

Synthesis and Characterization of Silver Nanoparticles using microorganism Ecoli and its Applications

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Abstract: *For the manufacture of silver nanoparticles, multiple methods, including chemical simplification with different natural and inorganic decreasing agents, physicochemical reduction, electrochemical procedures, and radiolysis, are employed. Silver nanoparticles are the single most manufacturer-identified material that can be used in all nanotechnology products. They can be used in food packing polymers to enhance the shelf lifespan. The present review is aimed at different types of in nanotechnology, a nano speck is defined as a small object or a speck that acts as a whole unit in terms of its conveyed properties. The physical and chemical properties of nanomaterials can alter from those of the same material in colossal bulk class; nano subatomic particles have one attribute in the reach of 1 to 100 nm. These are utilized in nutrition handling, surgical, promotional material, wound dressing, computing devices, recollection implements, water purifiers, textiles, cosmetics, and contact lens. Silver nanoparticles are the unity most producer-identified material that can be used in all the nanotechnology products. They can be used in food packing polymers to enhance the shelf life of food. Silver nanoparticles in the range from 1 to 100 nm are widely used in industrial applications as catalysis, electronics, and photonics, and they have unique properties such as optical, electrical, and magnetic characteristics that can be used as antimicrobial, biosensor textile, cosmetics, composite fibers, and electronic components and to amend shelf life of food substances. (1) When reacting with bacteria, silver nanoparticles adhere to both the cell wall and cell membrane and inhibit replication, leading to cell death. When silver dissolves in the cytosol, it ionizes to engender nanoparticles that increase the bactericidal activity (2).*

Keywords: Biosynthesis, Silver Nanoparticles, Engineered Escherichia Coli, Metallothionein

I. INTRODUCTION

Over the time period of silver-based compounds were used as no toxic inorganic antibacterial agents owing to their biocidal properties in many applications such as wood preservatives, water purification in hospitals, in wound or burn dressing, antimicrobial activity and so on. In fact, silver ions and their related compounds have low toxicity toward animal cells but present a high toxicity to microorganisms like bacteria and fungi. The recent advances in the field of nanoparticle synthesis have a strong impact in many scientific areas and the synthesis of silver nanoparticles has also followed this tendency. These unique properties of nanomaterials have spurred numerous investigations and applications in electronics, nanomedicine, biomaterials, energy, and food. In fact, silver-based compounds are much cheaper than gold based one; moreover, silver nanoparticles are now considered as an important class of nanomaterials. They are presently mainly used as catalyst (3) Environmentally friendly synthesis methods are becoming more and more popular in chemistry and chemical technologies. This trend has several origins, including the need for greener methods counteracting the higher costs and higher energy requirements of physical and chemical processes. For this reason, scientists search for cheaper methods of synthesis. The other reason is that conventional methods for nanoparticle synthesis usually require harmful reductants such as sodium borohydride or hydrazine and many steps in the synthesis procedure including heat treatments, often producing hazardous by-products. In order to reduce the environmental impact of nanoparticle synthesis, greener routes have been investigated for overtime period. (4)

II. SYNTHESIS METHODS

2.1. Material Approach

The production of tube furnaces by Silver Particles Nano has some disadvantages; however, since tube ovens occupy a more extended area, they consume a lot of push while raising environmental temperatures from around. It will require a long time for the source material to achieve thermal stability. A standard tube oven needs over several kilowatts of energy and several 10 minutes of preheating to ensure a constant operating temperature. Silver nanoparticles were additionally generated with precision optical maser removal of metallic mass materials (5,6) In essence, the physical synthesis of Silver Particles Nano's customarily leverages the physical energies to create Silver Particles Nano's with proximately restricted size distribution. The physical methodology may sanction significantly copious amounts of sampling of Silver Particles Nano in one procedure, which is also the most excellent subsidiary method for producing Silver Particles Nano powder. Nevertheless, primary expenditures for equipment investment should be taken into account.

