



Bacterial Synthesis of Nanoparticles: Current Trends in Biotechnology and Biomedical Fields

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Abstract

On estimation scales ranging from 0.1 nm to 100 nm, the nanoscale is part of the capacitance components of the physical-synthetic and natural environment. Dimensionality, morphology, structure, uniformity, and agglomeration are all used to classify nanoparticles. Its functionality and effect on the environment and species are influenced by its shape and morphology. The priority research is to determine the effects of nanoparticles on any biological entity that is necessary when designing nanotechnology-based biotechnological and biomedical products. Bacteria have a remarkable ability to reduce metal ions, making them one of the most promising candidates for nanoparticle biosynthesis. Nanoparticles have been researched in the biomedical field for antimicrobial, biosensor, diagnostic imaging, and drug delivery applications. These natural technologies appear to be capable of producing stable nanoparticles with well-defined dimensions, morphologies, and compositions by optimizing reaction conditions and selecting the best bacteria. This work includes a list of the most commonly used microorganisms and associated Nanoparticles, as well as a discussion of current biotechnology and biomedical developments.

Keywords: Nanoparticle; Bacterial; Biotechnology; Biomedical Application

Introduction to Nanoparticles

Nanoscience and Nanotechnology due to its potential effects on many scientific areas such as oil, medicine, pharmaceutical industries, electronics, and space industries, has sparked a lot of interest in recent years [1]. Tiny structures and materials, ranging in size from a few nanometres to less than 100 nanometres, are the focus of this technology. Due to their high surface-to-volume ratio, nanoparticles (NPs) have distinct and substantially altered chemical, physical, and biological properties as compared to most of the same chemical composition [2]. Biosensors and catalysts, as well as optics, antimicrobial operation, device transistors, electrometers, chemical sensors, and wireless electronic logic and memory schemes, all benefit from the shape and size based properties of NPs [3,4].

The nanoscale is part of the capacity components of the physical-synthetic and natural world on estimation scales ranging from 0.1 nm to 100 nm [5]. In general, as particle size decreases, the proportion of surface to volume increases, increasing the reactivity of the particles. Particles of different components exhibit extraordinary physical, compound, and natural properties at the nanoscale. NPs have a lower softening point, electrical resistivity, particular heat, diffusivity, flexibility, and mechanical quality than typical coarse-grained partners, as well as improvements in their electromagnetic and synergistic properties. With several developments, researchers are attempting to decode these NP-related properties [6].

Dimensionality, morphology, structure, uniformity, and agglomeration are all used to classify NPs. Its functionality

and toxic impact on the environment and humans are influenced by its shape and morphology [7].

NPs are graded as one-, two-, or three-dimensional depending on their dimensionality. Thin films used in electronics and sensor applications are examples of one-dimensional NPs. Carbon nanotubes, which are two-dimensional NPs, have a high adsorption capacity and stability. Dendrimers and quantum dots are found in three-dimensional NPs [8-10]. NPs may have a flat, circular, or crystalline structure depending on their morphology. They can also be used in single or composite form. Oxide nanoparticles, sulphides nanoparticles, and magnetic nanoparticles are the three forms of NPs [11].

The need for a comprehensive study of NPs is due to the considerable amount of scientific research, which has shown that their properties differ significantly from similar macro particles and from the substances from which they were obtained [12]. These properties depend on their composition, nature, shape, size, charge, structural features, and both the surface of the nanoparticle itself and the surface modifier molecules (if used), the preparation methods, and the process parameters [13]. There are many aspects of these biological methods that can be detected and then manipulated [14]. Nano biotechnologies, based on the use of bacteria, contribute to the reduction of NPs, have high commercial potential with the prospects of widespread use. The NPs obtained in this way show better biocompatibility due to the absence of adsorbed toxic substances. Much of the experimental research has been conducted to create diagnostic and therapeutic nanosheets [15]. Specialists of various profiles – material scientists, chemists, biologists, physicians, and veterinarians – are involved in this problem [12-16]. The purpose of these studies is to create NPs, not as a final product, but as a feedstock for biocompatible substances. Compared to conventional pathway NPs, biosynthetic NPs have some unique properties and can be used without side effects in areas such as catalysis and degradation of organic pollutants [15].

Priority research is to determine the effects of the impact of NPs on any biological entity that is required when designing biotechnology products with the involvement of nanotechnology. Such studies can only be adequate with the consistent use of biomarkers – key systemic characteristics of a living organism (biochemical, physiological, immunological, etc.) [17]. Several basic mechanisms of contact interaction of metals in different chemical forms on the model of cells of microorganisms are described [18]. The mechanism of passive deposition of metals by a bacterial cell is based on the physical and chemical binding of the structural components of the cell membrane. Metabolism-dependent cumulation of metals is associated with the functioning of cellular enzyme

systems that provide metabolism and energy responses to the environment. Prokaryotic cells, due to their unique structural properties and metabolism, are able to actively interact with metals and can be a model system for studying the relationship between a cell and NPs [15].

