Korean J. Chem. Eng., **29**(12), 1771-1775 (2012)
DOI: 10.1007/s11814-012-0082-4

Antibacterial latex foams coated with biologically synthesized silver nanoparticles using *Magnolia kobus* leaf extract

Jae Yong Song, Eun-Yeong Kwon, and Beom Soo Kim†

Department of Chemical Engineering, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea
(*Received 18 May 2012 • accepted 31 May 2012*)

Abstract−Silver nanoparticles are used in many industries due to their disinfection and antibacterial properties. Biological methods of nanoparticle synthesis have been suggested as possible ecofriendly alternatives to chemical and physical methods. In this study, biologically synthesized silver nanoparticles using *Magnolia kobus* leaf extract were coated on the surface of latex foam products using dip coating (exposure to nanoparticle solution) or ultrasonic treatment. SEM image of the treated foam showed that silver nanoparticles were uniformly coated on the surface of latex foam. Antibacterial properties were tested by counting viable *Escherichia coli* cells after 24 h growth in shake flask cultures containing latex foams coated with silver nanoparticles. Foams coated with silver nanoparticles showed higher antibacterial activities compared with foams untreated. Smaller silver nanoparticles synthesized at higher temperature showed higher antibacterial activity due to the larger specific surface area and higher content of silver nanoparticles. The growth of *E. coli* decreased with increasing the concentration of silver nanoparticles. Ultrasonic treatment showed higher adsorption and lower desorption of silver nanoparticles to and from the foams compared with dip coating, resulting in higher antibacterial activity.

Key words: Silver Nanoparticles, Antibacterial Activity, Latex Foam, Biological Synthesis, *Magnolia kobus*

INTRODUCTION

Silver nanoparticles have been used extensively in many antibacterial fields [1-9]. The antibacterial activities of silver nanoparticles were found to relate to their shapes and sizes. Many studies have been reported to synthesize silver nanoparticles with controllable shape, size, and size distribution. These controls were achieved by a variety of physical, chemical, and biological methods. Chemical approaches are the most popular methods for the production of nanoparticles [10]. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticle synthesis that do not use toxic chemicals. Biological methods of nanoparticle synthesis using microorganism [11-14], enzyme [15], and plant or plant extract [16-18] have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale synthesis of nanoparticles [17].

Recently, we reported engineering approaches such as rapid synthesis of silver, gold, platinum, and bimetal nanoparticles using plant extracts and size and shape control of the synthesized nanoparticles [19-23]. Several plant leaf extracts were screened and compared for their synthesis of nanoparticles. The effects of reaction conditions such as temperature and composition of the reaction mixture were also investigated to control the size and shape of nanoparticles. Despite a number of recent reports on biological nanoparticle synthesis [24], studies on the evaluation of antimicrobial properties by applying the biologically synthesized silver nanoparticles to the final products are rarely reported.

In this study, we evaluated antibacterial characteristics of latex foams coated with silver nanoparticles synthesized using *Magnolia kobus* leaf extract. Latex foams are used where antimicrobial properties are required for human health such as in pillows and mattresses. By incorporating silver nanoparticles to the latex foam products, we expected to increase the antimicrobial activity of the products. Coating methods (dip coating or ultrasonic treatment) and the average sizes of silver nanoparticles (depending on synthesis temperature) were changed to investigate antibacterial activity and the degree of adsorption and desorption of silver nanoparticles to and from the foams.

EXPERIMENTAL

1. Materials

Silver nitrate ($AgNO_3$; 99.8% purity) was purchased from Samchun Chemical (Korea). Latex foams were supplied from Latex Korea Co. (Cheongju, Korea).

2. Synthesis and Characterization of Silver Nanoparticles

Magnolia kobus leaves were collected and dried for two days at room temperature. The plant leaf broth solution was prepared by taking 5 g of thoroughly washed and finely cut leaves in a 300 ml Erlenmeyer flask with 100 ml of sterile distilled water, and then boiling the mixture for 5 min before finally decanting it. They were stored at 4 °C and used within a week.

