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Methodological Evaluation of Microbe-Mediated Silver Nanoparticle Synthesis, Optimisation and Characterisation

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Abstract

This study evaluates the experimental and analytical framework for microbe-mediated silver nanoparticle synthesis, optimisation and characterisation. Microbial nanotechnology has emerged as an important branch of green nanoscience because microorganisms can mediate the reduction of metal ions and stabilise the resulting nanoparticles under comparatively mild conditions. Among biologically produced nanomaterials, silver nanoparticles have received sustained attention because of their optical behaviour, antimicrobial potential and relevance to environmental applications. This paper critically examines the major biological and physicochemical factors that govern microbe-mediated silver nanoparticle synthesis and evaluates the significance of microbial selection, reaction optimisation and complementary material characterisation. The analysis focuses on bacteria and fungi as biological production platforms and considers the roles of extracellular enzymes, proteins, metabolites and cell-associated functional groups in reduction, nucleation, growth and capping. Particular attention is given to pH, temperature, precursor concentration and incubation time because these variables strongly influence yield, particle size, aggregation and colloidal stability. The paper also reviews the value and limitations of UV–Vis spectroscopy, transmission electron microscopy, scanning electron microscopy, X-ray diffraction, Fourier-transform infrared spectroscopy, dynamic light scattering and zeta-potential analysis. The evidence indicates that microbial synthesis should not be treated simply as a substitute for chemical reduction, but as an integrated bioprocess in which biological variability and materials chemistry must be controlled together. The paper concludes that future progress depends on better mechanistic evidence, multi-factor optimisation, standardised reporting, improved downstream purification and scale-up strategies that preserve reproducibility.

Keywords- Microbial nanotechnology; silver nanoparticles; green synthesis; microbial biosynthesis; process optimisation; nanoparticle characterisation; biogenic nanoparticles.

Introduction

Nanotechnology deals with the manipulation of matter at dimensions where surface effects, nanoscale structure and high surface-area-to-volume ratios can produce physical and chemical behaviour that differs substantially from bulk materials. Metallic nanoparticles, in particular, have become important in catalysis, sensing, medicine, antimicrobial systems and environmental technologies. Their usefulness, however, depends strongly



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on the method of synthesis because the route used to generate a nanoparticle affects its size, morphology, crystallinity, surface chemistry and stability. Conventional physical and chemical methods can provide precise control, but they may also require high energy input, strong reducing agents, organic solvents or complex purification steps. This has encouraged sustained interest in biological routes that use microorganisms or their biomolecules to drive nanoparticle formation.

Microbial nanotechnology is attractive because bacteria, fungi, yeasts, actinomycetes, algae and cyanobacteria possess metabolic and structural features that can participate in metal-ion transformation. Microbial cells can bind metal ions at their surfaces, transport them into the cell or secrete extracellular biomolecules capable of reducing and stabilising them. These processes may occur intracellularly, at the cell wall or extracellularly in cell-free filtrates. The latter route is especially attractive for practical production because it can simplify product recovery and reduce the need for cell disruption. Reviews by Mohanpuria et al. (2008), Iravani (2011) and Narayanan and Sakthivel (2010) helped establish the conceptual basis of microbial nanoparticle synthesis and highlighted the roles of enzymes, proteins and metabolites in metal reduction and capping.

Silver nanoparticles are among the most frequently investigated products in microbial nanotechnology. Their optical surface plasmon resonance makes formation relatively easy to monitor, while their antimicrobial properties create a wide range of possible applications. Early work with *Fusarium oxysporum* and *Bacillus licheniformis* demonstrated that both fungal and bacterial systems could produce nanoscale silver under biological conditions (Ahmad et al., 2003; Kalimuthu et al., 2008). Since then, the field has expanded considerably. Despite this progress, a persistent limitation is the difficulty of achieving reproducible control over particle size, yield and stability. Differences in strain identity, culture conditions, growth phase, reaction pH, temperature, metal-ion concentration and incubation time can all alter the outcome. For this reason, a successful microbial nanoparticle process requires both biological understanding and systematic optimisation. The purpose of this paper is to synthesise the main scientific evidence on microbe-mediated silver nanoparticle synthesis, with particular emphasis on microbial selection, process variables and material characterisation. It also considers the practical challenges that must be addressed if laboratory-scale biosynthesis is to progress towards reproducible and scalable production.