Methodologies for Synthesis of NPs using Microbes

NPs are constantly being studied and developed [19]. Three methods are used to synthesize nanomaterials [20]. Lithography, pyrolysis, and vapor pressure are examples of physical methods. However, these strategies are very costly. Physical and chemical methods are important because they are low-productivity, energy-intensive, difficult to scale, often generate high levels of hazardous waste, and can involve the use of costly precursors [21]. Irradiation reduction, microemulsion reduction, and electrochemical reduction are also chemical methods, but chemical methods are very risky because this synthesis can also result in the presence of certain lethal chemical species adsorbed on the surface, which can have unfavourable effects in medical applications [22]. As a result, researchers have turned to biological systems for inspiration in the synthesis of NPs over the last year. Microorganisms have recently been discovered as potential environmentally friendly Nano factories, despite their numerous biotechnological applications, such as lethal metal remediation. It has been established that prokaryotes play an important role in the synthesis of NPs [23]. Adapted to their excess atmosphere and their ability to adapt to the original transmission situation, bacteria are a good selection for research [24]. The bacteria are also residual maturing, low-cost to produce, and convenient to handle. Oxygenation, incubation time, and temperature could all be easily regulated even during diffusion process. It was discovered that the pH of the incubation selection operation is uneven in the game of incompatible visualization and dressing NPs; nonetheless, since unconditional NP morphologies are needed for numerous technologies such as catalysts, antitumor agents, drug delivery, diagnostics, and antimicrobials, such transfer is common [22].

Bacteria are one of the best candidates for the synthesis of NPs as they have a remarkable ability to reduce heavy metal ions. Some bacterial organisms have adapted the ability to react to stresses such as the toxicity of heavy metal ions or metals by implementing complex defence mechanisms [7]. Furthermore, selected of these bacteria are capable of synthesizing inorganic materials [1]. Table 1 shows the majority of the bacterial species used in the biosynthesis of NPs, with various protocols and methodologies used among different authors and bacterial species.

Bacteria	Nanoparticles	Synthesis method
<i>Acinetobacter sp.</i> [25]	Fe ₃ O ₄	Extracellular
<i>Acinetobacter sp.</i> [26]	Si/SiO ₂	Extracellular
<i>Acinetobacter spp.</i> [25]	Fe ₂ O ₃	Extracellular
<i>Aquaspirillum Magnetotacticum</i> [27]	Fe ₃ O ₄	Intracellular
<i>Arthrobacter globiformis</i> [28]	Au	Extracellular
<i>Arthrobacter sp.</i> [28]	Au	Extracellular
<i>Azoarcus sp.</i> [29]	Se	Intracellular
<i>Bacillus amyloliquefaciens</i> [24]	CdS	Extracellular
<i>Bacillus cereus</i> [30]	Ag	Extracellular
<i>Bacillus flexus</i> [31]	Ag	Extracellular
<i>Bacillus licheniformis</i> [32]	Ag	Intracellular
<i>Bacillus licheniformis</i> [24]	Ag	Extracellular
<i>Bacillus licheniformis</i> [33]	Au	Not specified
<i>Bacillus licheniformis</i> [33]	Ag	Extracellular
<i>Bacillus licheniformis</i> [33]	Ag	Not specified
<i>Bacillus licheniformis</i> [34]	Ag	Extracellular
<i>Bacillus licheniformis</i> [35]	Ag	Extracellular
<i>Bacillus megaterium</i> [36]	Ag	Extracellular
<i>Bacillus methylotrophicus</i> [24]	Ag	Extracellular
<i>Bacillus persicus</i> [24]	Ag	Extracellular
<i>Bacillus pumilus</i> [24]	Ag	Extracellular
<i>Bacillus sp.</i> [37]	Ag	Extracellular
<i>Bacillus sphaericus</i> [37, pag. 11]	U, Cu, Pb, Al, Cd	Extracellular
<i>Bacillus spp.</i> [38]	Ag	Intracellular
<i>Bacillus subtilis</i> [39]	Au	Intracellular
<i>Bacillus subtilis</i> [32]	Au	Intracellular
<i>Bacillus subtilis</i> [40]	TiO ₂	Not specified
<i>Bacillus subtilis</i> [41]	Co ₃ O ₄	Extracellular
<i>Bacillus subtilis</i> [42]	Ag	Extracellular
<i>Bacillus subtilis</i> [43]	Ag	Extracellular
<i>Bacillus subtilis</i> [44]	Au	Intracellular
<i>Bhargavaea indica</i> [24]	Ag and Au	Extracellular
<i>Brevibacterium frigoritolerans</i> [24]	Ag	Extracellular
<i>Cladosporium cladosporioides</i> [45]	Ag	Extracellular
<i>Clostridium thermoaceticum</i> [46]	CdS	Extracellular
<i>Clostridium thermoaceticum</i> [46]	CdS	Extra/Intracellular
<i>Corynebacterium spp.</i> [47]	Ag	Intracellular
<i>Cupriavidus necator</i> [32]	Pd	Intracellular

