I.A.U
Karaj branch

Journal of
A p p l ied
C hemical
R esearch
jacr.kiau.ac.ir

Journal of Applied Chemical Research, 18, 2, 27-39 (2024)

Biosynthesis of Silver Nanoparticles using *Bacillus subtilis* Bacterium Cultured in Corn Steep Liquor and Evaluation of its Antibacterial Activity

Naeimeh Faridi Aghdam[1*], Shahram Moradi[1], Sirous Ebrahimi[2], Hamed Hamshehkar[3]

[1]Faculty of Chemistry, Islamic Azad University, North Tehran Branch, Tehran, Iran

[2]Biotechnology Research Centre, Faculty of Chemical Engineering, Sahand University of Technology, Tabriz, Iran

[3]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

(Received 20 Nov. 2023; Final revised received 18 Feb. 2024)

Abstract

In this study, the biosynthesis of silver nanoparticles was done using *Bacillus subtilis* bacterium cultured in corn steep liquor (CSL) nutrient. The biosynthesized nanoparticles were characterized by several techniques including FT-IR, XRD, UV-Vis, SEM, EDX, and TEM. The absorption spectrum of the nanoparticles indicated the maximum absorption at 436 nm. The SEM image confirmed the nanoparticles had polydisperse spherical morphology (~20nm). Also, the TEM image showed the nanoparticles had spherical or elliptical shape and the approximate diameter of the particles was between 10-20 nm. Morphological studies showed that the nanoparticles were completely separated and no aggregation was observed. Moreover, XRD studies confirmed that the produced nanoparticles were crystallized in the FCC crystal lattice. The antibacterial activity results indicated that the synthesized nanoparticles had significant effect against *Escherichia coli* bacteria, and the inhibition zone was equal to Gentamicin. So, the production of silver nanoparticles using green method is economically very economical, and can be a method for the production of silver nanoparticles in industrial scale.

Keywords: Bacteria, Corn steep, Green method, Silver nanoparticles.

[*]***Corresponding author****: Naeimeh Faridi Aghdam, Faculty of Chemistry, Islamic Azad University, North Tehran Branch, Tehran, Iran. Email: Faridi.aghdam@gmail.com.*

Introduction

Nanotechnology is defined as the synthesis, characterization and application of nanoscale materials. Metal nanoparticles, mainly silver nanoparticles, are broadly applied as biological sensor [1], optoelectronics [2], plasmonics [3], and catalyst [4]. Also, these nanoparticles were used at the agricultural and pharmaceutical industries [5, 6].

Silver nanoparticle can be synthesized using the chemical and physical methods such as microwave, laser ablation, electrochemical, thermal decomposition, and etc [7-9]. Although, all of these methods are successful in the synthesis of silver nanoparticles, but most of these techniques require the use of toxic and hazardous chemical materials as reducing and stabilizer agents. In addition, some of the mentioned techniques require harsh laboratory conditions and expensive tools.

Nowadays, there is an increasing number research devoted to the synthesis of nanoparticles employing green synthesis methods. In these methods, the plant, fungi, yeast, algal, and bacteria extracts are used as a safe reducing and stabilizer agent for the biosynthesis of nanoparticles. The green methods are eco-friendly, easy available, safe and cost-effective processes for the preparation of nanoparticles. Since the growth of bacteria is fast, they are usually used for the synthesis of nanoparticles [10, 11]. The bacterial silver nanoparticles can be simply coated with a protein/lipid capping, which reveal greater antibacterial activity than uncapped silver nanoparticles [12]. Also, the capped silver nanoparticles could confer physiological stability and solubility [13]. Klaus et al. [14] studied the synthesis of silver nanoparticles using *Pseudomona*. They indicated that the size of nanoparticles was increased up to 200 nm by increasing the concentration of silver ions up to 500 mM. The morphology and shape of nanoparticles have been investigated using EDXA method. Sintubin et al. [15] used several gram-negative and gram-positive bacteria such as *Lactococcus garvieue*, *Pediococcus pentasaceus*, and *Lactobacillus* spp. to reduce silver ions to nanoparticles. The UV-Vis spectroscopy results showed that the size of silver nanoparticles reduced with increasing pH value. Pallavi et al. [6] investigated the biosynthesis of silver nanoparticles using *Streptomyces hirsutus*. The synthesized silver nanoparticles were characterized by XRD, EDX, TEM, TGA, FTIR, and UV–Vis techniques. Then, The antibacterial activity against *Fusarium oxysporum*, *Candida glabrata*, *Alternaria alternata*, *Candida albicans*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* bacteria were studied. In another research, the bacterial strains acted as reducing agents as well as capping and stabilizing agents during the synthesis process. The silver nanoparticles had spherical and rod shape, with sizes of 20 to 50 nm [16].