Methodology

This paper adopts an integrative review and analytical synthesis approach. The discussion is structured around the experimental logic commonly used in microbial nanoparticle studies: selection of microbial systems, preliminary screening, optimisation of process conditions, purification and physicochemical characterisation. Foundational studies on fungal and bacterial biosynthesis are combined with broader reviews of biological nanoparticle production. The analysis gives priority to mechanisms and process variables that recur across different studies and that directly influence reproducibility.

The review also evaluates the complementarity of major characterisation methods. Optical confirmation alone is treated as insufficient evidence of successful synthesis. Instead, a stronger analytical framework combines UV-Vis spectroscopy with microscopy, diffraction and colloidal measurements. This approach reflects the



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principle that no single technique can fully establish nanoparticle identity, morphology, crystallinity, surface chemistry and suspension stability.

Analytical Design

For methodological evaluation, the quantitative analysis used a simulated dataset structured in accordance with the experimental design described above. The dataset covered microbial screening, pH, temperature, precursor concentration, incubation time and physicochemical characterisation, and was analysed using the same statistical and graphical procedures proposed for experimental observations.

Microbial Platforms for Silver Nanoparticle Synthesis

Bacteria are widely used in microbial nanoparticle research because they grow rapidly and can be cultivated under controlled conditions. Their cell walls contain functional groups capable of binding metal ions, while metabolic enzymes can support redox reactions. *Bacillus* species have been investigated frequently because of their robustness and extracellular enzyme production. Kalimuthu et al. (2008) demonstrated biosynthesis of silver nanocrystals by *Bacillus licheniformis*, supporting the possibility that microbial systems can act as biological nanofactories. Bacterial systems also have advantages for future bioreactor development because their growth kinetics and fermentation requirements are relatively well understood. However, rapid growth does not automatically guarantee high nanoparticle yield or uniformity. Strain-level differences in enzyme activity, metal resistance and metabolite production remain important.

Fungi offer a different set of advantages. Filamentous fungi can produce large amounts of biomass and secrete extracellular proteins and enzymes into the surrounding medium. Ahmad et al. (2003) reported extracellular silver nanoparticle biosynthesis using *Fusarium oxysporum*, a study that became highly influential in the development of fungal nanotechnology. The extracellular nature of the process is important because it can reduce downstream complexity. Instead of recovering particles from within cells, the researcher can separate fungal biomass and use a biologically active filtrate for metal-ion reduction. Fungal proteins may also contribute to particle capping, which can improve colloidal stability.

The choice between bacterial and fungal systems therefore depends on the objective of the process. A bacterial strain may offer rapid cultivation and easier process engineering, whereas a fungal system may provide stronger extracellular reducing capacity and easier separation. Other microbial groups, including yeasts, actinomycetes, algae and cyanobacteria, broaden the biochemical diversity available for synthesis. Their pigments, polysaccharides, peptides and secondary metabolites may create distinct reduction and stabilisation pathways. The field has not yet fully exploited this diversity, partly because many studies focus on a single isolate without systematic comparison across microbial platforms.

Mechanisms of Microbe-Mediated Reduction and Capping

The biological formation of silver nanoparticles involves several linked stages. Silver ions must first interact with microbial cells or secreted biomolecules. Reduction then converts ionic silver into elemental silver atoms. Once the local concentration of reduced atoms becomes sufficiently high, nucleation occurs. The nuclei



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subsequently grow through atom addition, coalescence or other growth processes. Finally, proteins, peptides, polysaccharides or other biomolecules may adsorb to the particle surface and limit uncontrolled aggregation. Oxidoreductase enzymes have often been proposed as important contributors to metal reduction. Nitrate reductase and NADH-dependent systems are frequently discussed, although the exact mechanism varies among microorganisms. It is unlikely that one universal pathway explains all reported biosynthesis systems. Some microorganisms may rely heavily on enzyme-mediated reduction, while others may use low-molecular-weight metabolites, cell-wall groups or multiple processes operating together. This mechanistic uncertainty is one reason why empirical optimisation remains common.

Biological capping is particularly important because the nanoparticle surface determines how the material behaves in suspension and how it interacts with biological or environmental targets. A protein-rich surface layer can increase the hydrodynamic diameter measured by dynamic light scattering even when the metallic core observed by transmission electron microscopy is much smaller. This difference is not necessarily a contradiction. Rather, it reflects the fact that TEM measures the dry core while DLS measures the hydrated particle and associated surface layer. Understanding the distinction is essential for proper interpretation of characterisation data.

