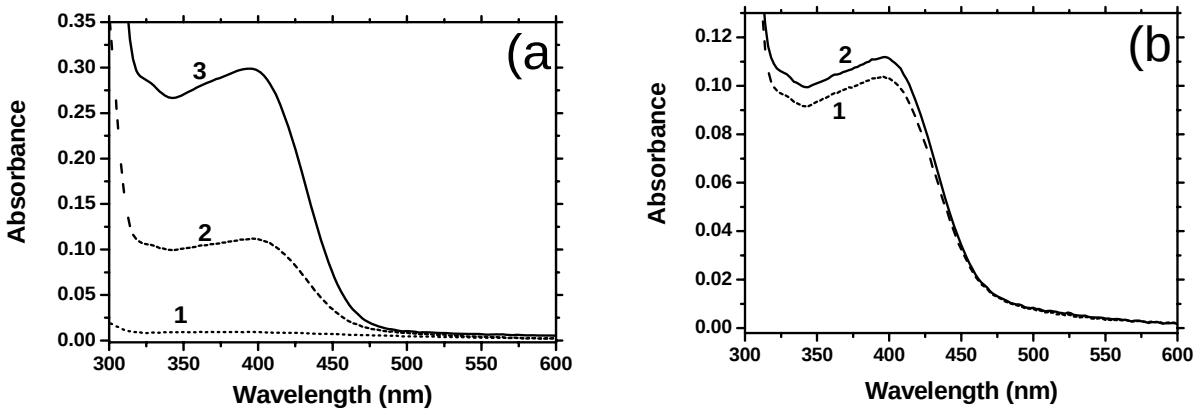

Figure 1. a) UV-Vis absorption spectra of silver nanoparticles formed after 1 hour of the reaction of different amounts (1, 5 and 10 ml, curves 1-3 respectively) of *Opuntia ficus indica* extract with 5 ml of 10^{-3} M aqueous solution of Ag NO_3. b) UV-Vis absorption spectra of silver nanostructures formed with 5 ml of *Opuntia ficus indica* extract and 5 ml of 10^{-3} M aqueous solution of $AgNO_3$ after 1 and 65 hours of the reaction (curves 1 and 2, respectively).

microorganisms and metals have been well documented [16] and the ability of microorganisms to extract and/or accumulate metals is already employed in biotechnological processes such as bioleaching and bioremediation [17].

Among the use of living organisms for nanoparticle synthesis, plants have found application particularly in metal nanoparticle synthesis. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell cultures. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracellularly using plants or their extracts and in a controlled manner according to their size, dispersity and shape. Plant use can also be suitably scaled up for large-scale synthesis of nanoparticles [18]. Noble metals, especially Au and Ag, have been extensible tested for the biosynthetic process assisted by plants, in order to obtain metallic nanoparticles with control over shape and size. A list of the metallic nanoparticles obtained by biosynthesis employing plants biomass or extracts can be checked in recent reviews, reported by Yadav *et al.* [7, 18] and Bali *et al*. [19].

In the case of the plants, the possibility to obtain metallic particles with nanometric dimension were explored just after, these organisms were employed for the remediation of metal-contaminated water [20] due to a growing necessity to develop environmentally friendly systems to retrieve metals. It has been shown that many plants can actively uptake and bioreduce metal ions from soils and solutions during the detoxification process, thereby forming insoluble complexes with the metal ion in the form of nanoparticles.

The first successfully report of synthesis of nanoparticles assisted by living plants appeared in 2002 when it was shown that gold nanoparticles, ranging in size from 2 to 20 nm, could form inside alfalfa seedlings [10]. Subsequently it was shown that alfalfa also could form silver nanoparticles when exposed to a silver rich solid medium. [21]