<i>Delftia acidovorans</i> [48]	Au	Extracellular
<i>Desulfobacteraceae spp.</i> [49]	ZnS	Not specified
<i>Desulfovibrio desulfuricans</i> [32]	Pd	Intracellular
<i>Desulfovibrio desulfuricans</i> [50]	Pd	Intracellular
<i>Desulfovibrio desulfuricans</i> [51]	Pd	Extracellular
<i>Enterobacter cloacae</i> [52]	Ag	Not specified
<i>Enterococcus faecium</i> [52]	Ag	Extracellular
<i>Escherichia coli</i> [53]	Cd	Intracellular
<i>Escherichia coli</i> [54]	Ag	Not specified
<i>Escherichia coli</i> [55]	Cu	Extracellular
<i>Escherichia coli</i> [56]	CdS	Intracellular
<i>Escherichia coli</i> [56]	CdS	Intracellular
<i>Escherichia coli</i> [56]	CdS	Intracellular
<i>Escherichia coli</i> [57]	CdTe	Extracellular
<i>Escherichia coli</i> [58, pag. 5]	Au	Intracellular
<i>Escherichia coli</i> [58]	Au	Intracellular
<i>Escherichia coli</i> [59]	Pt	Not specified
<i>Escherichia coli</i> [52]	Ag	Not specified
<i>Escherichia coli</i> [43]	Ag	Extracellular
<i>Escherichia coli</i> [60]	Pd, Pt	Extracellular
<i>Fusarium oxysporum</i> [61]	Ag	Extracellular
<i>G. sulfurreducens</i> [32]	Au	Extracellular
<i>Geobacillus sp.</i> [62]	Au	Intracellular
<i>Geobacillus sp.</i> [62]	Au	Intracellular
<i>Geobacter sulfurreducens</i> [63]	Pd	Extracellular
<i>Idiomarina spp.</i> [64]	Ag	Intracellular
<i>Klebsiella pneumoniae</i> [65]	Se	Intracellular
<i>Klebsiella pneumoniae</i> [65]	Se	Intracellular
<i>Klebsiella pneumoniae</i> [66]	Ag	Extracellular
<i>Klebsiella pneumoniae</i> [66]	Ag	Not specified
<i>Klebsiella pneumoniae</i> [52]	Ag	Not specified
<i>Klebsiella pneumoniae</i> [52]	Ag	Extracellular
<i>Lactobacillus acidophilus</i> [67]	Ag	Extracellular
<i>Lactobacillus acidophilus</i> [68]	Se	Not specified
<i>Lactobacillus casei</i> [67]	Ag	Extracellular
<i>Lactobacillus crispatus</i> [69]	TiO ₂	Extracellular
<i>Lactobacillus mindensis</i> [70]	Ag ₂ O	Not specified
<i>Lactobacillus reuteri</i> [67]	Ag	Extracellular
<i>Lactobacillus sp.</i> [32]	Au, Ag, Au-Ag alloys	Intracellular

<i>Lactobacillus sp.</i> [71]	TiO ₂	Extracellular
<i>Lactobacillus sporogens</i> [72]	ZnO	Intracellular
<i>Lactobacillus spp.</i> [73]	Ag	Extracellular
<i>Lactobacillus spp.</i> [74]	Au, Ag, Au-Ag	Intracellular
<i>Lactobacillus strains</i> [75]	Ag and Au	Intracellular
<i>Lactococcus garvieae</i> [52]	Ag	Extracellular
<i>Lysinibacillus sphaericus</i> [76]	Ag	Not specified
<i>M. morganii</i> [55]	Cu	Extracellular
<i>Magnetite gryphiswaldense</i> [77]	Magnetite	Intracellular
<i>Magnetospirillum gryphiswaldense</i> [78]	Fe ₃ O ₄ /Fe ₃ S ₄	Intracellular
<i>Magnetotactic bacterium</i> [79]	Fe ₃ O ₄	Intracellular
<i>Marinobacter pelagius</i> [80]	Au	Not specified
<i>Morganella psychrotolerans</i> [81]	Ag	Extracellular
<i>Morganella sp.</i> [82]	Cu	Intracellular
<i>Morganella sp.</i> [32]	Ag	Intracellular
<i>Morganella sp.</i> [83]	Ag	Extracellular
<i>Morganella sp.</i> [84]	Metallic Cu	Extracellular
<i>Mycobacterium psychrotolerans</i> [55]	Cu	Extracellular
<i>Myxococcus virescens</i> [85]	Ag	Extracellular
<i>Ochrobactrum sp.</i> [86]	Ag	Extracellular
<i>Pantoea agglomerans</i> [87]	Se	Intracellular
<i>Paracoccus denitrificans</i> [32]	Pd	Intracellular
<i>Penicillium fellutanum</i> [88]	Ag	Extracellular
<i>Planomicrobium sp.</i> [89]	TiO ₂	Extracellular
<i>Planomicrobium sp.</i> [40]	TiO ₂	Extracellular
<i>Plectonema boryanum</i> [90]	Ag	Intracellular
<i>Plectonema boryanum</i> [91]	Au	Intracellular
<i>Proteus mirabilis</i> [92]	Ag	Extra/Intracellular
<i>Pseudomonas aeruginosa</i> [32]	Au, Ag, Pd, Fe, Rh, Ni, Ru, Pt	Extracellular
<i>Pseudomonas aeruginosa</i> [53]	Au	Extracellular
<i>Pseudomonas aeruginosa</i> [93]	Ag	Extracellular
<i>Pseudomonas aeruginosa</i> [94]	Se	Intracellular
<i>Pseudomonas aeruginosa</i> [95]	Gold	Extracellular
<i>Pseudomonas aeruginosa</i> [95]	Au	Not specified
<i>Pseudomonas aeruginosa</i> [95]	Au	Extracellular
<i>Pseudomonas aeruginosa</i> [96]	Au	Extracellular
<i>Pseudomonas alcaliphila</i> [97]	Se	Not specified
<i>Pseudomonas deceptionensis</i> [24]	Ag	Extracellular
<i>Pseudomonas fluorescens</i> [98]	Cu	Extracellular