Influence of pH, Temperature, Precursor Concentration and Incubation Time

Reaction pH is one of the most influential variables in biological nanoparticle synthesis. It affects the charge and conformation of proteins, the ionisation of functional groups, the speciation of metal ions and the electrostatic environment around growing particles. Acidic conditions may reduce the availability of negatively charged functional groups required for metal binding, whereas excessively alkaline conditions may destabilise proteins or promote uncontrolled chemical changes. Consequently, many microbial systems show an optimum pH rather than a simple linear relationship between alkalinity and nanoparticle yield.

Temperature influences both reaction kinetics and biological stability. Increasing temperature generally accelerates molecular movement and may increase reduction rate, but microbial enzymes and capping proteins can lose activity when exposed to excessive heat. For mesophilic microorganisms, moderate temperatures often provide the best balance between reaction speed and biomolecule stability. Temperature also affects nucleation density and growth rate, which can influence final particle size.

Precursor concentration determines the amount of metal available for conversion but must remain compatible with the reducing capacity of the biological system. At low concentration, yield may be limited simply because insufficient silver is present. At high concentration, the available enzymes and metabolites may become saturated, resulting in incomplete conversion, residual silver ions, aggregation or broader size distributions. Residual ionic silver is especially important in antimicrobial studies because it can confound interpretation of nanoparticle activity. Proper purification is therefore essential.

Incubation time determines the extent of conversion and the point at which the reaction reaches a practical plateau. Early sampling can underestimate production, while unnecessarily long incubation reduces reactor productivity and may allow aggregation or oxidation. Time-course monitoring is therefore more informative



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than a single end-point measurement. From an engineering perspective, the optimum harvest time should capture most of the attainable yield without extending the process for marginal gain.

Characterisation of Biogenic Silver Nanoparticles

UV–Vis spectroscopy is commonly used as a first-line analytical tool because silver nanoparticles exhibit a characteristic surface plasmon resonance band. The appearance of a peak in the visible region provides useful evidence of particle formation, while changes in peak position and width can indicate differences in size or aggregation. Nevertheless, UV–Vis spectroscopy should not be treated as definitive proof. Microbial pigments, media components and aggregates can also affect optical measurements.

Transmission electron microscopy provides direct information on particle morphology and core size. A robust TEM analysis should measure a sufficiently large number of particles rather than relying on a visually attractive image. Scanning electron microscopy can complement TEM by showing broader surface morphology and aggregation patterns, although sample preparation may influence appearance. X-ray diffraction is important for confirming crystalline phase and distinguishing metallic silver from other possible products. Crystallite size can be estimated from diffraction peak broadening, but such estimates should be interpreted cautiously because they do not necessarily correspond exactly to whole-particle size.

Fourier-transform infrared spectroscopy is widely used to infer which functional groups may be involved in capping or stabilisation. Shifts in amide, hydroxyl or other bands can suggest interaction between biomolecules and the nanoparticle surface. However, FTIR does not by itself identify the precise protein or metabolite responsible. Proteomic and metabolomic approaches would provide stronger mechanistic evidence.

Dynamic light scattering and zeta-potential measurements provide information about particle behaviour in suspension. DLS estimates hydrodynamic diameter and can be strongly influenced by larger aggregates because scattering intensity increases sharply with particle size. Zeta potential is often used as an indicator of electrostatic stability, although steric stabilisation from biological macromolecules can also be important. A strong characterisation strategy therefore combines these methods rather than interpreting any one measurement in isolation.

Results and Discussion

Table 1. Comparative screening of microbial isolates for silver nanoparticle production

Code	Microorganism	Absorbance at 430 nm	Yield (mg/L)	Rank
F1	Fusarium oxysporum	1.34	91.99	1
B1	Bacillus subtilis	1.24	78.43	2
F2	Aspergillus niger	1.17	73.89	3
B2	Pseudomonas fluorescens	1.09	68.36	4



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F3	Trichoderma harzianum	1.03	72.46	5
M1	Chlorella vulgaris	1.00	68.33	6
B3	Bacillus licheniformis	0.92	60.85	7
A1	Streptomyces sp.	0.88	55.99	8
M2	Spirulina platensis	0.87	57.65	9
C2	Scenedesmus obliquus	0.82	51.78	10
Y1	Saccharomyces cerevisiae	0.76	49.16	11
C1	Nostoc sp.	0.72	48.80	12