To the our knowledge, no study has been reported for the green synthesis of silver nanoparticles using the *Bacillus subtilis* bacterium cultured in corn steep liquor (CSL) nutrient. Therefore, the uses of Bacillus subtilis in the synthesis of silver nanoparticles is safe and environment-friendly as toxic chemicals are not used, cost-effective as they are less resource and energy-intensive, and uses the biological component itself as the capping and reducing agent, producing biocompatible products. The biosynthesied silver nanoparticles were characterized and studied by several techniques including Fourier transform Infrared (FT-IR) spectroscopy, X-ray powder diffraction pattern (XRD), UV-Vis spectroscopy, field emission scanning electron microscope (SEM), energy dispersive x-ray (EDX) and transmission electron microscopy (TEM).

Experimental

Preparing the culture medium

Corn steep liquor (CSL) was purchased from Glucosan Company (Qazvin, Iran). CSL was diluted 20 times with distilled water. The pH of the CSL was adjusted to 7 using NaOH. The CSL was placed inside the autoclave (120ºC, 1.5 Bar, and 15 min), and finally centrifuged (9000 rpm, 20 min) to separate solid materials.

Cultivation of bacteria in CSL

The *Bacillus subtilis* bacterium cells were obtained from the Pasteur Institute (Iran), and cultured in CSL medium (COD = 34000 mg/lit) at 37 °C with continuous agitation (250 rpm) for one day under aerobic condition. The growth of bacteria was observed by measuring the optical density (OD) at ƛ=600 nm.

Synthesis of silver nanoparticles

The silver nanoparticles were synthesized by addition 16.9 mg of silver nitrate to 100 ml of bacterial culture solution. The sample was incubated at a temperature of 37ºC, with continuous agitation, and under anaerobic condition. During this process, the sample was protected from light. After 50 hours, the supernatant was ultra-centrifuged (100000 rpm, 20 min) to dislodge silver nanoparticles. The synthesized silver nanoparticles were rinsed twice with Milli-Q water till the supernatant's conductivity reached to below 20 μs/cm.

Characterization of silver nanoparticles

The optical properties of the silver nanoparticles were recognized using UV–Vis spectrophotometer (UV-2000, Pharmacia, Biotech, England). XRD data of silver nanoparticles were collected using a Bruker AXS model D8-Advance diffractometer equipped with Cu-Kα radiation at λ= 1.5418 Å. The morphology and average particle size of silver nanoparticles were analyzed using SEM (KYKY-EM3200, USA), and TEM (Zeiss LEO 906, Germany, 200 kV). Also, The EDX was used for the determination of nanoparticle's purity. To record the FT-IR spectra of silver nanoparticles in the range of 400-4000 cm^{-1}, a Bruker spectrophotometer was applied. The samples were prepared in the form of KBr pellets Zeta Potential (Zetasizer Ver. 6.20, Malvern Instruments, England) analysis was applied to determine the stability.

Antibacterial activity of silver nanoparticles

The antibacterial activity of silver nanoparticles was performed using the disk diffusion method against *Escherichia coli*. Bacteria were cultured on Müller Hinton agar plate at 37ºC for 24 hours, and then spread on the plates containing Mueller Hinton Agar culture medium. The paper discs were sonicated in solutions with different concentrations of silver nanoparticles (0.25, 0.5 and 1 mM), and then placed on the plates. The plates were grown at 37ºC for 24 hours, and finally, the inhibition zone was measured.

Results and discussion

In this study, the silver nanoparticles were synthesized using the *Bacillus subtilis* bacterium cultured in corn steep liquor, and then their antibacterial activities were investigated.