<i>Pseudomonas fluorescens</i> [99]	Cu	Extracellular
<i>Pseudomonas fluorescens</i> [100]	Au	Extracellular
<i>Pseudomonas putida</i> [32]	Pd	Intracellular
<i>Pseudomonas sp.</i> [55]	Cu	Extracellular
<i>Pseudomonas sp.</i> [101]	Ag	Extracellular
<i>Pseudomonas spp.</i> [37]	Ag	Intracellular
<i>Pseudomonas stutzeri</i> [32]	Ag	Intracellular
<i>Pseudomonas stutzeri</i> [53]	Ag	Intracellular
<i>Pseudomonas stutzeri</i> [55]	Cu	Extracellular
<i>Pseudomonas stutzeri</i> [102]	Ag	Not specified
<i>Pseudomonas stutzeri</i> [103]	Ag	Intracellular
<i>Pseudomonas stutzeri</i> [104]	Au, Ag, Au-Ag	Intracellular
<i>Pyrobaculum islandicum</i> [32]	Au	Extracellular
<i>Pyrococcus furiosus</i> [32]	Au	Extracellular
<i>Rhodococcus sp.</i> [105]	Au	Intracellular
<i>Rhodococcus sp.</i> [106]	Au	Intracellular
<i>Rhodococcus sp.</i> [107]	Gold	Intracellular
<i>Rhodopseudomonas capsulata</i> [32]	Au	Extracellular
<i>Rhodopseudomonas capsulata</i> [108]	Au	Extracellular
<i>Rhodopseudomonas capsulata</i> [109]	Au	Extracellular
<i>Rhodopseudomonas capsulata</i> [110]	Au	Extracellular
<i>Rhodopseudomonas palustris</i> [111]	CdS	Intracellular
<i>S. oneidensis</i> [32]	Pd	Intracellular
<i>S. oneidensis</i> [112]	Pd	Intracellular
<i>Salmonella typhirium</i> [113]	Ag	Extracellular
<i>Shewanella algae</i> [114]	Au, Pt	Intracellular
<i>Shewanella algae</i> [115]	Au	Extra/Intracellular
<i>Shewanella algae</i> [91]	Au	Intracellular
<i>Shewanella oneidensis</i> [116]	UO ₂	Extracellular
<i>Shewanella oneidensis</i> [116]	U (IV)	Extracellular
<i>Shewanella sp.</i> [117]	AsS	Extracellular
<i>Staphylococcus aureus</i> [118]	Ag	Extracellular
<i>Stenotrophomonas maltophilia</i> [119]	Au	Intracellular
<i>Stereum hirsutum</i> [120]	Cu, CuO	Extracellular
<i>Streptomyces sp.</i> [121]	Ag	Extracellular
<i>Streptomyces sp.</i> [55]	Cu	Extracellular
<i>Streptomyces sp.</i> [122]	Mn and Zn	Intracellular
<i>Streptomyces sp.</i> [123]	Ag	Extracellular
<i>Streptomyces sp.</i> [124]	Ag	Extracellular

<i>Streptomyces spp.</i> [125]	Ag	Extracellular
<i>Thermoanaerobacter sp.</i> [126]	Cu	Extracellular
<i>Thermomonospora sp.</i> [106]	Gold	Extracellular
<i>Thermomonospora sp.</i> [106]	Au	Extracellular
<i>Thermomonospora sp.</i> [106]	Au	Extracellular
<i>Thermomonospora sp.</i> [127]	Au	Extracellular
<i>Thiobacillus thioparus</i> [128]	Fe ₂ O ₃	Not specified
<i>Trichoderma viride</i> [129]	Ag	Extracellular
<i>Verticillium luteoalbum</i> [130]	Au	Extra/Intracellular
<i>Weissella oryzaecastro</i> [24]	Ag	Intracellular

Table 1: The table reports the biosynthesis of NP from different microbial candidates, according to the method and procedures found in the literature.

The Biological Synthesis of Nanoparticles

NPs biosynthesis is carried out by culturing microorganisms in specific nutrient media containing the corresponding ions. Depending on the localization site, the synthesis of NPs by microorganisms, in particular bacteria, is classified into intracellular and extracellular [131]. Metal ions enter the bacterial cell through ion channels, through active transport, endocytosis, or penetration through the lipid membrane [132]. The process of intracellular synthesis involves the process of trapping, bio reduction, and capping of various NPs [133]. Extracellular synthesis consists of enzyme secretion, bio reduction and particle capping [10]. Most published reports [15] have argued that extracellular nanoparticle synthesis is preferable because low-flow and purification processes are easier compared to intracellular methods. Most published works [134] argue that extracellular nanoparticle synthesis is preferable because downstream and purification processes are easier than intracellular methods. The enzyme nitrate reductase is one of the most widely used enzymes, and it is believed to be involved for the synthesis of NPs such silver and gold NPs. Individual enzymes play an important role in the transport of electrons from donors to the positive metal ion during in the bio reduction process [135].

Extracellular Mechanism

As for extracellular biosynthesis, a general scheme can be followed. Due to the production of the extracellular reductase enzyme in the cell-free supernatant, enzymatic bio-reduction of metal ions occurs within the cell-free supernatant with the end result of NP formation within a cell-free supernatant. The test strain is grown in appropriate media and incubated at 37 °C on an orbital shaker at 150 rpm. The broth is centrifuged after incubation, and the supernatant is used to create NPs. The supernatant is transferred to separate

reaction vessels containing appropriate concentrations of metal ions and incubated for 72 hours [136]. The presence of NPs in the solution is indicated by a color shift in the reaction mixture, and the bio reduction of silver ions in the solution is controlled by sampling the aqueous solution and measuring the absorption spectrum with a UV-visible spectrophotometer. Silver's morphology and uniformity [137].