The next methodology associated with plants to produce metallic nanoparticles employs the dried biomass of the plants and a metallic salt, as bioreducing agent and precursor, respectively. The general procedure can be described in a general way as follow, the plant is dried (typically sun-dried), then is suspended in water and placed in an ultrasonic bath to homogenize the suspension. Finally the suspension is allowed to rest with the metallic ion aqueous solutions followed by a homogenization process. Silver or gold precursors at ambient temperature produces both silver nanoparticles (55–80 nm) and triangular or spherical gold nanoparticles, from sun-dried biomass of *Cinnamomum camphora* leaf when are incubated together [13]. The author´s indicate the marked difference of shape control between gold and silver nanoparticles was attributed to the comparative advantage of protective biomolecules. The polyol components and the water-soluble heterocyclic components were mainly responsible for the reduction of silver ions or chloroaurate ions and the stabilization of the nanoparticles, respectively. The processes were scaled-up successfully for biological production of silver nanoparticles by lixivium of sundried *Cinnamomum camphora* leaf in continuous-flow tubular microreactors [22]. Furthermore, silver nanoparticles (NPs) were rapidly synthesized by treating silver ions with a *Capsicum annuum* L. extract [14]. The reaction process was simple and convenient to handle, and was monitored using ultraviolet-visible spectroscopy (UV-vis). The reduction of silver ions and stabilization of the silver NPs was thought to occur through the participation of proteins.

Important results employing biomass have been reached by J. Ascencio research group, who have developed a procedure based on the use of the tannins of the biomass of *Medicago sativa* (alfalfa) to obtain Au nanorods [23], bimetallic nanoparticles [24, 25] and even lanthanide clusters [26, 27], and lately has also been demonstrated effective for the synthesis Zn nanoparticles [28], and iron oxide [wuestite ($Fe_{0.902}O$) and magnetite (Fe_3O_4)] clusters [29]. The method is based on the reduction of metal ions through biomass at controlled pH conditions to improve the size control of nanoparticles.

Here we present a simple green synthetic methodology of silver nanoparticles and the effect of the temperature over

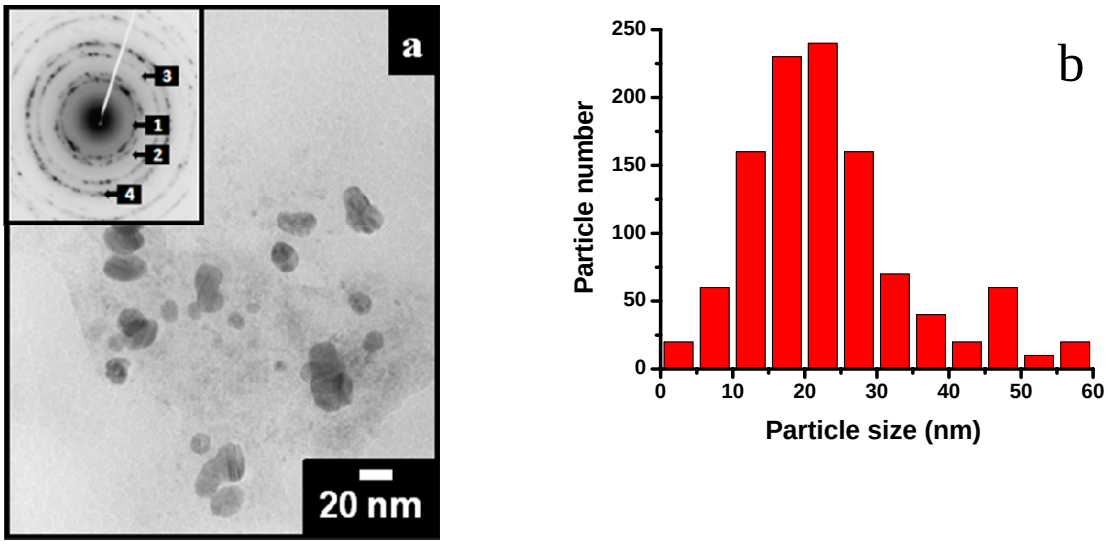

Figure 2. a) TEM image of silver nanoparticles synthesized with 5 ml of *Opuntia ficus indica* extract when reacting with 5 ml of 10^{-3} M aqueous solution of $AgNO_3$. Inset 2 a) Electron diffraction pattern of (a), which can be indexed on the basis of the FCC structures of silver. b**)** Size distribution histogram of the Ag nanoparticles synthesized with 5 ml of *Opuntia ficus indica* extract when reacting with 5 ml of 10^{-3} M aqueous solution of $AgNO_3$.

the morphology of the nanoparticles, which involves the *in-situ* reduction of aqueous Ag(I) ions employing *Opuntia ficus indica* aqueous extract as a reducing and capping agent,

2. Experimental

2.1 Materials.

Silver nitrate ($AgNO_3$) and 30% ammonia solution were all purchased from Sigma-Aldrich Chemicals. The above reagents were of analytical purity and were used without further purification.