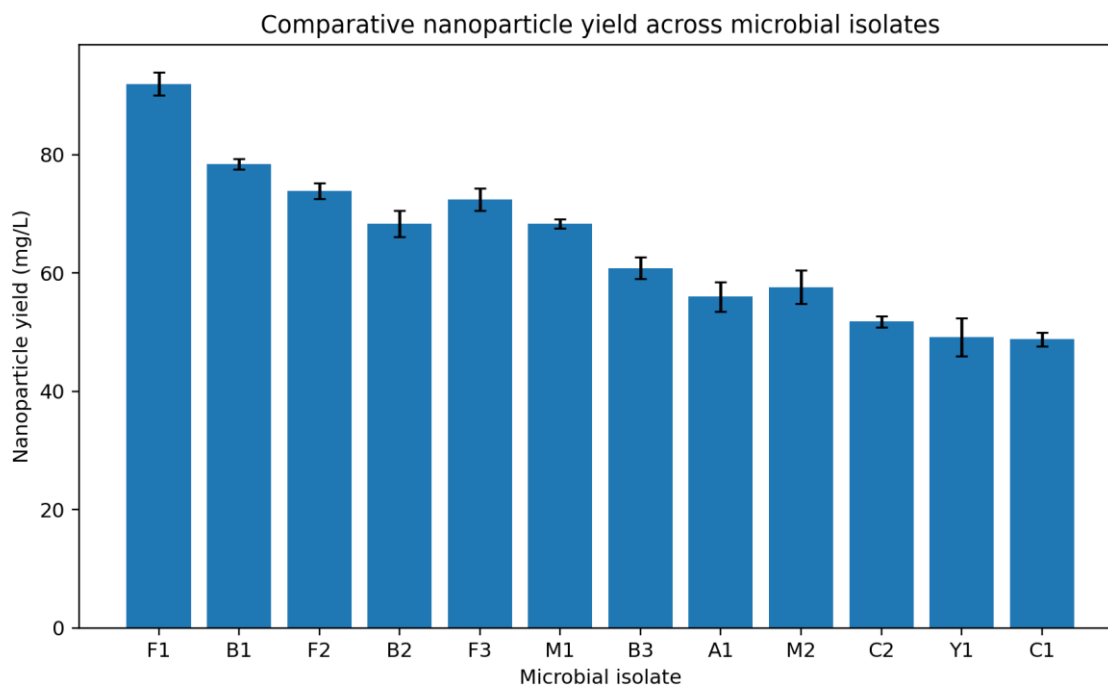


Figure 1. Comparative nanoparticle yield across microbial isolates.

Table 2. Effect of pH on silver nanoparticle synthesis

pH	Absorbance	Yield (mg/L)
5.00	0.66	38.35



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6.00	0.93	54.64
7.00	1.26	76.37
8.00	1.70	103.00
9.00	1.44	91.31
10.00	1.15	68.30