Intracellular Mechanism

As far as intracellular biosynthesis is concerned, the mechanism is different from the previous one, but it is always possible to follow a general scheme. Given the intracellular mechanism, an entrapment of positively charged metal ions occurs within the negatively charged cell wall, subsequently an enzymatic bio-reduction of the metal ions occurs within the cell wall with formation of nanoclusters and, as a final step, the NPs are widespread in the cell wall. The culture is grown in appropriate liquid media and incubated at the proper temperature on a shaker. The flask is held at a static temperature after incubation to enable the biomass to settle, after which the supernatant is discarded and sterile distilled water is added to wash the cells [137]. The flask is held at room temperature for 30 minutes to settle the biomass, after which the supernatant is discarded once more. The biomass is then centrifuged for 10 minutes to isolate it from the sterile distilled water. Wet biomass is exposed to 50 mL of sterilized aqueous metals solution at different dilutions and incubated on shaker at appropriate temperature until visual color change is detected. Silver NPs are formed when the color changes from pale yellow to brownish, gold NPs are formed when the color changes from pale yellow to pinkish, and manganese and zinc NPs are formed when the color changes from whitish yellow to yellow [138]. In summary, extracellular NP synthesis involves trapping metal ions on the cell surface and reducing them in the presence

of enzymes, while intracellular NP synthesis involves carrying metal ions into the microbial cell and creating NPs in the presence of enzymes [28]. Drug carriers for targeted delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, separation science, and magnetic resonance imaging have all allowed use of the biosynthesized NPs [139-141].

Learning, Issues, and Possibilities for Large-Scale Applicability

In vivo microbial nano biosynthesis, as well as the ability to track and adjust nanomaterial properties, pose a concrete potential for future growth and applications in biosensorics and biomedical fields [142]. Bacteria have shown the ability to synthesize nanomaterials both extracellularly and intracellularly. In terms of metal NP dispersity and purification, these mechanisms typically produce opposite advantages and disadvantages [143]. Extracellularly produced NPs are more polydisperse (having a wide range of sizes) than intracellularly

produced NPs. Extracellular nanomaterial processing, on the other hand, necessitates less downstream extraction/purification steps (e.g., ultrasound treatment and detergent use) [144]. As a result, the extracellular mediated synthesis mentioned for yeast and molds can greatly simplify purification steps while also allowing microorganisms to be reused for additional biosynthesis cycles [145]. However, the characterization and identification of the enzymes responsible for nano biosynthesis in molds is still incomplete [146]. Carbon dioxide (as a carbon source), light (as an energy source), inorganic nutrients, and water are all used in the photoautotrophic metabolism of microalgae and cyanobacteria. This condition lowers the cost of culture media (when compared to culture media used for the growth of bacteria, yeasts, and molds) and can help drive potential scaling-up from the laboratory to the industrial scale, including through the design and production of solar photobioreactors for the fixation (and reduction) of atmospheric CO₂ [142].

Cell Culture Conditions for the Synthesis of Nanoparticles

To avoid limiting the applications of microbial biosynthetic nanomaterials, the costs of culture media for microbial growth should be seriously considered for potential large-scale productions. Bacterial nanocellulose is one current example, whose applications are still limited to a few biomedical devices, owing to the high cost of culture medium [142,147]. Furthermore, for monitoring, tuning, and improving the characteristics of microbial biosynthesized nanomaterials, optimization and standardization of

microbial cell culture growth protocols and modifications to culture conditions are critical. The impact of physicochemical parameters in cell culture operational setup on nanomaterials biosynthesis has previously been discussed. I microbial cell concentration; (ii) precursor concentration; (iii) pH; and (iv) temperature are some of these variables. The optimal pH, temperature, and NaCl concentration for achieving high purity and high synthesis rate of cadmium selenide NPs by the bacterium *Pseudomonas aeruginosa* strain RB have been investigated. Surprisingly, the results of this study revealed that the best conditions for NP synthesis did not correspond to the best conditions for *Pseudomonas aeruginosa* strain RB development [148]. For the bacterium *E. coli* strain DH5, the effects of precursor concentration, temperature, and pH on silver nanoparticle synthesis and particle sizes have been identified [149]. Other recent examples include: I the temperature dependence of the size and monodispersed of silver NPs biosynthesis by the mold *Trichoderma viride*; and (ii) the temperature dependence of the size and monodispersed of silver NPs biosynthesis by the mold *Trichoderma viride* [150], (ii) the effect of yeast on the form and scale of gold nanostructures synthesized *Yarrowia lipolytica* strain NCIM 3589 by altering the proportion of cell concentration to precursor gold salt concentration, (ii) the effect of temperature on gold nanostructures release from the cell wall into the aqueous phase, and regulation of bacterial growth kinetics of *Morganella psychrobacterium* [81,142,151]. These results have been ascribed to the activation of enzyme (s) involved in biosynthetic pathway [142,152]. The optimization of frustule morphological properties (e.g., pore sizes and pore density) has been explored by adjusting operational parameters of the experimental setup (e.g., pH, salinity, temperature, nutrient concentration, precursor Si(OH)₄ concentration, and light regime), despite the fact that controlling the biomineralization process remains a challenge [153]. Researchers discovered that when *Coccolithus wailesii* frustules were exposed to sublethal nickel concentrations, their pore sizes changed [142]. It was recently reported that frustules or other biomineralized structures can be chemically modified in vivo. By adding given precursors to the culture medium at sublethal concentrations, these in vivo chemical modifications result in inorganic elements/compounds doping of biomineralized structures. *Pinnularia* sp. frustules or diatoms have been doped in some studies. Frustules of *Thalassiosira weissflogii* with titania (TiO₂) [154] by attaching Ge(OH)₄ or GeO₂ to the diatom culture medium at photobioreactor scale-productions, nano biosynthesis containing Si-Ge oxides nano comb in diatom *Nitzschia* and *Pinnularia* sp. [142]. In comparison to diatoms, the calcareous-based shell of marine protozoa foraminifera has not been thoroughly investigated for nanotechnological applications. The inclusion of fluorescent magnetite NPs within the calcite skeletal structure of the unicellular

organism foraminifer *Amphistrigina lesson* resulted in the in vivo preparation of a bionic material. The natural biomineralization mechanism of growth in the presence of magnetic NPs functionalized with a hydroxylated dextran shell was exploited in this in vitro synthetic approach [142].

