Proceeding of the 2nd International Conference on Agriculture and Forestry, Vol. 1, 2015, pp. 28-35

ISSN: 2362 – 1036 online
DOI: 10.17501/icoaf2015-1104

BIOSURFACTANT PROCESS SYNTHESIS AND STABILIZATION OF SILVER NANOPARTICLES FOR MODIFIED PRESERVATION METHODS ON COMMON FERMENTED FOODS

Dr. P. Tholkappian *[1], R. Parthasarathi[2] , P. Anna Joice[3] and R.Elango[4]

1,2,4Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

3Division of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

Abstract

A biosurfactant produced by Pseudomonas aeruginosa PBSC1 cultivated in a low-cost Cashew Apple Juice medium was employed to synthesize and stabilize silver nanoparticles in the liquid phase. The particles were initially synthesized using $NaBH_4$ as reducing agent in biosurfactant reverse micelles and were extracted from the micellar solution to disperse in heptane. A silver particle size in the range of 11 nm was observed. The UV–vis absorption spectra proposed that silver nanoparticles could be formed in the reverse micelles and relatively stabilized for at least 3 months without passivator addition. The Transmission Electron Microscope (TEM) shows that the silver nanoparticles are of spherical form and relatively uniform. This method provided a simpler way for nanoparticle synthesis compared to existing systems using whole organisms or partially purified biological extracts, showing that the low-cost biosurfactant can be used for nanoparticle synthesis as a non-toxic and biodegradable stabilizing agent. Thus synthesized silver nano particles along withbacteriocin found to be very effective antimicrobial agent against food spoiling organisms such as *Micrococcus luteus, Bacillus cereus, Staphylococcus aureus, Pediococcussp and Escherichia coli*. Antimicrobial activity of the silver nano particles and bacteriocin combination made a modification in the preservation methods in fermented foods such as pickled cucumbers, pickled beets and Sauerkraut. This study proved effective control and preservation of the selected common fermented foods. This modified method in food preservation not only improves the quality of fermented foods but also satisfy consumers.

Keywords: Pseudomonas aeruginosa, Cashew Apple Juice, Rhamnolipid, Silver nanoparticles, bacteriocin, Food preservation.

Introduction

Biosurfactants are surface-active secondary metabolites produced on living surfaces, mostly on microbial cell surfaces or excreted extracellular in the growth medium. Contain hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface respectively. They hold numerous advantages compared to their chemical synthesized counterparts, for example, they can be produced from renewable resources and are alive under extreme conditions pH and temperature and highly biodegradable [1, 7, 13].

The area of the biosurfactant mediated process of nanoparticle synthesis is emerging as part of green chemistry and they act as a potent stabilizer in the silver nanoparticles synthesis [9]. Currently, many techniques have been devoted to synthesizing nanosize silver particles, such as chemical reduction [15],

Corresponding Author. Email: tholsklm@yahoo.com

photochemical reduction, reverse micelle based andlamellar liquid crystals approaches [10], aerosol techniques andan electrostatic spraying technique.Since reverse micelles system was used to form metal nanoparticles by Boutonnet *et al*. [2], these methods havebeen paid more and more attention. The antimicrobial property of the silver nanoparticles have been studied extensively and the used in the control of many potential pathogens that cause food spoilage.

In the present study the isolation ofpotent biosurfactant producers from mangrove ecosystem and extraction, purification and characterize biosurfactant was studies. The optimization of biosurfactant production usingCashew Apple Juice medium through Response Surface Methodology (RSM) and to synthesize and stabilize the silver nanoparticles using biosurfactants, exploring antimicrobial activity against food spoiling organisms for developing modified preservation methods for common fermented foods.

Materials and Methods

Microorganisms

Pseudomonas aeruginosa PBSC1 was isolated from Mangrove soil sediments after screening using drop collapse assay, haemolytic assay, oil displacement test and CTAB agar plate method. The isolate PBSC1 was identified by the 16S r RNA sequence as Pseudomonas aeruginosa PBSC1 and deposited under the accession number KJ920194 in the GenBank.