Typically, 10 ml of leaf broth was added to 190 ml of 1 mM aque-

†To whom correspondence should be addressed.
E-mail: bskim@chungbuk.ac.kr

ous $AgNO_3$ solution for reduction of Ag^+ ions. The reactions were carried out in water bath at 30, 60, and 95 °C with reflux for 10 h, 5 h, and 30 min, respectively, to ensure almost complete conversion of silver ions to silver nanoparticles. The structures of nanoparticles and foams were analyzed by field emission transmission electron microscopy (FE-TEM, Tecnai F30 S-Twin, FEI) and scanning electron microscopy (SEM, Hitachi S-2500C). Silver concentrations were determined using inductively coupled plasma spectrometry (ICP, JY38Plus). Average particle sizes were measured using particle analyzer (NICOMP™ 380 ZLS).

3. Coating of Silver Nanoparticles on Latex Foams

Latex foams were washed, sterilized and dried before use. Latex foams were coated using two methods, dip coating (exposure to nanoparticle solution) or ultrasonic treatment. For dip coating, latex foam (2 cm×2 cm×2 cm) was added to three different concentrations of silver nanocolloidal solution at 0.1, 0.5, and 1 mM. The weight of latex foam was 5 g and the total volume of solution was 100 ml. The nanoparticle solution containing latex foam was placed in shaking incubator for 1 h at 30 °C. For ultrasonic treatment, 5 g of latex foam was added to 100 ml solution containing 1 mM $AgNO_3$ and 5% *Magnolia kobus* leaf broth. The solution was irradiated for 1 h with high intensity ultrasonic horn (Ti horn, 20 kHz, 130 W at 60% efficiency) under air. The resulting foams coated with silver nanoparticles using the two methods were dried in an oven for 24 h at 50 °C. The degree of coating on latex foams was determined by the difference of initial and residual concentrations of silver nanoparticle solution before and after coating, which were analyzed by ICP. The desorption of silver nanoparticles from foam was evaluated by measuring silver nanoparticle concentrations after adding 5 g of coated foams in a 250 ml Erlenmeyer flask that contained 100 ml distillated water and then shaking the whole solution at 200 rpm for 24 h. The experiments were performed in triplicate and all data were obtained based on the average values.

4. Antibacterial Activity

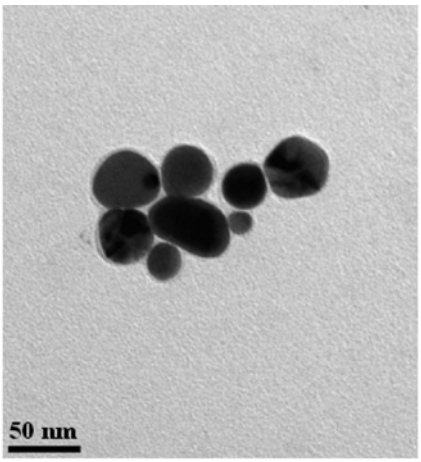
The antibacterial activity was tested against gram-negative *Escherichia coli* strain (ATCC 25922). A 100 ml of LB broth (yeast extract 5 g/L, tryptone 10 g/L, and NaCl 5 g/L) was added to 250 ml sterile flask and then inoculated with 1 ml of the *E. coli* culture solution grown on LB broth for 12 h at 37 °C. Five grams of sterile latex foams coated with silver nanoparticles were placed in the flasks and shaken for 24 h at 37 °C. Then, 100 µl of the cultured solution was transferred to a nutrient agar medium (Difco™ LB Agar). After 24 h of incubation at 37 °C, colonies of viable cells were counted with suitable dilution and reported as colony forming unit (CFU) per ml. Uncoated foams were used as control. Antibacterial activity was defined as the percentage of microbe reduction [9] and was calculated as follows:

$$\text{Antibacterial activity } (\%) = \left[1 - \frac{\text{CFU/ml(sample)}}{\text{CFU/ml(control)}}\right] \times 100$$