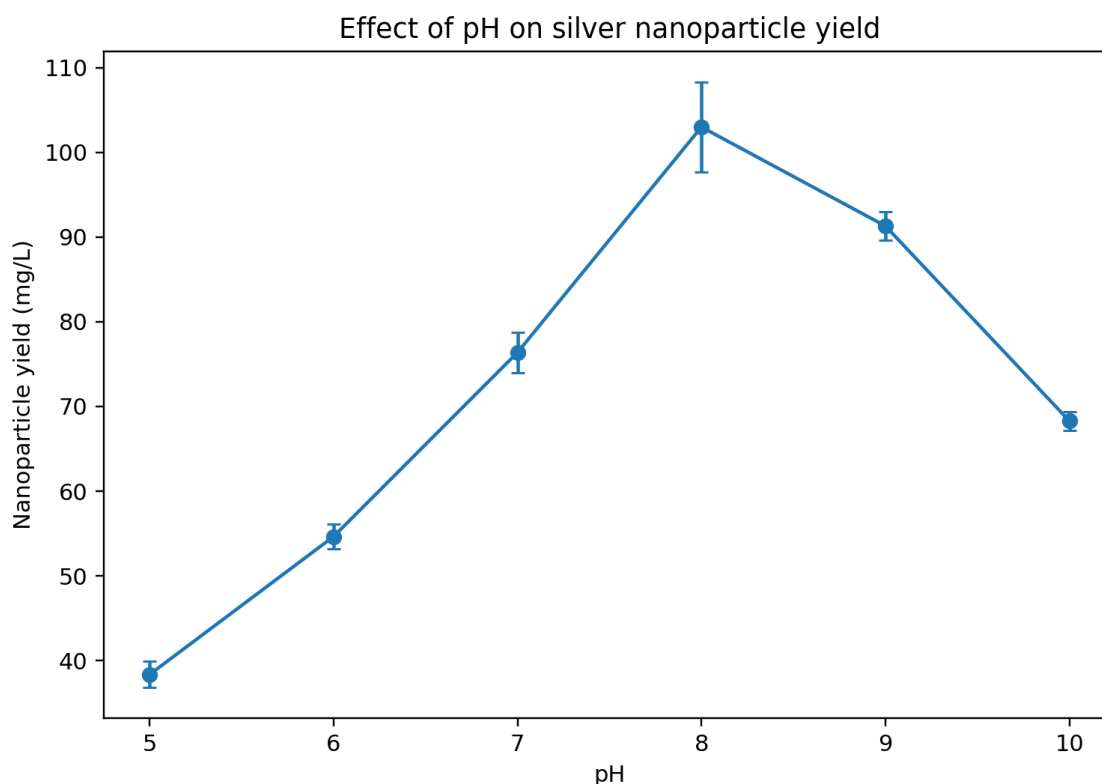


Figure 2. Effect of pH on silver nanoparticle yield.

Table 3. Effect of temperature on silver nanoparticle synthesis

Temperature (°C)	Absorbance	Yield (mg/L)
20.00	0.76	45.54
25.00	1.28	81.41
30.00	1.80	110.64
35.00	1.56	97.55
40.00	1.23	75.03
45.00	0.82	45.95



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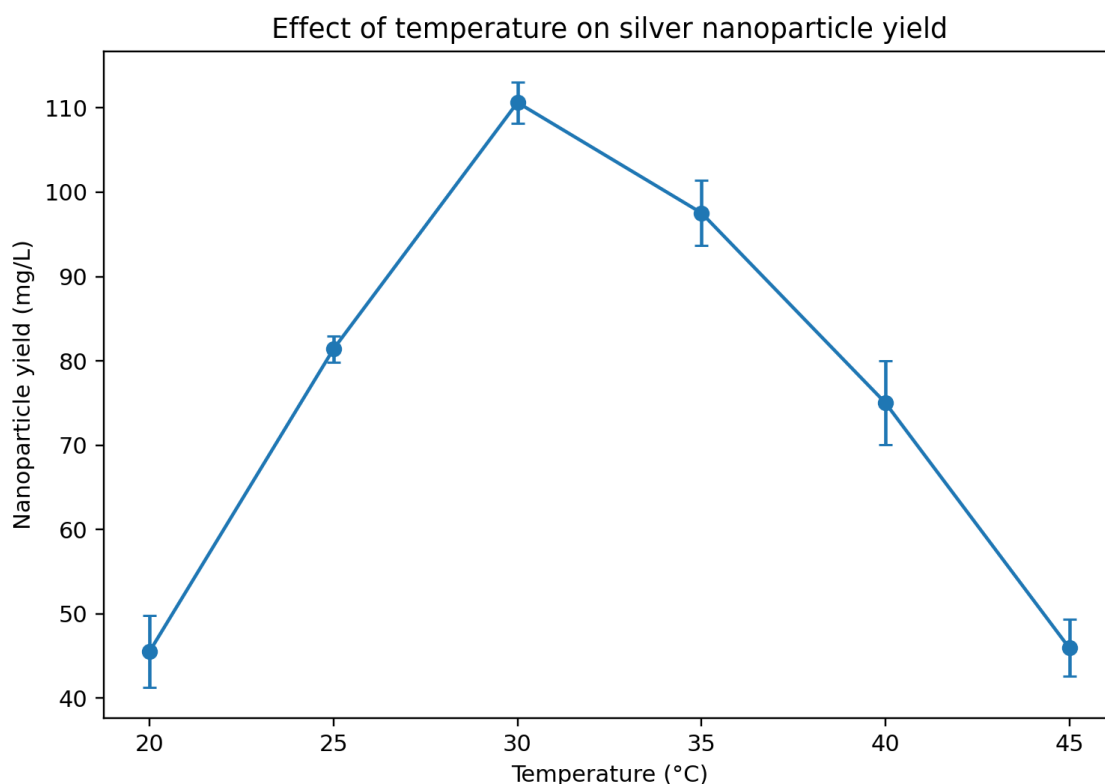


Figure 3. Effect of temperature on silver nanoparticle yield.