2.2 Opuntia ficus indica Extract Preparation.

A 30 g portion of *Opuntia ficus indica* cladodes was rinsed with de-ionized water and finely cut. Afterwards, it was boiled in 100 mL of de-ionized water for five minutes. The mixture was cooled and vacuum filtered. The resulting extract was used for further experiments.

2.3 Synthesis of silver nanoparticles using Opuntia ficus indica extract.

Three different volumes (1, 5 and 10 mL) of the extract were added to 5 mL of 10^{-3} M $AgNO_3$ solutions, followed by the addition of 2.5 mL of 30% ammonia solution. Then, the volume was mixed with de-ionized water, to a final volume of 50 mL.

2.4 UV-Vis absorbance spectroscopy.

UV-Vis spectroscopy of the Ag-nanoparticles in aqueous solution was performed at room temperature using a PerkinElmer (model: Lambda 650) UV-Vis spectrophotometer. The bioreduction (formation of nanoparticles) of Ag(I) in aqueous solution was monitored by following the plasmon resonance absorption band of the reaction mixture during 4 hours as a function of time.

2.5. Transmission electron microscopy (TEM).

Samples for TEM studies were prepared by placing a drop of the silver nanoparticles colloidal suspension on carbon-coated grids. TEM images were obtained by using a transmission electron microscope JEOL 2010.

3. Results and Discussion

Chemical composition of *Opuntia ficus indica* cladodes' are mainly water (92%), carbohydrates (~4-6%), proteins (~1%), vegetable fats (~0.2%), minerals (~1%) such as calcium and iron predominantly, and vitamins, mainly ascorbic acid (vitamin C) [30, 31]. On the other hand, it has been demonstrated by Sun et al. [32] that gold nanoparticles can be obtained directly and simply by reduction of Au(III) ions with ascorbic acid as reducing agent.

The carbohydrate fraction of the *Opuntia ficus indica* can be attributed mainly to the starch and soluble sugars contents. The cladodes showed higher starch contents (from 7 to 13/100 g of dry weight) than soluble sugars (from 6 to 2/100 g of dry weight), in contrast, protein and fats contents showed poorer quantities. Besides, cladodes exhibited also low pH values due to the presence of many organic acids such as: malic, citric and oxalic acids [33]. Carboxylic moieties show a great affinity towards the surface of the nanoparticles, for this reason nanoparticles can be stabilized through electrostatic interactions with carboxylic groups, as reported previously by K Yoosaf et al. [34].

