



# A Review on Biosynthesis of Zinc Oxide Nano Particles from Microbes

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## Abstract

Nanotechnology is a new technology that has the potential to revolutionize a variety of scientific sectors. Nanoparticles are single particles with a diameter of 1–100 nm that are part of nanomaterials. Nanoparticles have recently become a popular material for developing new cutting-edge applications in communications, energy storage, sensing, data storage, optics, transmission, environmental protection, cosmetics, biology, and medicine due to their important optical, electrical, and magnetic properties. Zinc oxide nanoparticles (ZNPs) are one of the most versatile nanomaterials due to their excellent chemical and thermal robustness. Physical and chemical approaches are available for the synthesis of ZNPs, but biological methods are found to be more eco-friendly and provide a better-defined size and morphology. From a functional and mechanistic standpoint, we have highlighted the possibilities of exploiting the microbial world as nano factories for the production of zinc oxide nanoparticles. Microorganisms have a lot of potential for the biosynthesis of nanoparticles because it is environmentally safe and does not require the use of dangerous chemicals. Microorganisms are inexpensive and do not require a lot of energy. They can also accumulate and detoxify heavy metals using reductase enzymes, which convert metal salts to metal nanoparticles with minimal polydispersity and a narrow size range. If properly utilized, the vast pool of bioresources (microbes and microbial enzymes) could aid biosynthesized NPs in becoming a game changer in the near future. An overview of zinc oxide nanoparticles and their biogenic synthesis is briefly discussed in this review.

## Keywords

Nanotechnology, Biosynthesis, Zinc oxide.

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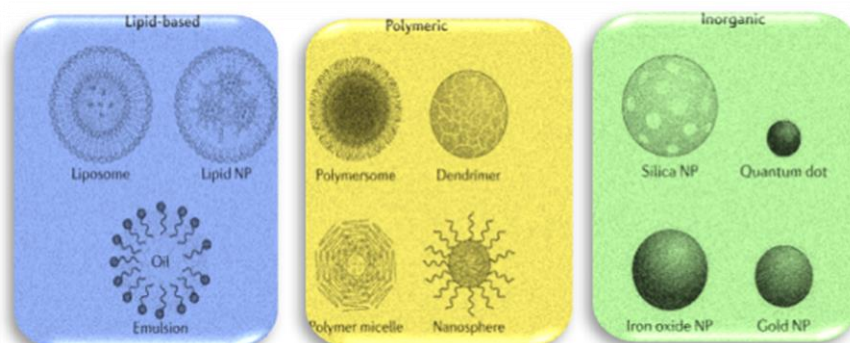
## INTRODUCTION

Nanotechnology has sparked a lot of attention in recent years because of its potential impact on a variety of industries, including energy, medicine, electronics, and space. Across the last decade, research in this sector has increased substantially all over the world. The main activity is the development of new nanometer-sized materials, such as nanoparticles, nanotubes, and nanowires [1]. Nanoparticles (NPs) are nanoscale materials with very small particle sizes ranging from 1 to 100 nm. Nano from the Greek word “nanos”, implies dwarf or

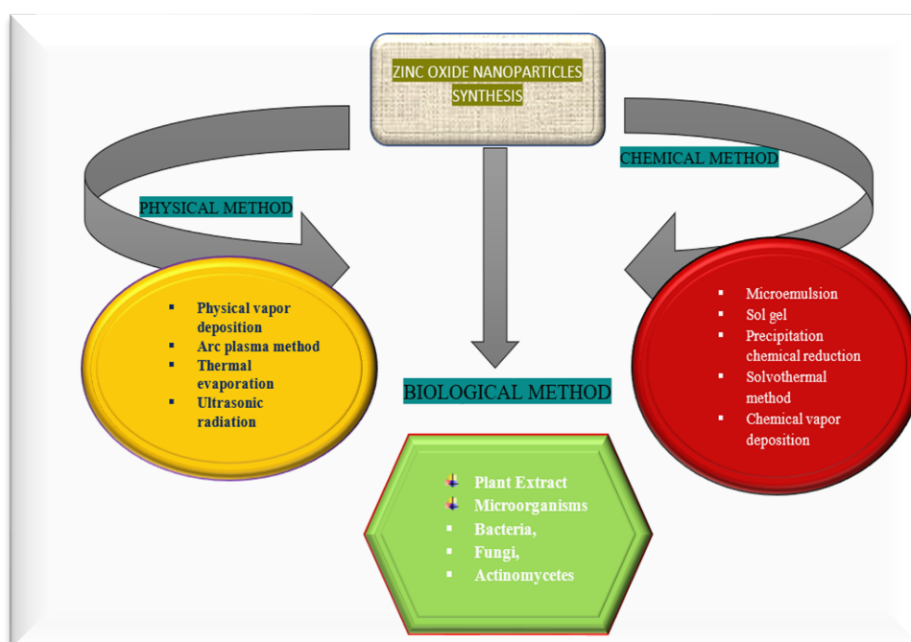
anything smaller [2]. Because of their minute size and high surface area to volume ratio, NPs have unique properties from their bulk counterparts, resulting in considerable variances in attributes [3]. Nanomaterials have better catalytic reactivity, thermal conductivity, nonlinear optical performance, and chemical stability due to their large surface area to volume ratio [4]. They're employed in a variety of processes, including material science, agriculture, food processing, cosmetics, medicine, and diagnostics [5]. Polymeric NPs, lipid-based NPs, inorganic NPs, bio-inspired NPs, and hybrid NPs, are

five broad groups of materials that can be classified based on their kind[6].( Figure 1)