Silver nitrate (1 mM) was added to the samples which had different optical density (0.5, 1 and 2), and were named CSL-A, CSL-B and CSL-C. The formation of silver nanoparticles was observed by color change from yellow to dark brown, and then monitored by UV-Visible spectroscopy, which recorded in the range of 200 to 600 nm (Figure 1). There have been previous reports of the formation of silver nanoparticles with strong and wide peaks in the range of 400 nm to 450 nm [17]. In this study, after 1h of incubation from the initiation of the reaction, the highest absorption peak intensity was observed at 436 nm, which indicates the formation of silver nanoparticles in a shorter time. Also, the maximum absorption shifted to longer wavelengths as the incubation time was increased. It was confirmed that with the increase in the size of the nanoparticles, it is not possible to polarize the nanoparticles homogeneously, so, increasing the size of the particles led to the red shift of SPR absorption, and the broadening of the absorption spectrum. In previous reports, when the silver nitrate was

reacted with *B. Subtilis* for 2h, a similar peak of silver nanoparticles was observed [18]. Also, Vigneshwaran et al. [19] reported that *Aspergillus flavus* in yeast malt could synthesis the silver nanoparticles at 72 hours.

For quantitative analysis, sampling of the reaction mixture was provided at different times, and in order to investigate the effect of biomass concentration on the production rate of silver nanoparticles, the adsorption rate of each sample was measured at corresponding wavelengths (λ_{max}) and the change curve has been exposed in Figure 2. The results showed that with increasing biomass concentration from 0.5 to 2, biosynthesis of silver nanoparticles increased up to two times. It can also be stated that by increasing the concentration of biomass to a value greater than 1, due to the trapping of nanoparticles by the biomass, Romance absorption does not increase.

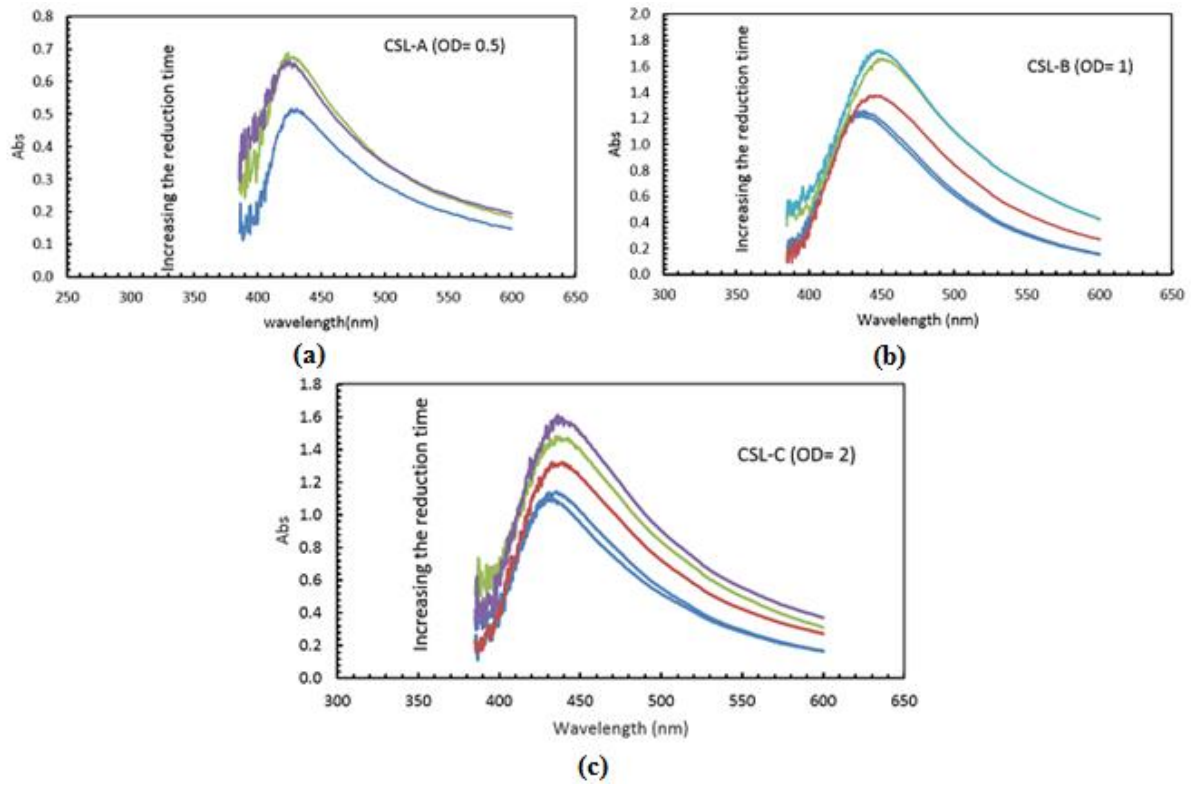

Figure 1. UV-Vis spectrum of silver nanoparticles, a) CSL-A; b) CSL-B; c) CSL-C.