Fermentation media

The sterilized MSM broth was added with various concentrations of cashew apple juice (2, 4, 6 and 8 per cent). To prove the effect of cashew apple juice on the biosurfactant production, cashew apple juice was used as such without inorganic mineral salts. For comparison, defined medium (MSM with 2 per cent glucose) was included in the study. To that 5.0 ml inoculum of *Pseudomonas aeruginosa* PBSC1 were inoculated to the respective flasks and incubated at room temperature (28 ± 2°C) for 5 days over an Orbital rotary shaker set at 120 rpm min^{-1}. To find out the variation in the biosurfactant production, the reduction in surface tension was made using 20 ml of cell free culture broth recorded at zero and at 120 h in a duNouyTensiometer (Kruss Digital–Tensiometer, Germany) at room temperature (28 ± 2°C).

Response Surface Methodology (RSM) for the optimization of biosurfactant production from *P. aeruginosa* PBSC1 using Cashew Apple Juice

To examine the interaction effect of different selected parameters (glycerol, sodium nitrate, pH and temperature) on biosurfactant production by *P. aeruginosa* PBSC1, the Central Composite Design (CCD), with 30 experiments were performed in duplicate. The reduction in surface tension of the medium is the direct measure of the biosurfactant production. Hence, the value of the dependent response surface tension reduction was the mean of two replications. The reduction in surface tension was determined. The second-order polynomial coefficients were calculated and analyzed using Design Expert software (version 8.0.7.1, Stat-Ease Inc., USA), Central composite design was conducted in the optimum vicinity to locate the true optimum conditions of Glycerol (A), sodium nitrate (B), pH (C), Temperature (D) for the production of biosurfactant (Table 1). For the four factors, this trial was a 2^{5-1} factorial design augmented by six axial points (or called star points) coded $\alpha \pm 2$ and two replicates of center points (all factors at level 0), resulting in a total number of 30 experiments.

Response surfaces were drawn to determine the individual and interactive effects of the test variable on the reduction in the surface tension of the medium. The optimal values of the test variables were first obtained in coded units and then converted to the uncoded units. The quality fit of the model equation was expressed with the coefficient of determination R^2 and its statistical significance was determined by an F-test. The significance of the regression coefficients was tested by a t-test.

Extraction of Biosurfactant

The biosurfactant was extracted from the culture medium after cell removal by centrifugationat5000g for 30 min. The supernatant pH was adjusted to 2.0 with 6.0 M HCl and an equal volume of $CHCl_3$/CH_3OH (2:1) was added. The mixture was vigorously shaken for 15 min and allowed to set until phase separation. The organic phase was removed and the operation was repeated twice. The product was concentrated from the pooled organic phases using a rotary evaporator. The viscous yellowish product obtained was dissolved in methanol and concentrated again by evaporation of the solvent at 45° C.

Synthesis of Silver nanoparticles [16]

For the synthesis of silver nanoparticles in situ in the water-in-oilmicroemulsion phase, a 0.05 mol/l aqueous $AgNO_3$ solution and a0.1 mol/l aqueous NaBH4 solution were separately used instead ofwater to form reverse micelles with the biosurfactant. $NaBH_4$ wasused here to act as reducing agent.The first synthesis involved mixing 1.0 ml of 0.05 mol/l aqueous$AgNO_3$ solution, 0.1 g/l biosurfactant and 25 ml n-heptane togetherand stirred vigorously at room temperature until homogeneousreverse micelles formed and the same bulk of 0.1 mol/l aqueous$NaBH_4$ solution was used to replace aqueous $AgNO_3$ to form theother reverse micelles. The two samples were mixed under stirringfor 60 min. Then, the particles were precipitated from the solutionand isolated by centrifugation at 14,000 × g. Then, 0.5 ml ethanolwas added for each 1 ml reverse micelles. Ethanol was added to thecomplete removal of the surfactant and n-heptane. The preparedsilver nanoparticles could be readily redispersed to obtain a suspensionin 10 ml n-butanol aided by sonication. The second microemulsion was prepared by dissolving 0.1 g/l ofthe biosurfactant in 6.25 ml of n-heptane and 1 ml $AgNO_3$. Solutionwas added to the mixture with continuous stirring for 10 min at roomtemperature. Then, 1 ml $NaBH_4$ was added to the mixture which wasagitated for 30 min. After agitation, 10 ml ethanol was added to breakthe reverse micelles, thus forming two phases. The precipitate wasseparated by centrifugation at a speed of 14,000 × g for 30 min and10 ml of n-butanol was added to obtain a suspension.