Application of Bacterial NPs

In the biomedical field, NPs have been studied for antimicrobial, biosensing, imaging and drug delivery applications [155]. Overall, some researchers are interested in NPs because their syntheses are less harmful to the environment, bacteria produce more evenly distributed NPs, and some of them can be easily biodegradable. Despite the fact that a number of studies have been performed on the use of NPs [156]. The emergence of drug-resistant bacteria and the rising incidence of hospital infection outbreaks has sparked renewed interest in NPs. NPs have been widely used in many fields due to their excellent antimicrobial resistance properties [157]. Each form of NP has its own collection of advantages and distribution patterns. Factors including average particle size, shape, specific surface area, and surface curvature affect behaviour and antibacterial mechanism. In reality, the use of nanoparticles in the fight against bacteria has resulted in a decrease in bacterial infection [158].

Antimicrobial uses of Biosynthesized Nanomaterials in Medicine

The emergence of antibiotic-resistant pathogenic strains has resulted in an increment in the amount of infectious diseases linked to these bacteria. Microbes develop resistance to antibiotics after being exposed to them repeatedly for several generations [159]. To resist antibiotics, they have evolved complex resistance mechanisms such as inactivation, alteration of the target site, and metabolic pathway transformation [160]. As a result, due to a lack of time, alternative antibiotics with strong bactericidal and bacteriostatic activities are requested. Microbial NPs have been discovered to have potent antibacterial properties. NPs' performance is likely due to their wider surface area, which allows for better contact with microorganisms. NPs stick to the cell membrane and then enter the cell by communicating with DNA, disrupting the replication process or attacking pathogens' respiratory chains. Pathogenic bacteria such as *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were found to have a similar bactericidal function as silver NPs obtained from the endophytic bacterium *Bacillus cereus* [161]. Another mechanism of NP antimicrobial activity is structural damage to cellular membranes caused by the forming of pits, which is accompanied by the degradation of cellular components and finally death [162]. Furthermore, when NPs are used in conjunction with traditional antibiotics,

their antimicrobial efficacy can be increased by a factor of ten. Antibiotics including certain nitrofurantoin, carbenicillin, and ciprofloxacin are shown to have a synergistic effect with AgNPs expressed by *R. stolonifer* against ESBL-strains of Enterobacteriaceae. Carbenicillin and ciprofloxacin saw 33.56 percent and 30.53 percent increases, respectively, while nitrofurantoin showed a 50 percent rise [159]. In another study, the antibiotics vancomycin, penicillin G, lincomycin, novobiocin, rifampicin, and oleandomycin were found to be more effective against pathogenic strains of *Vibrio parahaemolyticus*, *Bacillus cereus*, *Salmonella enterica*, *Escherichia coli*, *Bacillus anthracis*, and *Candida albicans* when combined with silver NPs provided by *Brevibacterium frigoriflorum* [10]. Despite the fact that several studies have revealed the antimicrobial ability of AgNPs generated by microbes, only a few studies have recorded antimicrobial activities of substances other than AgNPs. Additionally, the biosynthesis of titanium oxide NPs from *Planomicrobium* sp. was confirmed and identified, as well as their extensive antimicrobial activity against *B. Subtilis*, *K. planticola*, and *A. niger*. Antimicrobial properties of iron NPs produced by *Fusarium oxysporum* against *Bacillus*, *E. coli*, and *Staphylococcus* sps. The respiration process of pathogenic microorganisms was disrupted by small iron NPs that reduced oxygen supply [142]. Biosynthesized silver NPs have well-known antimicrobial properties against pathogenic microorganisms [142,159]. Other biologically synthesized NPs' antimicrobial activities and mechanisms of action, on the other hand, have yet to be identified.

Antitumor and Anticancer Properties of NPs

In recent years, cancer has been one of the leading causes of death all over the world. Cancer illnesses are a category of diseases characterized by uncontrollable growth of growth cells and delayed or absent apoptosis. Radiation, chemotherapy, and surgery are all traditional cancer treatments that have side effects [159]. Furthermore, early diagnostics and localized drug delivery to a specific organ are still in their infancy [163]. As a result of these limitations, the prevalence of different forms of cancers is increasing by the day. As a result, it is important to seek out logical solutions in order to solve these problems. Nanomedicine has been used to successfully detect tumors, administer targeted drugs, and treat them [134,163]. Because of their inherent advantages, biosynthesized NPs have the ability to promote molecular interactions and cross biological boundaries without harming healthy cells. Researchers tested the anticancer properties of platinum NPs made by *Saccharomyces boulardii* against A431 and MCF-7 cell lines in vitro. Breast cancer is one of the leading causes of death in women. Silver nanoparticles derived from *Cryptococcus laurentii* were found to have antitumor activity in both normal and cancer cell lines. Apoptosis, viability, and endocytic activity of cancer