2.2. Synthesis in Bacteria as a Medium

The Silver Particles Nanos generated are pretty stable, and this process benefits from other ways because the organism employed here is a nonpathogenic bacterium. The biological approach provides various resources in which silver nanoparticles are produced and is viewed as a process of synthesis of nanoparticles having benefits over standard synthetic chemical routes and an ecologically friendly gliding path and as an initial cost strategy. In addition, Kalishwaralal et al. 2008 noted the Silver Particles Nano's synthesis by reducing the aqueous Ag^+ ions and *Bacillus lichen* form is supernatant culture (7)

2.3. Observing Cell Growth

To examine whether the expression of *C. albicans* MT in *E. coli* affects cell growth, we monitored cell growth along time of cultivation. 50 μ L of the glycerol stock cells containing the *C. al-* *bicans* MT gene inserted in pUC19 plasmid (pUC-Met) was inoculated in 10 mL of LB in the presence of 100 μ g/mL carbenicillin and cultured overnight at 37 °C in a shaker incubator. Cells were spun down and resuspended in 100 mL of LB with 100 μ g/mL carbenicillin. Optical density at 600 nm (OD) was monitored each hour after resuspension until OD reached between 0.6 and 0.8, then OD was measured at 24 h, 48 h and 72 h. It has been shown that MT expression was induced and upregulated when microorganisms are under heavy metal stress [8,9,10,11]. To further investigate whether the expression of *C. albicans* MT in *E. coli* impact the bacterial tolerance to heavy metal, we cultivated the bacteria in the presence of 0.2 mM $AgNO_3$. 0.2 mM of $AgNO_3$ was added to the cells after OD reached between 0.6 and 0.8. Cells were further cultivated at 37 °C for 17–19 h with a continuous shake.



Figure: Broth Medium(A) and Synthesized Nano particles(B)

OD was measured again before cells were collected by centrifugation at 4000 \times g for 10 min. *E. coli* cells transformed with pUC19 empty vector (pUC19-Vec) was used as control. The viability of the cells after they grew overnight in the

presence of 0.2 mM AgNo₃ was estimated by plating the cells from the overnight culture on agar plates. The overnight bacterial culture was diluted with LB medium at a ratio of 1:1000, 1:10,000, 1:100,000 and 1:1,000,000, and 100 μL of each dilution was plated on agar plates in the absence of AgNo₃. Cells were allowed to grow overnight at 37 °C for 17 h to obtain colonies. Individual colonies were counted to estimate the viable cells after AgNo₃ challenge.

1.4. Process of Antibacterial Activity

Ag NPs with larger surface area provide a better contact with microorganisms [17]. Thus, these particles are capable to penetrate the cell membrane or attach to the bacterial surface based on their size. In addition, they were reported to be highly toxic to the bacterial strains and their antibacterial efficiency is increased by lowering the particle size [18]. Many arguments have been given to explain the mechanism of growth inhibition of microbes by Ag NPs but most convincing is the formation of free radical which has also been supported by the appearance of a peak at 336.33 in the electron spin resonance (ESR) spectrum of Ag NPs [22]. The free radical generation is quite obvious because in a living system they can attack membrane lipids followed by their dissociation, damage and eventually inhibiting the growth of these microbes [23].



Fig- (A) Microbial growing tubes, (B) Tubes after the nanoparticles addition

It conclude that the Synthesized Nanoparticles shows the Antibacterial Activity

UV Spectroscopy Graph

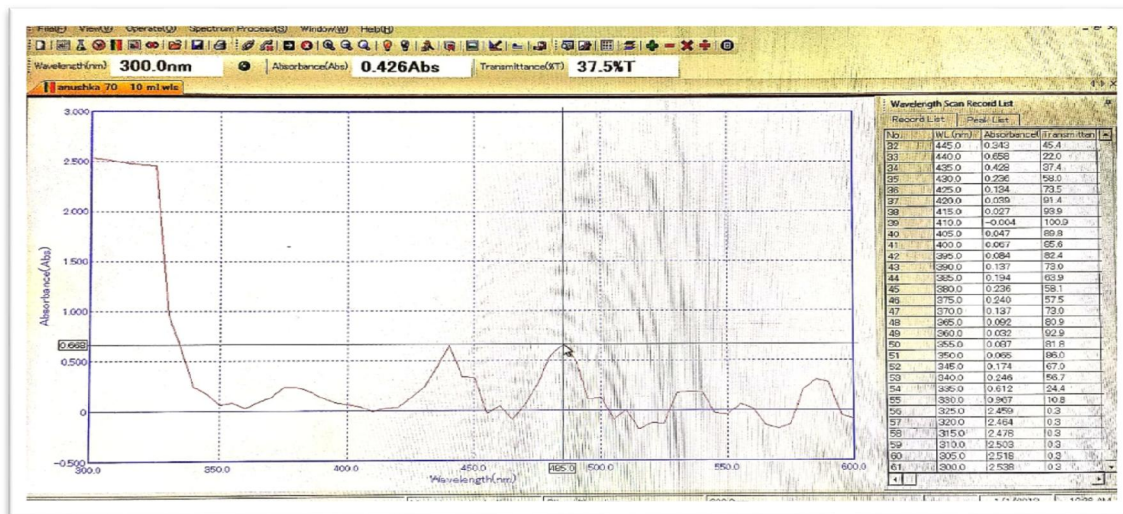


Figure: UV visible spectra Silver Nano Particles

Sr. No.	Wavelength	Absorption	Transmittance
1	440nm	0.658	22.00
2	445nm	0.343	45.4
3	435nm	0.428	37.4