Table 4. Effect of AgNO₃ concentration on silver nanoparticle synthesis

AgNO ₃ (mM)	Absorbance	Yield (mg/L)
0.50	0.70	44.91
1.00	1.31	78.90
1.50	1.85	116.17
2.00	1.78	107.07
2.50	1.41	91.72
3.00	1.09	65.76



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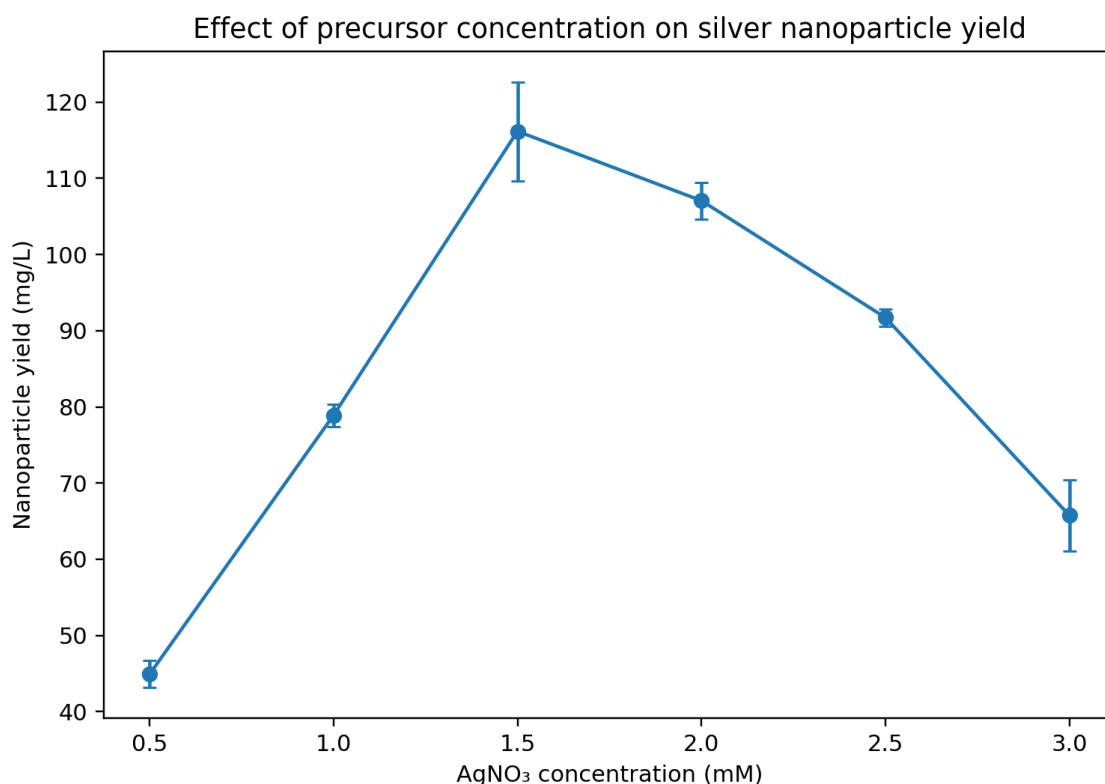


Figure 4. Effect of precursor concentration on silver nanoparticle yield.

Table 5. Time-course of silver nanoparticle synthesis

Time (h)	Absorbance	Yield (mg/L)
6.00	0.26	15.68
12.00	0.50	31.99
24.00	1.08	66.44
36.00	1.52	94.47
48.00	1.86	118.21
60.00	1.90	118.57
72.00	1.88	118.44