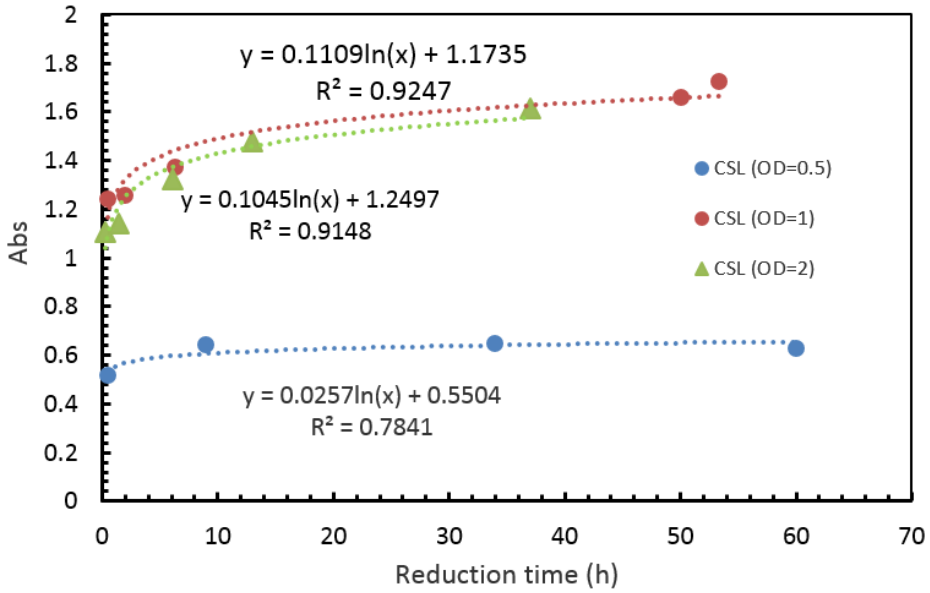

Figure 2. The effect of biomass concentration on the production rate of silver nanoparticles in CSL culture medium.

FT-IR spectroscopy

The FT-IR spectrum of synthesized nanoparticles has been shown in Figure 3. In the FT-IR spectrum, the broad and strong absorption peak at 3435 cm^{-1} revealed the absorption band of O-H and N-H. The peak at 1466 cm^{-1} could be assigned to the C=C stretching vibrations of aromatic rings. The peak at 2924 cm^{-1} could be attributed to the stretching vibrations of C-H of alkane, while the peak at 1741 cm^{-1} was related to the presence of C=O bend, due to the presence of carbonyl compounds. The vibrations at 1372 and 1173 cm^{-1} was also assigned to the -C-N asymmetric stretching and C-O stretching, respectively. So, The FT-IR spectrum of silver nanoparticles showed some of the *Bacillus subtilis* extract peaks such as C=O, C-O, -C-N [20], that indicated that these groups are effective in the reduction of the silver ions to silver nanoparticles.

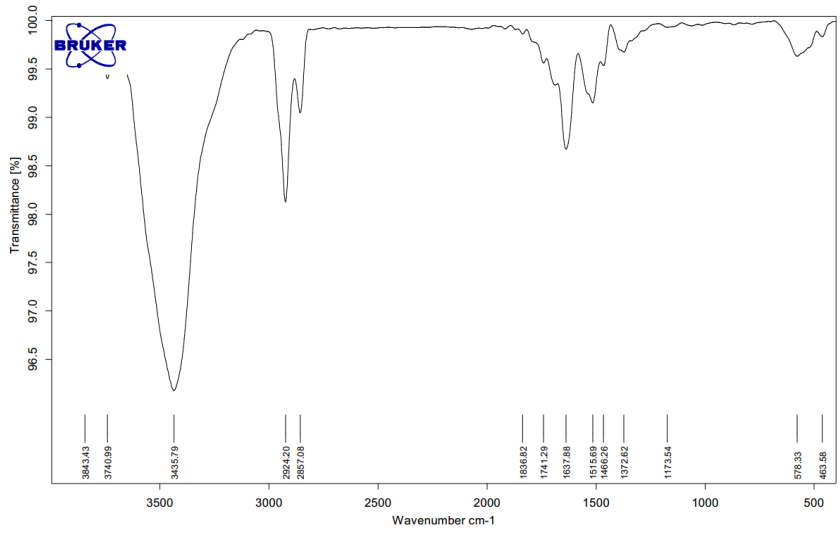

Figure 3. FT-IR spectrum of nanoparticles synthesized in CSL feed in the presence of B. Subtilis bacteria.