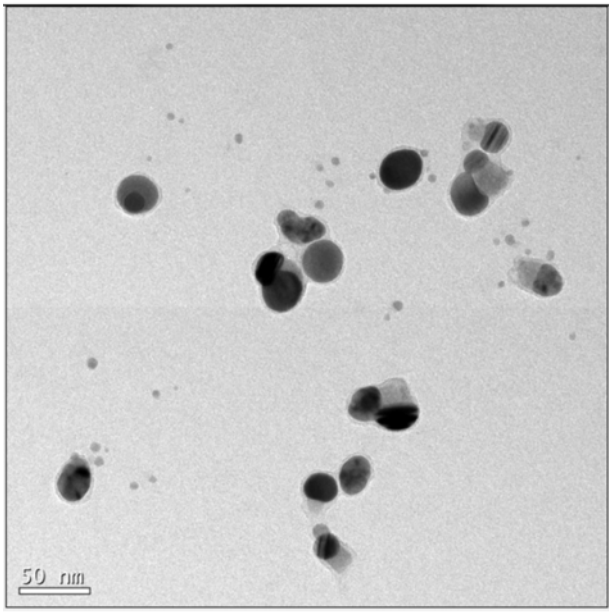
RESULTS AND DISCUSSION

1. Adsorption and Desorption Characteristics of Silver Nanoparticles

In our previous report, we screened several plant leaf extracts and compared their synthesis of silver nanoparticles by monitoring the conversion using UV-visible spectroscopy [19]. The synthesis rate was highest with *Magnolia kobus* leaf broth as reducing agent. Only 11 min was required for more than 90% conversion at 95 °C. SEM images of silver nanoparticles formed with 1 mM $AgNO_3$ and 5% *Magnolia kobus* leaf broth at 30 °C (Fig. 1(a)) and at 95 °C (Fig. 1(b)) show that particle sizes decrease with reaction temperature. It is also shown that the synthesized silver nanoparticles are surrounded by a thin layer of some capping materials and thus were stable in aqueous solution for a long time, which is another advantage using plant extracts as reducing agent compared with using chemical reducing agents that require additional stabilizer. Further characterization data on the synthesized silver nanoparticles were reported in our previous study [19].



(a)



(b)

Fig. 1. TEM images of silver nanoparticles formed with 1 mM $AgNO_3$ and 5% *Magnolia kobus* leaf broth (a) at 30 °C and (b) at 95 °C.

Table 1 summarizes adsorption and desorption characteristics of silver nanoparticles according to synthesis temperature and coating method. The average particle size could be controlled by changing

Table 1. Adsorption and desorption characteristics of silver nanoparticles with coating method and synthesis temperature

Coating method	Dip-coating			Ultrasonic treatment
Synthesis temperature (°C)	30	60	95	-
Average particle size (nm)	50	30	16	25
Coated amounts (mg/5 g foam)	6.1	6.1	7.1	13
Silver nanoparticle content (%)	0.12	0.12	0.14	0.25
Desorption after washing (%)	1.2	1.2	0.99	0.41