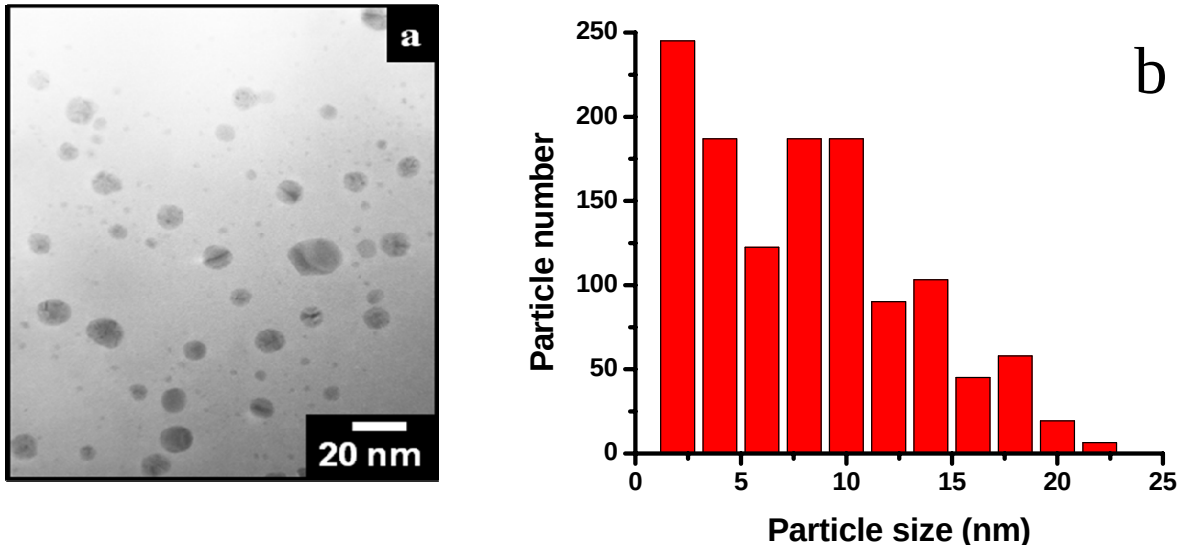

Figure 3. a) TEM image of Ag nanoparticles formed after 24 hours of the bioreduction, using 5 ml of *Opuntia ficus indica* extract and 5 ml of 10^{-3} M aqueous solution of $AgNO_3$ at 60°C. **b)** Histogram showing the Ag nanoparticles size distribution obtained at 60°C.

Opuntia ficus indica is considered as a great source of natural antioxidant compounds due to the content of polyphenols, higher than 900 mg/100 g of dry matter, which are mainly responsible for an antioxidant activity [33]. This type of compounds plays an important role during metal ion reduction processes in biosynthetic methods, as stated in previously reported [13]. Although a more detailed study is required to establish a detailed mechanism of formation and stabilization of the silver nanoparticles, we can assume that hydroxyl and carboxylic groups present in the biomolecules of the *Opuntia ficus indica* extract play an important role in the Ag(I) ions reduction and can also control the size and the stability of the nanostructures thus formed.

When *Opuntia ficus indica* aqueous extract is combined with 10^{-3} M aqueous silver nitrate, solution color changes from pale yellow to dark yellow due to the formation of silver nanoparticles and as a consequence of the surface plasmon resonance absorption.

The UV-Vis absorption spectra of silver nanoparticles, formed after 1 hour of reaction using different quantities of *Opuntia ficus indica* extract, are shown in Fig. 1(a) Spectra 3, 2 and 1 correspond respectively to 10, 5 and 1 ml of *Opuntia ficus indica* extract used as bioreducing agent. Spectra 2 and 3 show a single absorption band centered at 398 nm. In contrast, spectrum 1 exhibits an insignificant intensity absorption band at 398 nm, this is due to an insufficient amount of reductive biomolecules for the Ag^{+1} reduction; consequently, a small number of silver nanoparticles are formed.

As described by Huang et al. [13], weaker binding of biomolecules with the nascent silver nanoparticles could lead to isotropic growth of the crystals and further formation of spherical nanoparticles.

In plant-mediated synthesis, the control of the size of silver nanoparticles has been proposed to be time-reaction dependent [35]. Basically, the longer the reaction time, the larger the sizes and the nanoparticles change from polycrystalline to single crystalline. Fig. 1(b) shows UV-Vis spectra after 1 and 65 hours or reaction time. The fact that the spectra are very similar indicates that the particles have essentially the same size and shape (spheroids). This behavior takes place only when 5 ml of *Opuntia ficus indica* extract are used as the reducing agent. However, when this quantity is doubled, the corresponding UV-Vis band disappears after 65 hours, and silver micro-particles are deposited in the reaction solution as a result of a coalescence process of nanoparticles, and probably due to the poor efficacy of *Opuntia ficus indica* extract capping biomolecules in stabilizing the silver nanoparticles through time, thus increasing the size of Ag crystals.

TEM analysis reveals that silver nanoparticles are predominately ellipsoids, as can be observed in Fig. 2, micrograph (a) and histogram (b) correspond to silver nanoparticles formed after 24 hours of reaction, using 5 ml of 10^{-3} M of aqueous solution $AgNO_3$. Nanoparticles sizes are between 8 and 50 nm, with an average of 23 nm ± 5 nm.