SEM and TEM of silver nanoparticles

SEM image of biosynthesized silver nanoparticles is shown in Figure 4. The SEM image confirmed that the nanoparticles had polydisperse spherical morphology in the size range of ~20nm. According to the TEM image (Figure 5), the nanoparticles had spherical or elliptical shape, and the approximate diameter of the particles was between 10-20 nm. The morphological studies showed that the nanoparticles were completely separated and no aggregation was observed.

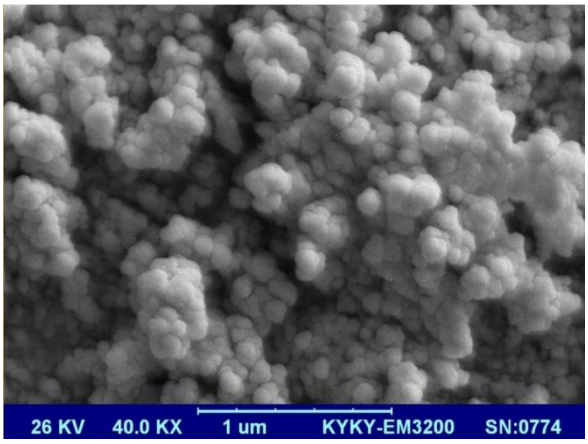

Figure 4. TEM image of nanoparticles synthesized in CSL feed in the presence of B. Subtilis bacteria.

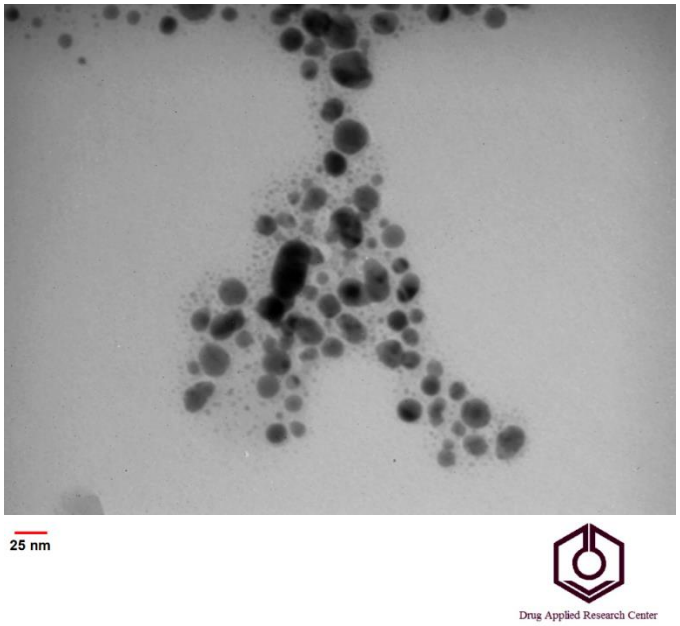

Figure 5. SEM image of nanoparticles synthesized in CSL feed in the presence of B. Subtilis bacteria.

XRD pattern of silver nanoparticles

The XRD pattern of the biosynthesied silver nanoparticles is shown in Figure 6. As observed, XRD profiles of the biosynthesied nanoparticles revealed Bragg's diffractions with the Miller indexation for the diffractions of (100), (200), and (311) planes at 2θ values of 1.32°, 46.70°, 76.00°, respectively. Compared to the XRD pattern of commercial silver nanoparticles, some unknown weak peaks were observed at 2θ=84.70°, 67.20° and 57.10° for biosynthesized nanoparticles, which most likely related to *B. Subtilis* compounds adsorbed on the silver nanoparticles surface [21]. Finally, the crystallite size of nanoparticles was estimated by Debye-Scherrer equation. The crystallite size of biosynthesied nanoparticles was determined about 18.2 nm based on the (100) plane.