the reaction temperature and decreased from 50 nm at 30 °C to 16 nm at 95 °C. The average size of silver nanoparticles formed by ultrasonic treatment was ca. 25 nm. For dip coating, the calculated silver nanoparticle contents were 0.12-0.14% of foam-nanoparticle composite. The nanoparticle content was higher at 95 °C of synthesis temperature than at 30 and 60 °C, suggesting higher incorporation of smaller particles to foams. Ultrasonic treatment increased the particle content to 0.25% of the composite. The mechanism of the strong adhesion of silver nanoparticles to substrates was discussed based on the point melting of the substrate due to the high rate and temperature of the silver nanoparticles thrown to the solid surface by sonochemical microjets [4]. Sonochemical irradiation has been proven as an effective method for the synthesis of nanophased materials [25] as well as for the deposition and insertion of nanoparticles on/into the fabrics [4]. The desorption characteristics of silver nanoparticles from the coated foam was investigated to evaluate laundering durability of the composite. For dip-coating, the loss of silver nanoparticles from foam ranged 0.99-1.2% of initial coated amounts after shaking for 24 h in distilled water. Silver nanoparticles synthesized at 95 °C showed slightly lower desorption of nanoparticles from foam. Ultrasonic treatment also decreased the loss of silver nanoparticles to 0.41% due to the stronger adhesion of silver nanoparticles to substrates compared with dip coating. Although adsorption and desorption depend on the permeability of silver nanoparticles into foam, the permeability would be the same because the two coating methods do not affect the internal structure of foam.

The incorporation of silver nanoparticles to latex foam was also observed by color change of the coated foams. The apparent colors of foams changed from white for the uncoated (Fig. 2(a)) to yellowish-brown for the coated (Fig. 2(b)) due to the silver nanoparticles incorporated. The color of the ultrasonically treated foam (Fig. 2(c)) turned into deep-brown, indicating higher content of silver nanoparticles in the composite. The color of silver nitrate solution remained unchanged during the ultrasonic treatment, suggesting

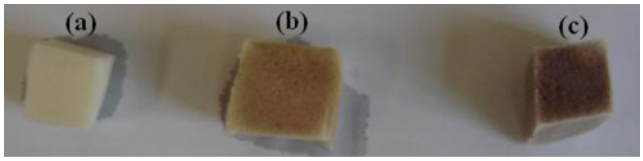


Fig. 2. Photographs of foams with coating method. (a) Untreated, (b) dip-coated with silver nanoparticles formed with 1 mM $AgNO_3$ and 5% *Magnolia kobus* leaf broth at 95 °C, and (c) ultrasonically treated with 1 mM $AgNO_3$ and 5% *Magnolia kobus* leaf broth.

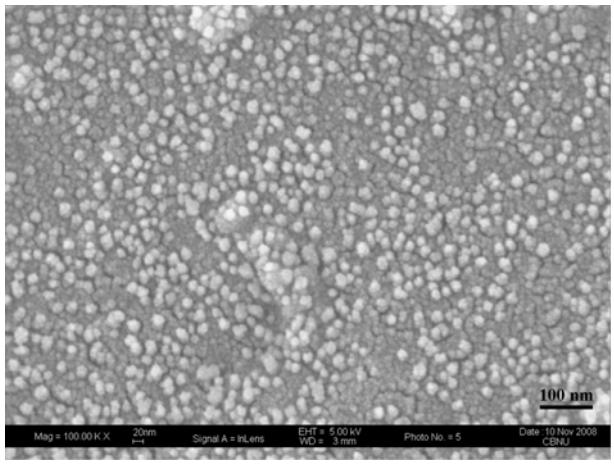


Fig. 3. SEM image of foam containing silver nanoparticles after ultrasonically treated with 1 mM $AgNO_3$ and 5% *Magnolia kobus* leaf broth.

that silver nanoparticles were coated on the pores of foam together with their synthesis. Fig. 3 is a representative SEM image of latex foam after coating with silver nanoparticles by ultrasonic treatment with 1 mM $AgNO_3$ and 5% *Magnolia kobus* leaf broth. It is shown that silver nanoparticles of approximately 25 nm are evenly coated on the surface of latex foam. Other foams coated by dip coating also showed uniform distribution of silver nanoparticles within the matrix. Although the covered area of silver nanoparticles appears to be large, the range of silver nanoparticle content was only 0.12-0.25% of the composite, indicating that silver nanoparticles were only coated on the surface of latex foam.