cell lines were all affected by biosynthesized AgNPs [159]. Besides, efficacy of AgNPs was found to be proportional to endocytosis activity of cancer cells [159]. Selenium is a trace material with cancer-fighting properties. The anticancer properties of selenium nanorods (SeNrs) derived from *Streptomyces bikiniensis* were discovered in MCF-7 and Hep-G2 human cancer cells. The anticancer activity of these compounds was thought to be due to the mobilization of chromatin-bound copper, followed by pro-oxidant action, and the death of HepG2 and MCF-7 cells [105]. In another study, gold NPs developed by *Streptomyces cyaneus* were tested in vitro against human liver and breast carcinoma cells, HEPG-2 and MCF-7, respectively, for anticancer activity. AuNPs mediated cytokinesis detention, induced mitochondrial apoptosis, localized nuclei of cells, causing DNA impairment, and stimulated mitochondrial apoptosis [164]. Biologically synthesized NPs have been shown to have anticancer activity in vitro in a number of studies, but their commercialization remains a mystery. Furthermore, defining toxicity and immune response prior to their use for diagnostic and treatment purposes is crucial.

In Drug Delivery Systems, NPs are used to Transport Drugs

Targeted drug delivery, drug bioavailability, bioactivity, and stability have all been found to be more efficient with NPs. Water-soluble polymers, such as natural antibodies and synthetic polymers, nanospheres of natural or synthetic polymers, liposomes consisting of vesicles with bilayer walls, polymeric micelles, and emulsions stabilized by amphipathic surface coatings are examples of drug delivery nano-vehicles [159]. The surface properties and small size of NPs help pharmacologically active compounds penetrate all of the human body's cellular compartments at the fastest possible time. When drugs form conjugates with NPs by encapsulation or different linker molecules, toxicity can be regulated. They help patients by reducing the toxic side effects of drug carrier systems while maintaining therapeutic effects. Unique targeting and biodistribution, biocompatibility, and protection are all advantages of NPs over traditional drug delivery systems [165]. Biosynthesized ZnO NPs loaded with anthraquinone by *Rhodococcus pyridinivorans* demonstrated concentration-dependent cytotoxicity against HT-29 colon carcinoma cells in a dose-dependent manner, suggesting that they could be used as an anticancer drug delivery vehicle [166]. Hydrophilic NPs have the potential to improve drug absorption and diffusion within cells. The anticancer drug doxorubicin was conjugated to multi-shaped gold NPs extracted from the fungus *Helminthosporium solani*. The conjugated drug was taken into HEK293 cells more easily and had cytotoxicity equivalent to doxorubicin. [167]. Another research used gadolinium oxide nanoparticles made by the thermophilic fungus *Humicola sp.* to bioconjugate

with the anticancer drug Taxol to improve its effectiveness against antitumor cells [159]. Similarly, *Humicola*-derived gold NPs conjugated with doxorubicin have been used in hepatic cancer care as a targeted drug delivery system [168]. Biogenic nanocrystals have been revealed to be promising drug delivery vehicles, but their toxicity to other cellular compartments and biocompatibility must be assessed first.

In The Field of Diagnosis

Infectious disease prevalence is one of the leading causes of morbidity and mortality in developing countries [159]. Though infectious diseases begin in a small area, they can rapidly spread from one region to another, leading to a global pandemic. Rapid disease detection necessitates the use of cutting-edge diagnostic technology [9]. Culturing microorganisms, microscopy, biochemical experiments, immunoassays, and molecular diagnostics are all traditional methods of disease detection and diagnosis. Nonetheless, these approaches are inconvenient, time-consuming, and exhausting. Nanomaterials include pathogen detection tools that are fast, precise, reliable, easy to use, and ultrasensitive. Metallic nanoparticles, fluorescent nanoparticles (quantum dots and dye-loaded NPs), and magnetic nanoparticles have all been successfully used to photograph, monitor, and classify a variety of pathogens [141]. The role of biosynthesized nanomaterials in diagnostics, on the other hand, is poorly understood. The ability of *Candida albicans*-mediated biosynthesized gold nanoparticles to probe liver cancer cells by binding with liver cancer cell surface-specific antibodies was investigated in a study. The antibody-conjugated gold particles bind specifically to the surface antigens of affected cells, allowing them to differentiate between normal and cancer cells without a doubt [9]. The use of microbially synthesized nanomaterials in diagnostics is still in its early stages, and further progress in this area will provide a more viable future prospect.

Conclusion

Specific resistance mechanisms, such as efflux pumps, metal efflux systems, metal inactivation and complexation, metal permeability and the lack of specific metal transport systems, alteration of solubility and toxicity by changes in the redox state of metal ions, and extracellular precipitation of metals, can help bacteria survive and develop in stressful situations and volatilization of toxic metals by enzymatic reactions [1]. The stability and aggregation of biosynthesized NPs, crystal growth control, form, scale, and size distribution are the most significant problems encountered with bio-based approaches. Furthermore, biologically synthesized NPs are more polydisperse than chemically synthesized NPs. Important parameters that regulate organism development, cellular activities, and enzymatic processes can be optimized

to control the properties of NPs [15,152]. As a result, more detailed research is needed to understand the exact reaction mechanisms and identify the enzymes and proteins involved in the biosynthesis of NPs. The use of bacteria to synthesize NP on a large scale is appealing because it eliminates the need for dangerous, harmful, and costly chemical materials in the synthesis and stabilization processes. These natural technologies appear to be capable of producing stable NPs with well-defined sizes, morphologies, and compositions by optimizing reaction conditions and selecting the best bacteria [1,22]. The most significant drawbacks of bacteria-based NP biosynthesis are the time-consuming purification steps and a lack of knowledge of the mechanisms. Controlling the shape and size of the particles, as well as achieving monodispersity in the solution process, are two of the most common challenges in NP biosynthesis [1]. Before this biological approach becomes a viable and competitive alternative for the industrial synthesis of NP, it appears that many significant technological challenges must be addressed. Increasing the scale of manufacturing at the production level is one of the most difficult challenges. Furthermore, little is known about the mechanistic aspects of NP biosynthesis, and knowledge of these aspects is needed for the efficient and logical production of NP biosynthesis. The following are some of the important factors to consider in a well-defined NP production process:

- **Collection of the greatest bacteria:** The researchers focused on some essential intrinsic properties of the bacteria, such as growth rate, enzymatic activities, and biochemical pathways, in order to choose the best candidates. The selection of a good candidate for nanoparticle development should be based on the application for which the NPs will be used. It may be necessary, for example, to synthesize NP in smaller sizes or with complex forms, or it may be necessary to synthesize NP in a shorter amount of time.
- **Range of the biocatalyst state:** Biocatalysts, which can be whole cells, crude enzymes, or distilled enzymes, are the key agents in the synthesis of NPs. It appears that using the cell's culture supernatant or cell extract will speed up the reaction. These NPs, on the other hand, did not demonstrate long-term stability. Furthermore, in the case of intracellular derived NPs, the release of NP from cells was a significant aspect that could be calculated. The majority of the responses responsible for NP synthesis tend to be bio-reductions. We need stoichiometric amounts of coenzymes (e.g., NADH, NADPH, FAD, etc.) in bio reduction processes. Since whole cells are more costly, they are favored because the coenzymes can be recycled in the pathways into live whole cells.

The conditions are ideal for cell growth and enzyme activity. Larger quantities of enzymes are needed, which

can be found by synthesizing more biomass. As a result, optimizing the rising conditions is extremely important. It is essential to optimize nutrients, inoculum size, pH, light, temperature, buffer power, and mixing speed. Induction of the responsible enzymes appears to be essential as well. Subtoxic levels of substrates or associated mixtures present from the start of growth will boost activity. When using whole cells and raw enzymes, the harvest time is crucial. Then, as the organism grows, enzyme activity can need to be regulated.

- **Finest reaction environments:** Harvesting the cells to eliminate unnecessary residual nutrients and metabolites to prevent harmful reactions and provide a safer means of analysis is ideal. Yield and speed of development are critical factors to consider when using bacteria to synthesize NPs on a large scale. Then, in the reaction mixture, we must optimize the bio reduction conditions. The substrate concentration, biocatalyst concentration, electron donor concentration, exposure time, pH, temperature, buffer power, mixing speed, and light must all be optimized. Complementary factors such as visible light or microwave irradiation, as well as boiling, were used to influence the morphology, duration, and rate of the reaction. Highly stable NPs with ideal dimensions and morphologies tend to be obtained by optimizing these essential parameters. Furthermore, the purification, isolation, and stabilization of the NPs generated are critical, and challenges must be overcome in this region. Researchers concentrated their efforts on identifying the best reaction conditions and cellular pathways involved in metal ion bioreduction and NP synthesis.
- **Extraction and purification procedures:** The extraction and purification of metal NPs developed by bacteria for future applications has not been well studied, but research is underway to determine the best methods. Additional processing steps, such as ultrasonic treatment or reaction with suitable detergents, are needed to release the intracellularly formed NPs. The recovery of precious metals from mining waste and metal leachate may be hampered by this. Biomatrix metallic NPs have the potential to serve as catalysts in a variety of chemical reactions. This will make it easier to keep NPs in bioreactors. To extract NPs produced by cells, physic-chemical methods such as freeze-thaw, heating processes, and osmotic shock can be used. However, it appears that these methods will disrupt the structure of NPs, resulting in accumulation, precipitation, and sedimentation. This can alter the shape and size of NPs as well as interfere with their proper properties. Enzymatic lysis of intracellular NP-containing microbial cells can also be used, but this process is costly and unsuitable

for large-scale industrial NP production. Surfactants and organic solvents appear to be viable options for NP extraction and stabilization, but these chemicals are toxic, costly, and risky. It should be noted that in the case of extracellular NP development, the centrifuge can be used to extract and purify the NPs, but aggregation can occur.

- **Maintenance of the formed NPs:** Even after many weeks at room temperature, the NPs provided by these biocompatible methods maintained an interesting permanency without aggregation. The microorganisms' secreted proteins and enzymes may be responsible for the NPs' stability. As a result, it appears that these environmentally friendly methods can be used to make highly stable NPs.

The laboratory technique is being scaled up to an industrial scale. Improved NP biosynthesis can be achieved by optimizing reaction conditions. When considering critical aspects such as types of organisms, hereditary and genetic properties of organisms, optimal conditions for cell growth and enzymatic activity, optimal reaction conditions, and selection of the biocatalyst, biological protocols could be used for highly stable and well characterized NP synthesis. Changing the reaction conditions above can be used to determine the size and morphology of the NPs. Crop culture, seed inoculation into biomass, cell harvesting, NPs synthesis by adding metal ions to cells, cell separation by filtration, cell homogenization to separate NPs products, NPs stabilization, product formulation, and quality control are all steps in the industrial-scale synthesis of metal NPs using biomass.

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