Extraction of the Synthesized Nano Particles

The synthesized nano particles are collected by the Process of the centrifugation. In which the synthesized Particles Are filled in the micro centrifuge tubes and spined at the speed of 5000rpm for 3-5 min duration. After that the silver nano particles get Accumulate at the bottom of the tube that are visible by the naked eye that accumulated particle are dried thoroughly under the IR light or shade dry process. this process can take about the 2-3 days. after this process the collection of the nano particles at one place is done. after that they are converted into particle size



Figure: Synthesized Nano Particles

FTIR Process

In this Process the FTIR Device Is used the Following Are the Steps Of the FTIR

Preparation of the KBr Pellets

These pellets are prepared by the Process of the Hydraulic Press equipment. The Drug is thoroughly crushed and the Poured in the press and set for the 1000psi for the 3min only. This process is very delicate. More pressure and also more time can cause the pellets to break

FTIR Reading

Then the prepared pellets are Place at the FTIR Equipment Carefully and the Scanning process is done and the results are Observed.

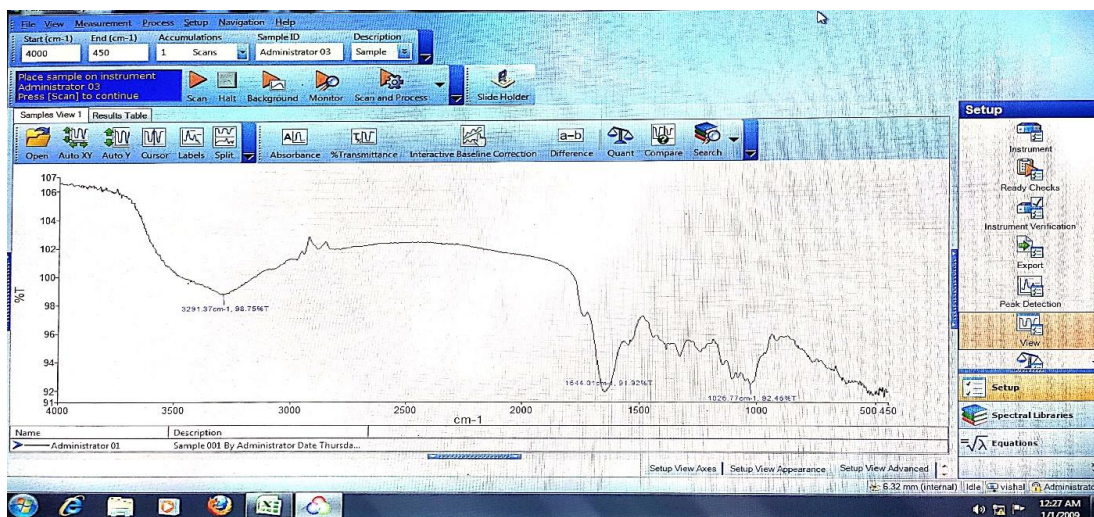


Figure: FTIR Graph of the Nano Particles



Figure: KBr Pellets

Sr. No.	Reading Peaks	Functional Groups Presents
1	3299.61cm ⁻¹ ,57.12% T	Alkyne C-H Stretch
2	2929.08cm ⁻¹ ,63.14% T	Methyl C-H Asym
3	1655.09cm ⁻¹ ,57.26% T	Alkyne C-H Stretch C=C-C Aromatic Ring Quinone or Conjugated ketones
4	1546.44cm ⁻¹ ,61.83% T	Aliphatic Nitro Compounds
5	1400.72cm ⁻¹ ,65.10% T	Phenol, Alcohol, Oh Bend Organic Sulphate