2. Antibacterial Property of Foams Containing Silver Nanoparticles

These foams containing silver nanoparticles were used to test antibacterial activity against *E. coli*. Fig. 4(a) to f are photographs

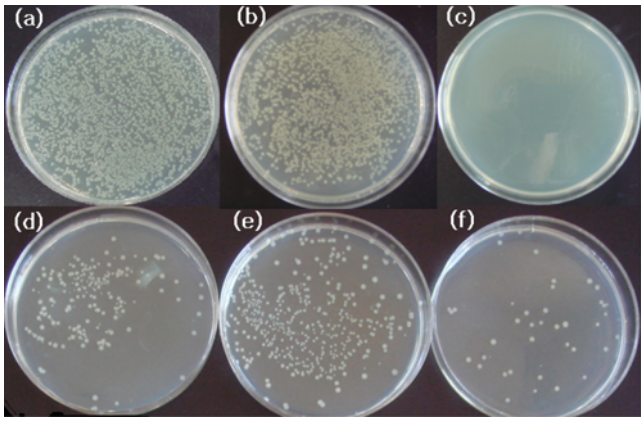


Fig. 4. Photographs of *E. coli* cultures grown with foams (a) untreated, (b) treated with only 5% *Magnolia kobus* leaf broth, (c) ultrasonically treated with 1 mM $AgNO_3$ and 5% *Magnolia kobus* leaf broth, and (d)-(f) dip-coated with silver nanoparticles formed with 1 mM $AgNO_3$ and 5% *Magnolia kobus* leaf broth at 30, 60, and 95 °C respectively. *E. coli* cells were grown in LB media containing latex foams for 24 h at 37 °C and then 100 μl of cultured solution was transferred to agar medium and incubated for another 24 h.

Table 2. Antibacterial activity of foams with coating method, synthesis temperature, and dipping concentration of silver nanoparticles

Coating method	Control (untreated)	Treated with only 5% leaf broth	Dip-coating					Ultrasonic treatment
Synthesis temperature (°C)	-	-	30	60	95	95	95	-
Silver nanoparticles concentration (mM)	-	-	1	1	1	0.5	0.1	1
Colony count (CFU/ml)	20,630	19,980	1,520	1,950	450	2,480	4,530	0
Antibacterial activity (%)	0	3.15	92.6	90.5	97.8	87.9	78.0	100

of *E. coli* cultures grown with foams after various treatments. The antibacterial characteristics of foams containing silver nanoparticles are summarized in Table 2. The colony count of untreated latex foam was 20,630 CFU/ml (Fig. 4(a)), which was much lower than that of untreated cotton (1.2×10^7 CFU/ml), indicating that latex foam itself originating from natural rubber trees has antimicrobial activity. When foams were treated with only 5% *Magnolia kobus* leaf broth without silver nanoparticles, the colony count was 19,980 CFU/ml (Fig. 4(b)). The value was similar to that of untreated latex foam, suggesting that *Magnolia kobus* leaf broth does not contribute to the antibacterial activity. The antibacterial activity of dip-coated foams with silver nanoparticle solution synthesized at 30, 60, and 95 °C was 92.6, 90.5, and 97.8, respectively (Fig. 4(d) to (f)). When dip-coating concentrations of silver nanoparticle solution synthesized at 95 °C were 0.1, 0.5, and 1 mM, the antibacterial activity was 78.0, 87.9, and 97.8%, respectively. The antibacterial activity of latex foams coated with silver nanoparticles using ultrasonic treatment was 100% as shown in Fig. 4(c), where no colonies were formed. The above results show that the growth of *E. coli* was significantly suppressed by foams coated with silver nanoparticles compared with latex foams untreated or treated with only leaf broth without silver nanoparticles. Smaller nanoparticles synthesized at 95 °C showed higher antibacterial activity due to the larger specific surface area and higher content of silver nanoparticles. The growth of *E. coli* decreased with increasing the dip-coating concentrations of silver nanoparticle solution. Foams coated with silver nanoparticles using ultrasonic treatment showed highest antibacterial activity probably due to the high content and strong adhesion of silver nanoparticles.