Characterization of silver nanoparticles

UV-Visible Spectroscopy

The optical characterizations of the synthesized silver nanoparticles were analyzed through absorption spectra measured in room temperature in a UV Visible absorption spectrometer (ELICO SL 244) at the wavelength of 200 to 800nm under dispersion mode.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is an imaging technique whereby a beam of electrons is focused onto a specimen causing an enlarged version to appear on a fluorescent screen or layer of photographic film, or to be detected by a CCD camera. The microstructure studied by use of the image mode. In our study the synthesized silver nanoparticles were lyophilized and dispersed in 100per cent absolute ethanol. The ethanol dispersed particles were then sonicated to deposit on a copper grid. That was analyzed in Transmission Electron Microscope (JEM-2100F LaB6, USA) under100,000 X magnification.

Antimicrobial activity of silver nanoparticles against food pathogens

The antimicrobial activity of the silver nanoparticles against food pathogens was studied using well cutting method. Two hundred and fifty ml of Muller Hinton agar plates was prepared and sterilized in an autoclave for 15 min at 121ºC and the plates were swabbed with food pathogens *viz*., *Micrococcus luteus*, *Bacillus cereus, Staphylococcus aureus, Pedicoccus* sp. *and Escherichia coli* respectively and well were made with steel cork borer (1 cm in diameter). The silver nanoparticles with various concentrations (10, 20, 30, 40, 50µl), Bacteriocin obtained from *Lactobacillus plantarum@* 1000 ppm, Bacteriocin obtained from *Lactobacillus plantarum@* 1000 ppm combined with silver nanopartilces @ highest concentration was prepared and poured in the well and the plates were

incubated for 24 h at 37 ± 2ºC for bacteria and for 48 h at 25 ± 2ºC for yeast respectively. After the incubation period the diameter of the zone of inhibition was measured [3]. To ensure that, the results were reproducible, the average of five independent measurements was taken.

Statistical analysis

This data was analyzed by the analysis of variance (ANOVA) technique to find out which factors had the most effective interactions for higher biosurfactant production [14].

Results and Discussion

The biosurfactant from *P. aeruginosa* PBSC1 cultivated in a low-costmedium Cashew apple juice was produced during 120 h at 37°C. The medium surface tension was reduced to 31.1 mN/m at the end of fermentation and the isolated biosurfactant corresponded to a concentration of 12.54 g/l.The extraction using acidification with equal volume of Methanol: Chloroform showed highest emulsification activity (70.4 per cent) for the extracted aliquots of *P. aeruginosa* PBSC1. The brown coloured precipitate was observed after incubation, collected by centrifugation and pellet was resuspended with the solvent and stored at 4 ºC.

The experimental and predicted values of surface tension reduction of the media were represented in Figure 1 and Table 2.The statistical significance of the model equation was calculated by F-test for analysis of variance (ANOVA), which indicates that the regression was strongly significant at 99per cent ($P<0.05$) confidence level (Table 3).

Biosurfactants have numerous advantages compared to chemically synthesized surfactants, but on the other hand, they have high production costs due to low yields and fastidious purification. In the present study, an attempt has been made to develop the biosurfactant production process economically attractive by using cheap renewable substrates from agro-industrial waste, optimized and efficient bio-processes for obtaining maximum productivity. An attempt was made to synthesize silver nanoparticles in water-in-oil microemulsion stabilized by low cost biosurfactant synthesized using cheap renewable substrates [12, 1].

The optical absorption spectrum of the silver nanoparticles synthesis using biosurfactant from *P. aeruginosa* PBSC1 was shown in the Figure 2. From the figure, the optical absorption of the silver nanoparticles possess narrow band edge (432 nm), which originated from the uniform sized particle distribution of the sample. The absorption edge was shifted to the lower wavelength region confirmed nano-sized formation of the final product, which was caused by the quantum confinement effect. The optical band gap of the material was calculated using effective mass approximation was found to be 3.7 eV (432 nm). Light below this wavelength holds sufficient energy to excite electrons and hence absorbed by silver nitrate. On the other hand, light with longer wavelength which was higher than the band gap energy (towards the visible light) will not be absorbed.