A typical selected area diffraction pattern is shown in the inset of Fig. 2(b). Main Diffraction rings can be indexed as (111), (200), (220) and (311) reflections (indicated by numbers 1, 2, 3 and 4 respectively), corresponding to a FCC structure of silver. For the 1 and 10 ml of biomass extract, the average particle size was 9 and 53 nm, respectively (no pictures shown due to space limitation).
Fig. 3(a) shows silver nanoparticles after 24 hours of the bioreduction process, with the same amount of silver ions and volume of reducing agent used previously in Fig. 2(a) but kept at 60°C instead of at room temperature. Average particle size is reduced to 8 nm, and the particles are quite spherical.

5. Conclusions

A rapid, cost-effective and eco-friendly approach to obtaining stable silver nanoparticles in aqueous media, and at room conditions, has been established, employing *Opuntia ficus indica* extract as the reducing agent. The amount of reducing agent and temperature used during the synthesis has a deep effect on the size and morphology of the nanoparticles, resulting in an unsophisticated mode to manipulate those characteristics and, thus, to enhance their possible applications in biomedical, pharmaceutical and biotechnology areas. However, the specific mechanism of reduction, nucleation and grow model of silver ions and nanoparticles respectively, when interact with biomolecules, remains as a big challenge to be reached. Despite of more detailed studies are required to fully understand the difference in nanoparticles morphology and size when a temperature increment is applied during the biosynthesis, temperature is factor that can control the size and morphology in order to obtain a narrow size distribution of the particles synthesized by biological approach.

Acknowledgments

We acknowledge UNAM for financial support through the grant number IN108908. Rafael Vilchis Nestor acknowledges UNAM for a postdoctoral fellowship from DGAPA-UNAM program. Miguel Camacho acknowledges COMECYT for financial support through the Fondo Mixto Estado de México-Conacyt (FOMIX) program Grant Number 86644. Finally, we also like to thank Francisco Ruiz and Sidney Jimenez for their technical support.