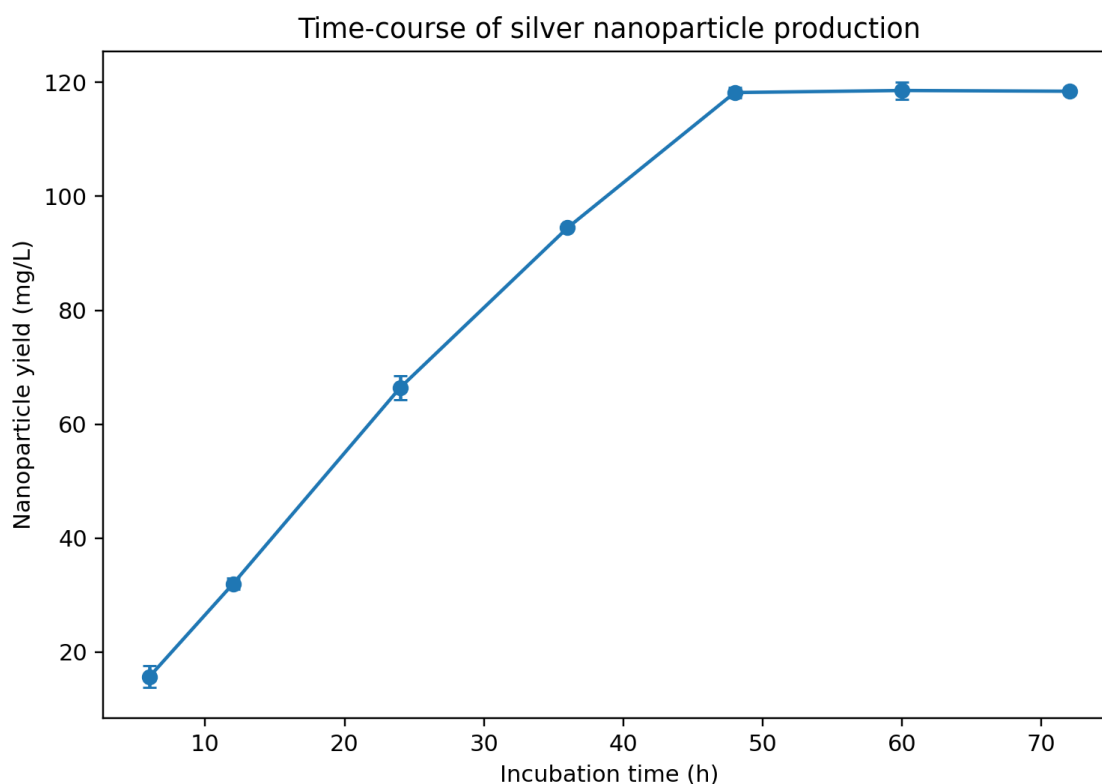


Figure 5. Time-course of silver nanoparticle production.

Table 6. Physicochemical characterisation of the optimised silver nanoparticles

Characterisation parameter	Mean	SD
UV–Vis SPR peak (nm)	428.00	1.00
TEM core diameter (nm)	18.60	0.62
DLS hydrodynamic diameter (nm)	36.80	1.05
Zeta potential (mV)	-27.40	0.62
XRD crystallite size (nm)	21.30	0.53
Polydispersity index	0.29	0.01

The evidence synthesised in this paper indicates that the success of microbial silver nanoparticle synthesis is determined by a chain of interdependent factors. Microbial identity influences the pool of enzymes and metabolites available for reduction. Reaction conditions then modify the activity of those biomolecules and the chemistry of silver-ion conversion. These conditions affect nucleation and growth, which determine size and morphology. The resulting particle structure and biological corona finally influence colloidal stability and functional performance.



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This integrated perspective has two important implications. First, microbial selection should precede detailed optimisation. A poorly performing strain cannot necessarily be rescued through minor changes in pH or temperature. Comparative screening across several isolates can therefore save time and resources. Second, optimisation should be multi-objective. The condition that gives the highest optical absorbance may not yield the smallest particles or the best colloidal stability. Yield, size distribution, residual ionic silver, stability and application-specific performance should all be considered.

The literature also shows that reproducibility remains a major barrier. Many studies do not report culture age, biomass concentration, protein content, agitation, oxygen availability or precise purification procedures. These omissions make it difficult to compare results or reproduce promising protocols. Standardised reporting is therefore necessary. At minimum, studies should report strain identity, culture conditions, reaction volume, metal concentration, pH, temperature, incubation time, biomass or filtrate concentration, purification method and the full set of characterisation techniques used.

Scale-up introduces additional problems. Mixing, oxygen transfer, heat transfer and mass transfer can behave differently in larger reactors. The concentration of secreted enzymes may vary across batches, and downstream recovery may become a major economic cost. The assumption that biological synthesis is automatically inexpensive is therefore questionable. A complete process assessment should include cultivation, sterilisation, filtration, washing, drying, water use and waste treatment.