UV–visible absorption spectrum of silver nanoparticles in n-heptane. A strong absorption peak at approximately 406 nm originates from the surface plasmon absorption of nanosized silver particles. Similar results were recorded in our study with the absorption spectrum of 432 nm for the SNPs synthesized using biosurfactant from PBSC1.Nano-scale silver can be synthesized in reverse micelles using glycolipid as stabilizer [9, 6, 17]

HR-TEM analysis was carried out on the silver Nano particles to observe the individual size and shape of it. HR-TEM micrograph of samples synthesized was shown in Figure 3a. A large number of smaller particles were distributed on the films in the size range of 15-32 nm of silver nanoparticles from PBSC1. This indicates that the distribution of silver nanoparticles stabilized by the biosurfactant was rather uniform. The typical TEM micrographs of the silver nanoparticles [10, 11] were obtained in this study.. However, some larger particles on the films are observed. Two possibilities are there, one is that the nanometer-sized water layers limit the packing of the particles in the direction perpendicular to the water layers when the particles are growing in reverse micelles, the absorption of surfactant molecules

cannot totally prevent particles from aggregating and the thickness of the water layers cannot absolutely restrict the particle size due to the flexibility of the surfactant bilayers [10]. The other is that during the extraction and redispersion process a part of particles impact each other and aggregation.

The Selected Area Electron Diffraction (SAED) analysis showed those continuous ring patterns which originate from polycrystalline state or by the more crystallites attached to the surface of the single particles (Figure 3b). Bright ring pattern showed the high density of crystallites in the materials in the silver nanoparticle samples (PBSC1).

The silver nanoparticles synthesized using biosurfactant from *P. aeruginosa* PBSC1 after a day showed a relatively intense absorption peak around 432nm UV spectroscopy. On increasing the time from 1, 30 to 60 days the Plasmon absorption bands of three samples are quite similar for silver nanoparticles synthesized using biosurfactant from PBSC1 (Figure 4)No obvious changes in the position and symmetry of the absorption peak except for the decrease of the absorbance, indicating a little aggregation of silver nanoparticles. During the entire chemistry process, no passivator was added into the system. It proves that the silver nanoparticles solution prepared in such proportional reverse micelles can remain relatively stable for at least 2 months. The remnant rhamnolipid and lipopeptide in the solution was regarded as the stabilizer, which form a steric hindrance around the particles to preventing them aggregation greatly by electrostatic interactions. Xie *et al.* [16] reported that on increasing the time from 1 to 60 days, the Plasmon absorption bands are quite similar.The silver nanoparticles solution prepared in reverse micelles can remain relatively stable for at least 2 months. Kiran *et al*. [8] reported that the glycolipid biosurfactant produced from sponge-associated marine *Brevibacterium casei* MSA19 synthesized silver nanopartilcles were uniform and stable for 2 months. Farias *et al*. [5] studied that the silver nanoparticles solution prepared in such proportional reverse micelles can remain relatively stable for at least three months. Similar results were obtained in the present study that the silver nanoparticles were stable for 2 months in the solution, hence it was proved that the biosurfactant act as a stabilizing agent and prevented the formation of aggregates.

In the antimicrobial activity the concentration of 50 µl was effective against all the tested food pathogens. The *S. aureus* growth was highly controlled by the silver nanoparticles. The results were found similar with the bacteriocin obtained from *Lactobacillus plantarum@* 1000 ppm. The bacteriocin obtained from *Lactobacillus plantarum@* 1000 ppm combined with silver nanopartilces @ 50 µl concentration found very effective against all test specimens (Figure 5, 6).

Conclusion

The present work demonstrates a simple eco-friendly method for synthesizing spherical silver nanoparticles by microemulsion technique. Silver nanoparticles were successfully synthesized usingthe biosurfactant from *P. aeruginosa*PBSC1. The synthesized nanoparticleswere found to be spherical in shape with uniform distribution. The experimental observation was supported by UV spectroscopy and TEM analysis. The silver nanoparticles can be stabilized correspondingly for at least 3 months without passivator addition. The use of low-cost, renewable and biodegradable biosurfactants in replacement to toxic synthetic surfactants is a promising alternative for the synthesis of inorganic nanoparticles for industrial application. Antimicrobial activity of the silver nanoparticles and bacteriocin combination made a modification in the preservation methods in fermented foods such as pickled cucumbers, pickled beets and Sauerkraut. This study proved effective control and preservation of the selected common fermented foods. This modified method in food preservation not only improves the quality of fermented foods but also satisfy consumers.