Several factors have been reported to influence silver nanoparticle toxicity like particle size, shape, crystallinity, and capping agents, as well as solution pH, ionic strength, specific ions and ligands, and macromolecules [26]. Smaller particle sizes tend to enhance antibacterial properties because there is a large number of atoms on the surface available to interact with bacteria or to release a higher amount of silver ions with decreasing size [27,28], which is consistent with our data. Higher stability produces higher antibacterial properties because non-stable nanoparticles tend to form aggregates, and thus surface area is reduced [29]. It was also reported that biologically synthesized silver nanoparticles had a greater bactericidal activity than did chemically synthesized silver nanoparticles [30,31]. The nanoparticle surface coating appeared to play a prominent role in toxicity, suggesting that stable nanoparticles synthesized using plant extracts may have an advantage in antibacterial applications compared with chemically synthesized nanoparticles.

Currently, the mechanism of antimicrobial action of silver nanoparticles is only partially understood. Several studies propose that silver nanoparticles attach to the surface of cell membrane disturbing permeability and respiration function of the cell [32,33]. The damage to the cell may be caused by the interaction of silver nanoparticles with phosphate or sulfur-containing compounds such as DNA. The three most common mechanisms of toxicity proposed by Marambio-Jones and Hoek [26] are (1) uptake of free silver ions followed by disruption of ATP production and DNA replication, (2) generation of reactive oxygen species by silver nanoparticles and silver ions, and (3) direct damage to cell membranes by silver nanoparticles. Since the action of silver nanoparticles is not species-dependent, silver nanoparticles can possibly be used for the treatment of multidrug resistant bacteria. Recently, there is a concern that silver nanoparticles may be harmful to human health. However, no sufficient information is available on the adverse effects of nanoparticles on human health. Further research is required to fully understand the mechanisms of silver nanoparticle toxicity and to safely exploit the tremendous antimicrobial properties of silver without negatively impacting human health and the environment.

In summary, biologically synthesized silver nanoparticles using *Magnolia kobus* leaf extract were coated on the surface of latex foam for possible use as ecofriendly antibacterial products. Smaller nanoparticles synthesized at 95 °C showed higher adsorption and lower desorption to and from the foams, resulting in higher antibacterial activity against *E. coli*. Foams coated with silver nanoparticles using ultrasonic treatment showed 100% reduction of *E. coli* growth compared with untreated foams due to the high content and strong adhesion of silver nanoparticles.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2006375).

REFERENCES

1. Y. Zhang, H. Peng, W. Huang, Y. Zhou and D. Yan, *J. Colloid Interface Sci.*, **325**, 371 (2008).
2. M. Fondevila, R. Herrer, M. C. Casallas, L. Abecia and J. J. Ducha, *Anim. Feed Sci. Technol.*, **150**, 259 (2009).
3. Y. Li, P. Leung, L. Yao, Q. W. Song and E. Newton, *J. Hosp. Infect.*, **62**, 58 (2006).
4. I. Perelshtein, G. Applerot, N. Perkas, G. Guibert, S. Mikhailov and A. Gedanken, *Nanotechnol.*, **19**, 245705 (2008).
5. N. Durán, P. D. Marcato, G. I. H. De Souza, O. L. Alves and E.