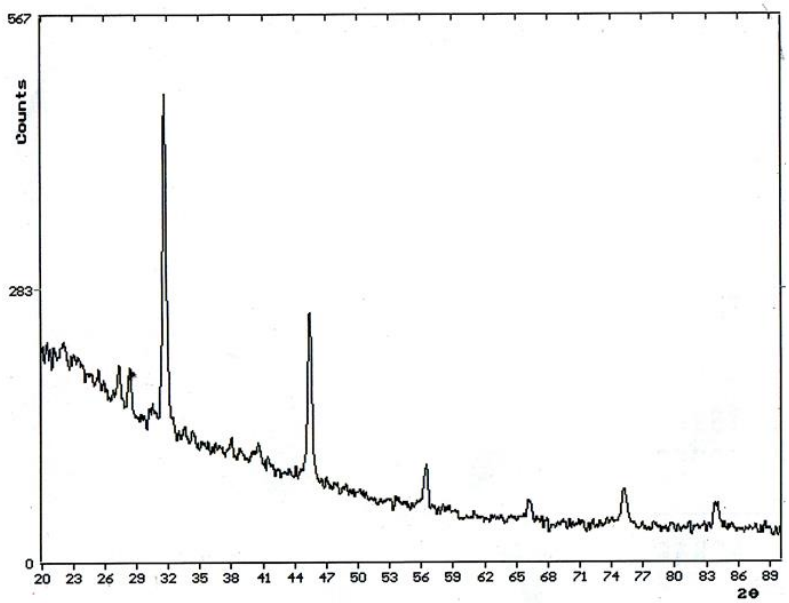

Figure 6. XRD spectrum of nanoparticles synthesized in CSL feed in the presence of B. Subtilis bacteria.

DLS analyses of silver nanoparticles

Particle size distribution and zeta potential of silver nanoparticles were determined by DLS (Figure 7). The particle size distribution curve showed that the produced silver nanoparticles are polydisperse (PDI= 0.698), and the particles had an average diameter of 15.96 nm. At experimental condition, the zeta potential of the synthesized nanoparticles was found to be less than -11.0 mV. This high value approved the repulsion among the silver nanoparticles, and thus increased in stability of the nanoparticles formulation [22]. The negative potential value might be due to the possible capping of the bio-organic compounds of *B. Subtilis* [23]. Also, negative-negative repulsion led to proper distribution of nanoparticles.