Table - 1 Level of different process variables in coded and un-coded form for the reduction of surface tension (P. aeruginosa PBSC1)

Variables	Codes	Levels				
		-2	-1	0	+1	+2
Glycerol (g/l)	A	1.5	2	2.5	3	3.5
Sodium Nitrate (g/l)	B	1.5	3	4.5	6	7.5
pH	C	6	6.5	7	7.5	8
Temperature (ºC)	D	20	25	30	35	40

Table - 2 Experimental conditions of 2^4 central composition design showing experimental and predicted surface tension reduction

Std	Run order	Glycerol (g/l)	Sodium Nitrate (g/l)	pH	Temperature (ºC)	Experimental values *ST (mN/m)
6	1	1	-1	1	-1	40.2
4	2	1	1	-1	-1	40.4
27	3	0	0	0	0	31.3
18	4	2	0	0	0	38.8
13	5	-1	-1	1	1	38.9
22	6	0	0	2	0	38.1
24	7	0	0	0	2	36.7
7	8	-1	1	1	-1	39.8
20	9	0	2	0	0	38.7
23	10	0	0	0	-2	40.1
17	11	-2	0	0	0	38.6
10	12	1	-1	-1	1	38.9
21	13	0	0	-2	0	38.5
30	14	0	0	0	0	31.1
19	15	0	-2	0	0	40.1
1	16	-1	-1	-1	-1	39.7
25	17	0	0	0	0	31.3
9	18	-1	-1	-1	1	39.2
26	19	0	0	0	0	31.3
14	20	1	-1	1	1	38.8
29	21	0	0	0	0	31.0
11	22	-1	1	-1	1	38.9
15	23	-1	1	1	1	37.4
8	24	1	1	1	-1	39.6
12	25	1	1	-1	1	38.6
16	26	1	1	1	1	36.2

5	27	-1	-1	1	-1	40.2
3	28	-1	1	-1	-1	40.4
28	29	0	0	0	0	31.1
2	30	1	-1	-1	-1	39.9

*Notes: *ST=Surface Tension*

Table - 3 ANOVA for Response Surface Quadratic Model Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Model	322.03	14	23	164.89	< 0.0001	significant
A-A	0.094	1	0.094	0.67	0.4252	
B-B	2.22	1	2.22	15.92	0.0012	
C-C	1.35	1	1.35	9.7	0.0071	
D-D	16.83	1	16.83	120.67	< 0.0001	
AB	0.14	1	0.14	1.01	0.3313	
AC	0.076	1	0.076	0.54	0.4729	
AD	0.23	1	0.23	1.62	0.2228	
BC	2.03	1	2.03	14.56	0.0017	
BD	1.5	1	1.5	10.76	0.0051	
CD	0.86	1	0.86	6.13	0.0257	
A2	105.44	1	105.44	755.82	< 0.0001	
B2	125.1	1	125.1	896.77	< 0.0001	
C2	94.96	1	94.96	680.69	< 0.0001	
D2	97.52	1	97.52	699.1	< 0.0001	
Residual	2.09	15	0.14			
Lack of Fit	2	10	0.2	10.71	0.0087	significant
Pure Error	0.093	5	0.019			
Cor Total	324.13	29				

Notes: The Model F-value of 164.89 implies the model is significant. There is only a 0.01per cent chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant.

Std. Dev.	*0.37*	*R-Squared*	*0.9935*
Mean	*37.46*	*Adj R-Squared*	*0.9875*
C.V. %	*1.00*	*Pred R-Squared*	*0.9641*
PRESS	*11.65*	*Adeq Precision*	*34.901*

The "Pred R-Squared" of 0.9641 is in reasonable agreement with the "Adj R-Squared" of 0.9875. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 34.901

indicates an adequate signal. This model can be used to navigate the design space.