Esposito, *J. Biomed. Nanotechnol.*, **3**, 203 (2007).
6. R. Yoksan and S. Chirachanchai, *Mater. Chem. Phys.*, **115**, 296 (2009).
7. S. M. Lee, K. C. Song and B. S. Lee, *Korean J. Chem. Eng.*, **27**, 688 (2010).
8. H. Jiang, S. Manolache, A. C. L. Wong and F. S. Denes, *J. Appl. Polym. Sci.*, **93**, 1411 (2004).
9. V. Ilić, Z. Šaponjić, V. Vodnik, B. Potkonjak, P. Jovančić, J. Nedeljković and M. Radetić, *Carbohydr. Polym.*, **78**, 564 (2009).
10. W.-S. Seo, T.-H. Kim, J.-S. Sung and K.-C. Song, *Korean Chem. Eng. Res.*, **42**, 78 (2004).
11. T. Klaus, R. Joerger, E. Olsson and C.-G. Granqvist, *Proc. Natl. Acad. Sci. U.S.A.*, **96**, 13611 (1999).
12. Y. Konishi, K. Ohno, N. Saitoh, T. Nomura, S. Nagamine, H. Hishida, Y. Takahashi and T. Uruga, *J. Biotechnol.*, **128**, 648 (2007).
13. B. Nair and T. Pradeep, *Cryst. Growth Des.*, **2**, 293 (2002).
14. S. Gurunathan, K. Kalishwaralal, R. Vaidyanathan, D. Venkataraman, S. R. K. Pandian, J. Muniyandi, N. Hariharan and S. H. Eom, *Colloids Surf. B*, **74**, 328 (2009).
15. I. Willner, R. Baron and B. Willner, *Adv. Mater.*, **18**, 1109 (2006).
16. J. L. Gardea-Torresdey, J. G. Parsons, E. Gomez, J. Peralta-Videa, H. E. Troiani, P. Santiago and M. Jose-Yacaman, *Nano Lett.*, **2**, 397 (2002).
17. S. S. Shankar, A. Rai, A. Ahmad and M. Sastry, *J. Colloid Interface Sci.*, **275**, 496 (2004).
18. S. P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad and M. Sastry, *Biotechnol. Prog.*, **22**, 577 (2006).
19. J. Y. Song and B. S. Kim, *Bioprocess Biosyst. Eng.*, **32**, 79 (2009).
20. J. Y. Song, H.-K. Jang and B. S. Kim, *Process Biochem.*, **44**, 1133 (2009).
21. J. Y. Song, E.-Y. Kwon and B. S. Kim, *Bioprocess Biosyst. Eng.*, **33**, 159 (2010).
22. J. Y. Song and B. S. Kim, *Korean J. Chem. Eng.*, **25**, 808 (2008).
23. J. Y. Song and B. S. Kim, *Korean Chem. Eng. Res.*, **48**, 98 (2010).
24. C. Krishnaraj, E. G. Jagan, S. Rajasekar, P. Selvakumar, P. T. Kalaichelvan and N. Mohan, *Colloids Surf. B*, **76**, 50 (2010).
25. A. Gedanken, *Sonochem.*, **11**, 47 (2004).
26. C. Marambio-Jones and E. M. V. Hoek, *J. Nanopart. Res.*, **12**, 1531 (2010).
27. C. Carlson, S. M. Hussain, A. M. Schrand, L. K. Braydich-Stolle, K. L. Hess, R. L. Jones and J. J. Schlager, *J. Phys. Chem. B*, **112**, 13608 (2008).
28. O. Choi and Z. Hu, *Environ. Sci. Technol.*, **42**, 4583 (2008).
29. L. Kvitek, A. Panacek, J. Soukupova, M. Kolar, R. Vecerova and R. Prucek, *J. Phys. Chem. C*, **112**, 5825 (2008).
30. A. K. Suresh, D. A. Pelletier, W. Wang, J.-W. Moon, B. Gu, N. P. Mortensen, D. P. Allison, D. C. Joy, T. J. Phelps and M. J. Doktycz, *Environ. Sci. Technol.*, **44**, 5210 (2010).
31. J. J. Antony, P. Sivalingam, D. Siva, S. Kamalakkannan, K. Anbarasu, R. Sukirtha, M. Krishnan and S. Achiraman, *Colloids Surf. B*, **88**, 134 (2011).
32. V. K. Sharma, R. A. Yngard and Y. Lin, *Adv. Colloid Interface Sci.*, **145**, 83 (2009).
33. M. Rai, A. Yadav and A. Gade, *Biotechnol. Adv.*, **27**, 76 (2009).