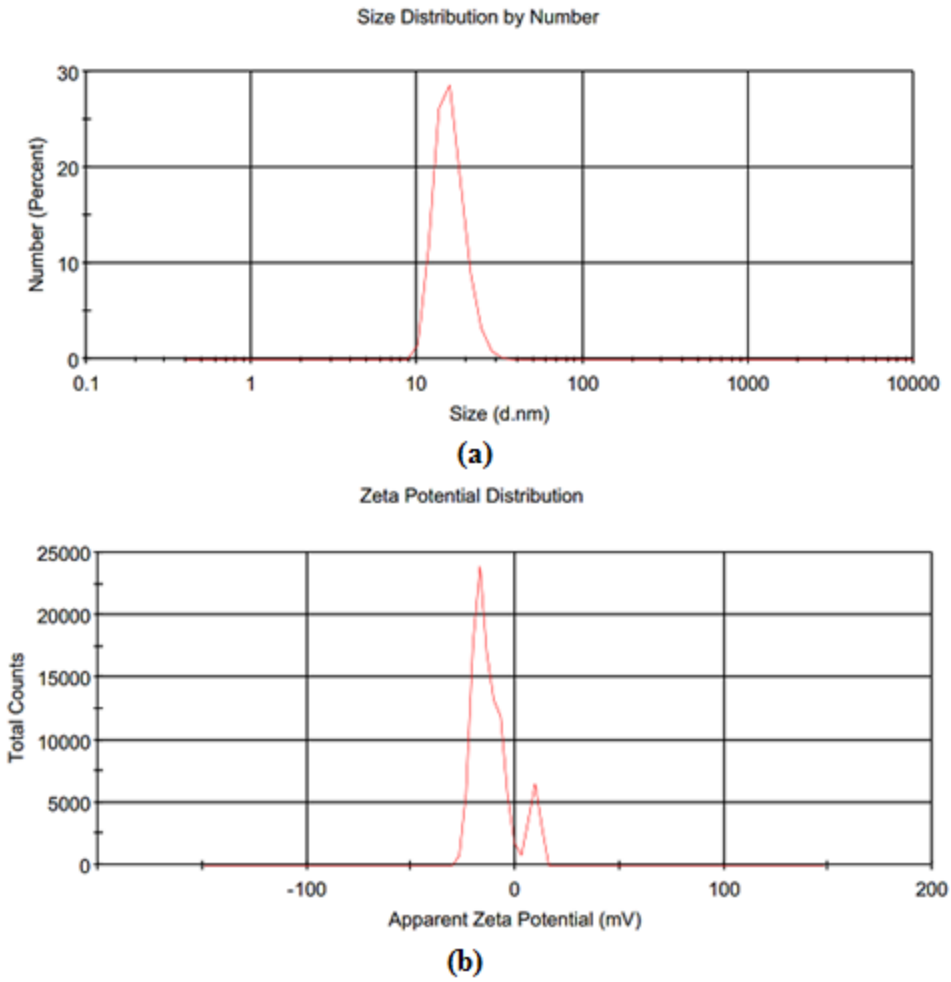

Figure 7. DLS synthesized nanoparticles in CSL feed. a) particle size distribution; b) Zeta potential.

Antibacterial activity of synthesized silver nanoparticles

Table 1 shows the average inhibition zone of three different concentrations of silver nanoparticles (CSL-A). With increasing the nanoparticles concentration, the inhibition zone increased. According to the results, the synthesized nanoparticles had significant effect against gram-negative bacteria such as *E. coli*, and the inhibition zone of NB-M-A with a concentration of 1 mM of silver nitrate was equal to Gentamicin. The negative zeta-potential of silver nanoparticles attacks gram-negative bacteria [24].

Table 1. Average inhibition zone obtained from three different concentrations of silver nanoparticles.

	Sample	Silver nanoparticles (mM)	Inhibition zone (cm)
1		0.25	7±0.10
2	CSL-A	0.5	9±0.20
3		1	11±0.10

Conclusion

The use of *Bacillus subtilis* bacterium cultured in corn steep liquor is a very economical method for the production of silver nanoparticles on a large scale and eliminates the costs associated with sterilization. The synthesized nanoparticles were identified and analyzed using different methods such as SEM, TEM, EDX, UV-Vis, FT-IR, and DLS. Morphological and particle size studies have shown that the synthesized nanoparticles were spherical and had a size of about 10 to 25 nm. It was also concluded that the resulting nanoparticles had an FCC crystal lattice. The nanoparticles were completely distributed and no aggregation was observed. Examining the antibacterial activity of synthesized nanoparticles against *E. Coli* bacteria showed that the bactericidal activity of nanoparticles was equal to gentamicin. As a result, this method is safe and environment-friendly as toxic chemicals are not used, cost-effective as it is less resource and energy-intensive, and uses the biological component itself as the capping and reducing agent, producing biocompatible products.

References

1. Lee KS, El-Sayed MA. Gold and silver nanoparticles in sensing and imaging: sensitivity of plasmon response to size, shape, and metal composition. The Journal of Physical Chemistry B. 2006;110(39):19220-19225.

2. Jeong SH, Choi H, Kim JY, Lee TW. Silver-based nanoparticles for surface plasmon resonance in organic optoelectronics. Particle & Particle Systems Characterization. 2015;32(2):164-175.

3. Cobley CM, Skrabalak SE, Campbell DJ, Xia Y. Shape-controlled synthesis of silver nanoparticles for plasmonic and sensing applications. Plasmonics. 2009;4(2):171-179.

4. Khan FU, Chen Y, Khan NU, Khan ZUH, Khan AU, Ahmad A, Wan P. Antioxidant and catalytic applications of silver nanoparticles using Dimocarpus longan seed extract as a

reducing and stabilizing agent. Journal of Photochemistry and Photobiology B: Biology. 2016;164:344-351.

5. Phull AR, Abbas Q, Ali A, Raza H, Zia M, Haq IU. Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of Bergenia ciliata. Future Journal of Pharmaceutical Sciences. 2016;2(1):31-36.

6. Pallavi SS, Rudayni HA, Bepari A, Niazi SK, Nayaka S. Green synthesis of Silver nanoparticles using Streptomyces hirsutus strain SNPGA-8 and their characterization, antimicrobial activity, and anticancer activity against human lung carcinoma cell line A549. Saudi Journal of Biological Sciences. 2022;29(1):228-238.