1.5. Review on Silver Nanoparticles as Drug Delivery Vehicles

Traditionally, gold and other molecules [12,13] have been used in nanoparticle-predicted drug delivery applications, but the use of silver has been restricted due to the complexity in synthesis; when functionalized using the classic salt aging procedure, there was a reduction in durability, and there were worries regarding silver toxicity in the past. Clinical application of silver nanoparticles as beneficial antibacterial treatments in wound care and also current in vivo studies showing that systemic exposure to silver nanoparticles is safe to have sparked interest in biomedical research involving silver nanoparticles. In a 2008 rat study, it was revealed because even in the diagnosis of severe oral Gaps at doses larger than 300 mg/kg/day during 28 days, there was a greater preponderance of above 300 mg/kg/day throughout 28 days [14], and there was only a minor induction of secondary indicators of liver injury. Reports of the safety and absence of side-effects associated with silver nanoparticles administered at “moderate” doses may instill greater preponderant confidence in the suitability of Gaps for in vivo studies that aim to lower the threshold of effective Gaps daily dosage by integrating their drug load potential and electromagnetic field research amplifying properties. Enhanced biocompatibility of Silver Particles Nanos as a result of surface alteration, as well as outstanding optical characteristics [15,16], has increased the suitability of Silver Particles Nanos for drug delivery applications.

III. APPLICATIONS

3.1. Protein Sensing

Tung N.H reported that silver nanoparticles labeling could be used in protein sensing studies by liquid electrode plasma-atomic emission spectrometry (LEP-AES). This technique is suitable for on-site portable analysis because plasma gas and the high-power source are not required. Proposed detection method could have a wide variety of promising applications in metal nanoparticle-labeled biomolecule detection.

3.2. Hospitals

Duran et al. prepared silver nanoparticles by using *Fusarium oxysporum* and studied their antimicrobial effect when incorporated in cotton fabrics against *Staphylococcus aureus*. They found that fabric incorporated with silver

nanoparticles have significant antibacterial activity. They proposed that clothes with silver nanoparticles are sterile and can be useful in hospitals to prevent or to minimize infection with pathogenic bacteria such as *Staphylococcus aureus*.

3.3. Fabrication of Antennas

Alshehri et al. have prepared two samples: the first was fabricated from the nano-metallic silver, and the second consists of micrometer-sized grains. Both types were prepared using thick-film fabrication process. The material involved in sample preparation was fine metal powder, an inorganic binder-like metal oxide, and an organic vehicle that evaporates during the initial drying stages. Both the samples were characterized for the electrical performances. They found that in the lower-frequency range, both types of conductors (samples) behave similarly with electrical loss but increase approximately linearly with increased frequency range (from 0.1 dB/mm/GHz up to 80 GHz), but above 80 GHz frequency, the silver nanoparticle-fabricated sample showed lower electrical loss, and this behavior continues up to the above whole frequency range. The lower level of the loss from the silver nanoparticle conductors and the overall trend in loss per wavelength do not depend significantly on frequency. Therefore, it has been concluded that the silver nanoparticle-fabricated conductors show a less electrical loss at high-frequency range which in turn attributed to lower surface roughness found in the nanoparticles due to better packing and may open opportunities for low-temperature fabrication of antennas and for sub-THz metamaterials with improved performance.

3.4. Agricultural and Marine

Silver nanoparticles synthesized by Guilger et al. using fungus *Trichoderma harzianum* were evaluated for cytotoxicity and genotoxicity against fungus *Sclerotinia sclerotiorum* which is responsible for the agricultural disease white mold and found that nanoparticles showed potential against *Sclerotinia sclerotiorum*, inhibiting sclerotium germination and mycelial growth. The study suggests that silver nanoparticles can be a new alternative in white mold control [78]. Babu et al. have synthesized silver nanoparticles in vitro using marine bacteria *Shewanella algae bangaramma* and found that the synthesized nanoparticles have both larvicidal and bactericidal activities and no mortality in control; in addition to this, the maximum values of LC₅₀ and LC₉₀ with 95% confidential limit [4.529 mg/ml (2.478–5.911), 9.580 mg/ml (7.528–14.541)] were observed with third instar larvae of *Lepidiotia mansueta* (Burmeister). It was found that the mortality of larvae was significantly increased in all the concentrations ($P < 0.0001$) in all the exposed groups. The bactericidal activities of the silver nanoparticles were determined against some of the bacterial species which followed the following order: *Vibrio cholera* < *Roseobacter* spp. < *Alteromonas* spp. It has been concluded that the synthesized silver nanoparticles had effective larvicidal and antifouling activities and can be effectively used in the agricultural and marine pest control [19].