References

[1]. S. Eustis, H. Y. Hsu, M. A. El-Sayed, J. Phys. Chem. B. **109**, 4811(2005).
[2]. F. Mafune, J. Kohno, Y. Takeda, T. J. Kondow, Phys. Chem. B. **106**, 7575(2002).
[3]. L. Rodríguez-Sánchez, M. C. Blanco, M. A. López-Quintela, J. Phys. Chem. B. **104**, 9683(2000).
[4]. G. Zhang, D. J. Wang, Am. Chem. Soc. **130**, 5616(2008).
[5]. M. Treguer, C. Cointet, H. Remita, J. Khatouri, M. Mostafavi, J. Amblard, J. J. Belloni, Phys. Chem. B. **102**, 4310(1998).
[6]. P. T. Anastas, M. M. Kirchhoff, Acc. Chem. Res. **35**, 686(2002).
[7]. P. Mohanpuria, N. K. Rana, S. K. Yadav, J. Nanopart. Res. **10**, 507(2008).
[8]. R. Joerger, T. Klaus, C. G. Granqvist, Adv. Mater. **12**, 407(2000).
[9]. K. C. Bhainsa, S. F. D'Souza, Colloids and Surfaces B: Biointerfaces. **47**, 160(2006).
[10]. J. L. Gardea-Torresday, J. G. Parsons, E. Gomez, J. Peralta-Videa, H. E. Troiani, P. Santiago, J. Yacaman, Nano. Lett. **2**, 397(2002).
[11]. A. R. Vilchis-Nestor, V. Sánchez-Mendieta, Marco A. Camacho-López, Miguel A. Camacho-López, J. A. Arenas-Alatorre, Mater. Lett. **62**, 3103(2008).
[12]. S. Shankar, A. Ahmad, M. Sastry, Biotechnol Prog. **19**, 1627(2003).
[13]. J. Huang , Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Y. Wang, W. Shao, N. He, J. Hong, C. Chen, Nanotechnology **18,** 105104(2007).
[14]. S. Li, Y. Shen, A. Xie, X. Yu, L. Qui, L. Zhang, Q. Zhang. Green Chem. **9**, 852(2007).
[15]. S.S. Shankar, A. Rai, B. Ankamwar, A. Singh, A. Ahmad, M. Sastry, Nature Materials **3**, 482(2004).
[16]. T. J. Beveridge, M. N. Hughes, H. Lee, K. T. Leung, R. K. Poole, I. Savvaidis, S. Silver, J. T. Trevors. Advances in Microbial Physiology, **38**, 177(1997).
[17]. Jean-Marc Bollag, Tawna Mertz, and Lewis Otjen. Bioremediation through Rhizosphere Technology, Chapter 1: Role of Microorganisms in Soil Bioremediation, 1994, ACS Symposium Series, Volume 563, pp. 2-10.
[18]. V. Kumar and S. K. Yadav. Journal of Chemical Technology and Biotechnology **84-2**, 151(2009).
[19]. R. Bali, N. Razak, A. Lumb and A.T. Harris. The synthesis of metallic nanoparticles inside live plants. Proceedings of the 2006 International Conference on Nanoscience and Nanotechnology (ICONN'2006), pp. 224-227.
[20]. G. Gamez, K. Dokken, I. Herrera, J. G. Parsons, K. J. Tiemann, and J. L. Gardea-Torresdey. Chemical processes involved in Au(III) binding and bioreduction by alfalfa biomass. Proceedings of the 2000 Conference on Hazardous Waste Research.
[21]. Jorge L. Gardea-Torresdey, E. Gomez, J. R. Peralta-Videa, Jason G. Parsons, H. Troiani, and M. Jose-Yacaman. Langmuir, **19**, 1357(2003).
[22]. J. Huang, L. Lin, Q. Li, D. Sun, Y. Wang, Y. Lu, N. He, K. Yang, X. Yang, H. Wang, W. Wang and W. Lin. Ind. Eng. Chem. Res. **47**, 6081(2008).
[23]. J. Gardea-Torresday, K. Tiemman, G. Gamez, K. Dokken, S. Tehuacanero, M. Jose-Yacaman J. Nanoparticles Res. **3,** 475(2001).
[24]. J. A. Ascencio, Y. Mejia, H. B. Liu, C. Angeles, G. Canizal. Langmuir **19**, 5882(2003).
[25]. P. S. Schabes-Retchkiman, G. Canizal, R. Herrera-Becerra, C. Zorrilla, H. B. Liu, J. A. Ascencio. Optical Materials, **29**, 95(2006).
[26]. J. A. Ascencio, A. C. Rodríguez-Monroy, H. B. Liu, G. Canizal. Chem.Lett., **33,** 1056(2004).
[27]. Jorge A. Ascencio, Ana C. Rincon, and Gerardo Canizal. J. Phys. Chem. B **109**, 8806(2005).
[28]. G. Canizal, P. S. Schabes-Retchkiman, U. Pal, Hong Bo Liu, J. A. Ascencio. Materials Chemistry and Physics **97**, 321(2006).
[29]. Raúl Herrera-Becerra, Cristina Zorrilla, and Jorge A. Ascencio. J. Phys. Chem. C **111**, 16147(2007).
[30]. D. Guzmán-Loayza, J. Chávez, Rev. Soc. Quím. Perú, **73**, 41(2007).
[31]. N. Salim, C. Abdelwaheb, C. Rabah, B. Ahcene, African Journal of Biotechnology. **8**, 1623(2009).
[32]. K. Sun, J. Qiu, J. Liu, Y. Miao, J. Mater. Sci, **44,** 754(2009).
[33]. M. A. Ayadi, W. Abdelmaksoud, M. Ennouri, H. Attia, Industrial Crops and Products **30,** 40(2009).
[34]. K. Yoosaf, B. I. Ipe, C. H. Suresh, K. G. Thomas, J. Phys. Chem. C **111**, 12839(2007).
[35]. V. Kumar, S. Kumar, J. Chem. Technol. Biotechnol. **84**, 151(2008).