7. Jensen TR, Malinsky MD, Haynes CL, Van Duyne RP. Nanosphere lithography: tunable localized surface plasmon resonance spectra of silver nanoparticles. The Journal of Physical Chemistry B. 2000;104(45):10549-10556.

8. Oseguera-Galindo DO, Machorro-Mejia R, Bogdanchikova N, Mota-Morales JD. Silver nanoparticles synthesized by laser ablation confined in urea choline chloride deep-eutectic solvent. Colloid and Interface Science Communications. 2016;12:1-4.

9. Quintero-Quiroz C, Acevedo N, Zapata-Giraldo J, Botero LE, Quintero J, Zárate-Triviño D, Pérez, VZ. Optimization of silver nanoparticle synthesis by chemical reduction and evaluation of its antimicrobial and toxic activity. Biomaterials Research. 2019;23(1):1-15.

10. Hulkoti NI, Taranath TC. Biosynthesis of nanoparticles using microbes—a review. Colloids and surfaces B: Biointerfaces. 2041;121:474-483.

11. Jain AS, Pawar PS, Sarkar A, Junnuthula V, Dyawanapelly S. Bionanofactories for green synthesis of silver nanoparticles: Toward antimicrobial applications. International Journal of Molecular Sciences. 2021;22(21):11993.

12. Sanyasi S, Majhi RK, Kumar S, Mishra M, Ghosh A, Suar M, Goswami L. Polysaccharide-capped silver Nanoparticles inhibit biofilm formation and eliminate multi-drug-resistant bacteria by disrupting bacterial cytoskeleton with reduced cytotoxicity towards mammalian cells. Scientific Reports. 2016;6(1):1-16.

13. Javed R, Zia M, Naz S, Aisida SO, Ao Q. Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends and future prospects. Journal of Nanobiotechnology. 2020;18(1):1-15.

14. Klaus T, Joerger R, Olsson E, Granqvist CG. Silver-based crystalline nanoparticles, microbially fabricated. Proceedings of the National Academy of Sciences. 1999;96(24):13611-13614.

15. Sintubin L, De Windt W, Dick J, Mast J, Van Der Ha D, Verstraete W, Boon N. Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. Applied Microbiology and Biotechnology. 2009;84(4):741-749.

16. John MS, Nagoth JA, Ramasamy KP, Mancini A, Giuli G, Miceli C, Pucciarelli S. Synthesis of bioactive silver nanoparticles using new bacterial strains from an antarctic consortium. Marine Drugs. 2022;20(9):558.

17. Jyoti K, Baunthiyal M, Singh A. Characterization of silver nanoparticles synthesized using Urtica dioica Linn. leaves and their synergistic effects with antibiotics. Journal of Radiation Research and Applied Sciences. 2016;9(3):217-227.

18. Azarkhalil MS, Keyvani B. Synthesis of silver nanoparticles from spent X-ray photographic solution via chemical rreduction. Iranian Journal of Chemistry and Chemical Engineering (IJCCE). 2016;35(3):1-8.

19. Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP, Balasubramanya RH. Biomimetics of silver nanoparticles by white rot fungus, Phaenerochaete chrysosporium. Colloids and Surfaces B: Biointerfaces. 2006;53(1):55-59.

20. Filip Z, Herrmann S, Kubat J. FT-IR spectroscopic characteristics of differently cultivated Bacillus subtilis. Microbiological research. 2004;159(3):257-262.

21. Abdallah BB, Landoulsi A, Chatti A. Combined static electromagnetic radiation and plant extract contribute to the biosynthesis of instable nanosilver responsible for the growth of microstructures. Journal of Saudi Chemical Society. 2018;22(1):110-118.

22. Rao YS, Kotakadi VS, Prasad TNVKV, Reddy AV, Gopal DS. Green synthesis and spectral characterization of silver nanoparticles from Lakshmi tulasi (Ocimum sanctum) leaf extract. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2013;103:156-159.

23. Parameshwaran R, Kalaiselvam S, Jayavel R. Green synthesis of silver nanoparticles using Beta vulgaris: Role of process conditions on size distribution and surface structure. Materials Chemistry and Physics. 2013;140(1):135-147.

24. Amro NA, Kotra LP, Wadu-Mesthrige K, Bulychev A, Mobashery S, Liu GY. High-resolution atomic force microscopy studies of the Escherichia coli outer membrane: structural basis for permeability. Langmuir. 2000;16(6):2789-2796.