3.5 Water Treatment

Dankovich prepared silver nanoparticles in a paper using microwave irradiation. Antibacterial activity and silver release from the silver nanoparticle sheets were assessed for model *Escherichia coli* and *Enterococcus faecalis* bacteria in deionized water and in suspensions that also contained with various influent solution chemistries, that is, with natural organic matter, salts, and proteins. The paper sheets containing silver nanoparticles were effective in inactivating the test bacteria as they passed through the paper. The resultant silver nanoparticle paper is just as effective for inactivating bacteria during percolation through the sheet; the silver nanoparticle papers effectively purify water contaminated with bacteria. Hence, in conclusion, the paper incorporated with silver nanoparticles by microwave has been used for the purification of contaminated water. Park et al. developed micrometer-sized silica hybrid composite decorated with silver nanoparticles, that is, AgNP-SiO₂ (to prevent the inherent aggregation of silver nanoparticles and easy recovery from environmental media after utilization), and evaluated them for antiviral activity using bacteriophage MS2 and murine norovirus (MNV) models. Results revealed their potential, and it was concluded that developed silver nanoparticles (AgNP-SiO₂) can be efficiently used in disinfection processes for inactivation of various waterborne viruses. Abu-Elala et al. investigated the effect of chitosan-silver nanocomposite on fish crustacean parasite, *Lernaea cyprinacea*, disease found in goldfish (*Carassius auratus*) aquaria during the spring season. Their results proposed that chitosan silver nanocomposite is efficient in parasitic control in ornamental glass aquaria

IV. RESULT

The Various application of silver nanoparticles are found that the silver nanoparticles are antimicrobial with the FTIR and UV Spectroscopy results The silver Nano particles By the E-coli bacteria is synthesized and Proven to Applicable.

V. CONCLUSION

It is revealed that silver nanoparticles have potential applications in therapeutics as well as in other physical fields. In therapeutics, researchers are seemed to be more focused on anticancer and antimicrobial evaluations. Green synthesis makes them eco-friendly and nonhazardous. Applications of silver nanoparticles are not limited to therapeutics only, they are equally covering physical fields too such as biosensors and antenna fabrication, conductive adhesives, in ink-jet printing, water treatment, solar cell optimization, protein sensing, etc. Rigorous research has been carried out and continued on this nanostructure. Therefore, the silver nanoparticle has the ability to be a lead nanoparticle of the future due to its wide variety of applications.

REFERENCES

- [1]. K. R. P. Biswas and D. Subhadip, "Effects and applications of silver nanoparticles in different fields," *International Journal of Recent Scientific Research*, vol. 6, pp. 5880–5883, 2015. View at: Google Scholar
- [2]. L. Malinger, S. D. Solomon, M. Baha dory, A. V. Jeyarajasingam, S. A. Rutkowsky, and C. Boritz, "Synthesis and study of silver nanoparticles," *Journal of chemical education*, vol. 84, no. 2, p. 322, 2007. View at: Publisher Site | Google Scholar
- [3]. S. A. Anuj and K. B. Ishnava, "Plant mediated synthesis of silver nanoparticles by using dried stem powder of *Tinospora Cordifolia*, its antibacterial activity and comparison with antibiotics," *International Journal of Pharma and Bio Sciences*, vol. 4, no. 4, pp. P849–P863, 2013. View at: Google Scholar
- [4]. P. T. Anastas and J. C. Warner, Oxford University Press, New York, NY, USA, 1998.
- [5]. J. S. Kim, E. Kuk, K. N. Yu et al., "Antimicrobial effects of silver nanoparticles," *Nanomedicine: Nanotechnology, biology and medicine*, vol. 3, no. 1, pp. 95–101, 2007. View at: Google Scholar
- [6]. N. Law, S. Ansari, F. R. Livens, J. C. Renshaw, and J. R. Lloyd, "Formation of nanoscale elemental silver particles via enzymatic reduction by *Geobacter sulfurreducens*," *Applied and environmental microbiology*, vol. 74, no. 22, pp. 7090–7093, 2008. View at: Google Scholar
- [7]. S. Prabhu and E. K. Poulose, "Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects," *International nano letters*, vol. 2, no. 1, pp. 1–10, 2012. View at: Google Scholar
- [8]. Calvo J., Jung H., Meloni G. Copper metallothioneins. *Int. Union Biochem. Mol. Biol.* 2017;69:236–245. doi: 10.1002/iub.1618. [PubMed] [CrossRef] [Google Scholar]
- [9]. Reddy M.S., Prasanna L., Marmeisse R., Fraissinet-Tachet L. Differential expression of metallothioneins in response to heavy metals and their involvement in metal tolerance in the symbiotic basidiomycete *Laccaria bicolor*. *Microbiology*. 2014;160:2235–2242. doi: 10.1099/mic.0.080218-0. [PubMed] [CrossRef] [Google Scholar]
- [10]. Oh K.B., Watanabe T., Matsuoka H. A novel copper-binding protein with characteristics of a metallothionein from a clinical isolate of *Candida albicans*. *Microbiology*. 1999;145:2423–2429. doi: 10.1099/00221287-145-9-2423. [PubMed] [CrossRef] [Google Scholar]
- [11]. Xu X., Duan L., Yu J., Su C., Li J., Chen D., Zhang X., Song H., Pan Y. Characterization analysis and heavy metal-binding properties of CsMTL3 in *Escherichia coli*. *FEBS Open Bio*. 2018;8:1820–1829. doi: 10.1002/2211-5463.12520. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
- [12]. M. N. Nadagouda, N. Iyanna, J. Lalley, C. Han, D. D. Dionysiou, and R. S. Varma, "Synthesis of silver and gold nanoparticles using antioxidants from blackberry, blueberry, pomegranate, and turmeric extracts," *ACS Sustainable Chemistry & Engineering*, vol. 2, no. 7, pp. 1717–1723, 2014. View at: Google Scholar
- [13]. C. K. Sathiya and S. Akilandeswari, "Fabrication and characterization of silver nanoparticles using *Delonix elata* leaf broth," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 128, pp. 337–341, 2014. View at: Google Scholar