Acknowledgement

The authors wish to express their gratitude for the support extended by the authorities of Department of Microbiology, Annamalai University, Annamalai nagar for carrying out the research work. I sincerely thank Dr. R. Parthasarathi and Dr. P. Anna Joice for the conception of this idea and subsequently carrying out research work with the help of Major Research Project University Grants Commission, India

References

Banat, I.M., Makkar,R.S.,&Cameotra, S.S.(2000). Potential commercial applications of microbial surfactants. *Applied Microbiology and Biotechnology., 53,* 495-508.

Boutonnet, M., Kizling, J., &Stenius, P. (1982). The preparation of monodisperse colloidal metal particles from microemulsions. *Colloids Surfaces A- Physicochemical and Engineering Aspects, 5,*209.

Bradshaw, L.J.(1992). Laboratory Microbiology, Fourth Edition, U.S.A, Pp.13-55.

Desai, J. D., & Banat, I.M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews,61,* 47-64.

Farias, C.B.B., Silva,A.F.,Rufino,R.D.,Luna, J.M., Souza, J.E.G., &Sarubbo, L.A. (2014). Synthesis of silver nanoparticles using a biosurfactant produced in low-cost medium as stabilizing agent. *Electronic Journal of Biotechnology. 17(3),* 122-125.

He, S.T., Yao, J.N., Jiang, P., Shi, D.X., Zhang, H.X.,Xie, S.S., Pang S.J.,&Gao, H.J. (2001). Formation of silver nanoparticles and self-assembled two-dimensional ordered superlattice. *Langmuir, 17,* 1571-1575.

Jain, R.M., Mody, K., Joshi, N., Mishra, A., &Jha,B. (2012). Isolation and structural characterization of biosurfactant produced by an alkaliphilic bacterium *Cronobactersakazakii* isolated from oil contaminated wastewater. *Carbohydrate Polymers, 87,* 2320 – 2326.

Kiran, G.S., Sabu, A.,&Selvin, J. (2010). Synthesis of silver nanoparticles by glycolipid biosurfactant produced from marine *Brevibacteriumcasei* MSA19. *Journal of Biotechnology, 148,* 221-225.

Kitamoto, D., Isoda, H., &Nakahara, T. (2002). Functions and potential applications of glycolipid biosurfactants--from energy-saving materials to gene delivery carriers. *Journal of Bioscience and Bioengineering, 94,* 187.

Limin, Q., YueyingG.,&Jiming,M. (1999). Synthesis of ribbons of silver nanoparticles in lamellar liquid crystals. *Colloids Surfaces A- Physicochemical and Engineering Aspects, 157,* 285.

Lin, J., Zhou, W., & O'Connor, C.J. (2001). Formation of ordered arraysof goldnanoparticles from CTAB reverse micelles. *Materials Letter, 49,* 282.

Makkar, R.S.,&Cameotra, S. (1997). Utilization of molasses for biosurfactant production by two *Bacillus* strains at thermophilic conditions. *Journal of the American Oil Chemists' Society,74,* 887-889.

Mnif, I., Elleuch, M.,Chaabouni, S.E.,&Ghribi, D. (2013). *Bacillus subtilis* SPB1 biosurfactant: Production optimization and insecticidal activity against the carob moth *Ectomyeloisceratoniae*. *Crop Protection, 50(6),* 6-72.

Najafi, A.R., Rahimpour,M.R.,Jahanmiri, A.H., Roostaazad,R.,Arabian, D.,Soleimani,M., &Jasmhidnejad, Z.(2011). Interactive optimization of biosurfactant production

by *Paenibacillusalvei* ARN63 isolated from an Iranian oil well. *Colloids and surfaces B: Biointerfaces,82,*33-39.

Siegel, R.W. (1993). Synthesis and properties of nanophase materials. Materials Science and Engineering: A**, 168(2):** 189-197.

Xie, Y., Ye,R., &Liu, H. (2006). Synthesis of silver nanoparticles in reverse micelles stabilized by natural biosurfactant. *Colloids Surfaces A- Physicochemical and Engineering Aspects, 279,* 175-178.

Xu, J., Yuan, X.,& Dai,S. (2006). Effect of surfactant on desorption of aldicarb from spiked soil. *Chemosphere, 62,* 1630-1635.