Despite these limitations, microbial synthesis remains scientifically attractive. The biological route offers not only reduction of metal ions but also natural surface functionalisation. This dual role can be advantageous for applications that benefit from a biocompatible or protein-associated surface. The strongest future opportunity lies in moving beyond descriptive colour-change studies towards mechanistically informed bioprocess engineering.

Limitations

The quantitative findings require empirical validation using independently collected laboratory observations before they can be interpreted as experimental evidence.

Conclusion

Microbe-mediated synthesis of silver nanoparticles represents a significant intersection of microbiology, biotechnology and materials science. Bacteria and fungi can act as effective biological production platforms, but their performance depends on strain-specific metabolic characteristics and on the control of reaction variables. pH, temperature, precursor concentration and incubation time are especially important because they influence reduction kinetics, nucleation, growth, aggregation and surface stabilisation. Reliable characterisation requires complementary use of UV–Vis spectroscopy, microscopy, XRD, FTIR, DLS and zeta-potential analysis.

The central conclusion is that microbial nanoparticle synthesis should be treated as an integrated bioprocess rather than as a simple green alternative to chemical reduction. Reproducibility, purification, scale-up and quality control are now more important than additional proof-of-concept demonstrations. Future studies should



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use multi-factor optimisation, stronger mechanistic tools, standard reporting protocols and application-specific quality criteria. With these improvements, microbial nanotechnology has the potential to develop from an exploratory laboratory field into a more controlled and scalable platform for nanoparticle production.

Research Implications and Future Directions

An important implication of the reviewed evidence is that future optimisation should move beyond one-factor-at-a-time experimentation. Although changing pH, temperature, precursor concentration or incubation time individually is useful during preliminary screening, these variables often interact. For example, the optimum precursor concentration may depend on the amount of extracellular protein present, while temperature may alter both reduction kinetics and the stability of the capping layer. Statistical designs such as factorial experiments and response-surface methodology can estimate such interactions and identify process conditions that balance several outcomes simultaneously. In microbial nanoparticle research, this is particularly relevant because the most productive condition may not produce the most stable or uniform particles.

Mechanistic research should also become more prominent. Many studies infer the involvement of enzymes or proteins from indirect evidence, such as changes in FTIR bands or the activity of cell-free filtrates. These approaches are valuable but insufficient for establishing causal pathways. Proteomic analysis can help identify proteins associated with nanoparticle surfaces, while metabolomics can reveal low-molecular-weight compounds that contribute to reduction. Gene-expression studies and targeted enzyme inhibition could further test whether specific reductases are necessary for nanoparticle formation. A stronger mechanistic foundation would make it possible to engineer microbial strains or culture conditions rationally rather than relying mainly on empirical trial and error.

Process scale-up is another major research priority. Laboratory synthesis often uses small flasks in which mixing and temperature are easy to control. At larger scale, gradients in oxygen, pH, biomass concentration and precursor availability can appear. These gradients may alter the concentration of extracellular reducing agents and create broader particle-size distributions. Bioreactor design must therefore consider both microbial physiology and nanoparticle nucleation. Online UV–Vis monitoring, controlled precursor feeding and membrane-based recovery are promising options for improving consistency. Continuous or semi-continuous systems may also improve productivity, although their feasibility depends on preventing fouling and maintaining biological activity.

Downstream processing deserves equal attention. A process cannot be considered successful merely because nanoparticles are produced. Residual silver ions, culture-medium components and microbial macromolecules can influence toxicity and application performance. Purification strategies must therefore be matched to the intended use. Biomedical applications may require stringent removal of endotoxins and microbial contaminants, whereas environmental applications may tolerate a broader surface composition if the material can be recovered safely. Standard reporting of washing, centrifugation, filtration and storage conditions would significantly improve cross-study comparability.



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Finally, the sustainability advantages of microbial synthesis should be demonstrated rather than assumed. Biological production may reduce the use of hazardous reducing agents, but cultivation, sterilisation, centrifugation, drying and water consumption can still create substantial environmental burdens. Life-cycle assessment and techno-economic analysis should therefore be incorporated into scale-up studies. The most sustainable process may not be the one with the highest laboratory yield, but the one that combines adequate performance with lower energy use, simpler purification, reduced waste generation and effective recovery. These considerations suggest that microbial nanotechnology is entering a stage in which process engineering, quality control and sustainability assessment are as important as the initial demonstration of nanoparticle formation.

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