- [14]. N. M. Shinde, A. C. Lokhande, J. S. Bagi, and C. D. Lokhande, "Biosynthesis of large area (30×30 cm²) silver thin films," *Materials science in semiconductor processing*, vol. 22, pp. 28–36, 2014. View at: Google Scholar
- [15]. E. Rodriguez-Leon, R. Iniguez-Palomares, R. E. Navarro et al., "Synthesis of silver nanoparticles using reducing agents obtained from natural sources (*Rumex hymenosepalus* extracts)," *Nanoscale research letters*, vol. 8, no. 1, pp. 1–9, 2013. View at: Google Scholar
- [16]. M. Vanaja, G. Gnanajobitha, K. Paulkumar, S. Rajeshkumar, C. Malarkodi, and G. Annadurai, "Phytosynthesis of silver nanoparticles by *Cissus quadrangularis*: influence of physicochemical factors," *Journal of Nanostructure in Chemistry*, vol. 3, no. 1, pp. 1–8, 2013. View at: Publisher Site | Google Scholar
- [17]. Krishnaraj RN, Berchmans S. In vitro antiplatelet activity of silver nanoparticles synthesized using the microorganism *Gluconobacter roseus*: an AFM-based study. *RSC Adv.* 2013;3:8953–9.
- [18]. Seshadri S, Prakash A, Kowshik M. Biosynthesis of silver nanoparticles by marine bacterium, *Idiomarina* sp. p R58–8. *Bull Mater Sci.* 2012;35:1201–5
- [19]. Babu MY, Devi VJ, Ramakritinan CM, Umarani R, Taredahali N, Kumaraguru AK Application of biosynthesized silver nanoparticles in agricultural and marine pest control. *Current Nanoscience.* 2014;10:1-9
- [20]. L. T. Jule, R. Krishnaraj, N. Nagaprasad, B. Stalin, V. Vignesh, and T. Amuthan, "Evaluate the structural and thermal analysis of solid and cross drilled rotor by using finite element analysis," *Materials Today: Proceedings*, vol. 47, 2021. View at: Google Scholar
- [21]. T. Amuthan, N. Nagaprasad, R. Krishnaraj, V. Narasimharaj, B. Stalin, and V. Vignesh, "Experimental study of mechanical properties of AA6061 and AA7075 alloy joints using friction stir welding," *Materials Today: Proceedings*, vol. 46, 2021. View at: Publisher Site | Google Scholar
- [22]. . Singh R, Wagh P, Wadhvani S, Gaidhani S, Kumbhar A, Bellare J, Chopade BA. Synthesis, optimization, and characterization of silver nanoparticles from *Acinetobacter calcoaceticus* and their enhanced antibacterial activity when combined with antibiotics. *Int J Nanomed.* 2013;8:4277–90.
- [23]. Duraisamy K, Yang SL. Synthesis and characterization of bactericidal silver nanoparticles using cultural filtrate of simulated microgravity grown *Klebsiella pneumoniae*. *Enzyme Microb Technol.* 2013;52:151–6.