**Figure 1-Different categories of nanoparticle.**



**Figure 2-Methods ZnO nanoparticles Synthesis**



The bottom-up and top-down approaches to nanomaterial production are the two most used methods.[7] The first make nanostructures out of atoms and molecules, which can be made through chemical synthesis, biological techniques, or controlled deposition and growth. The top-down strategy, on the other hand, refers to slicing or cutting bulk material to obtain nano-sized particles [8]. Physical nanoparticle synthesis methods such as “vapor condensation”, “interferometric” “lithography”, “physical” “fragmentation”, and “amorphous crystallization” necessitate a large amount of space for machine setup, expensive equipment, and high temperature and pressure conditions for the nanoparticle synthesis process. “Sol-gel” method, “solution” “evaporation” method, and reduction of precursor molecules like silver

nitrate ( $\text{AgNO}_3$ ), gold chloride ( $\text{HAuCl}_4$ ), and zinc acetate dihydrate ( $\text{C}_4\text{H}_6\text{O}_4\text{Zn} \cdot 2\text{H}_2\text{O}$ ) employing non-eco-friendly hazardous chemicals are some of the chemical approaches to nanoparticle manufacturing [4]. (Figure 2)

These types of preparations need a lot of energy and contain harmful and hazardous substances, which might cause biological problems [9]. Green technologies involving the integration of diverse plant components, bacteria, fungi, and algae have largely superseded physical and chemical approaches for the creation of nanostructures [10]. Microbes are thought to be nontoxic nanoparticle synthesis factories. Metal/metal oxide is formed by both prokaryotes and eukaryotes, particularly  $\text{ZnO}$ [11]. The manufacturing of nanoparticles in biological systems, with minimum environmental

dangers and simple and eco-friendly (biocompatible) production processes, has been chosen by researchers. Nanoparticle attributes (size, optical properties, electrical properties, and so on) can be created by altering biosynthetic factors like pH, temperature, substrate concentration and adjusting the substrate's exposure time [2]. After NPs are synthesized, they are characterized using a variety of microscopic and spectroscopic techniques to determine their unique physio-chemical properties. In biological applications, determining the exact morphological (size, shape, and particle distribution) and physicochemical features of NPs is crucial [12]. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), and high-resolution transmission electron microscopy (HR-TEM) are the most regularly utilized microscopic techniques to evaluate the size and morphological aspects of NPs [13] [14]. Whereas UV-visible spectroscopy (UV-Vis), energy dispersive spectroscopy (EDS), selected area electron diffraction (SAED), dynamic light scattering (DLS), X-ray diffraction (XRD), and X-ray photo-electron spectroscopy (XPS) were used to investigate the structure, composition, and crystallinity of synthesized NPs [15] [16].

### **Zinc oxide Nanoparticles**

Zinc (Zn) and its oxide are one of the most intriguing and promising metallic nanostructures (ZnO). Zinc is a moderately active element and a strong reducing agent; it can rapidly oxidize, generating zinc oxide, according to its reduction potential [17]. By employing microbial cells or enzymes, protein, and other molecular compounds in either an intracellular or extracellular pathway, both prokaryotes and eukaryotes, synthesize ZnO NPs [1]. Both metal and metal oxide nanoparticles have significant antioxidant and antibacterial capabilities, and are commonly employed for pathogenic microorganism detection and cancer progression diagnosis [18]. Zinc is a crucial nutrient in living beings. They are inorganic white powdery substance which is insoluble in water.

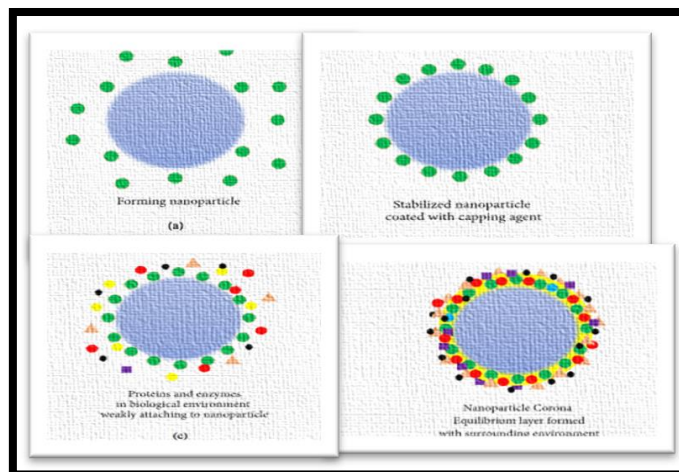
Zinc oxide is classed as a semiconductor in group II-VI in materials science, with a covalence that falls between ionic and covalent semiconductors. It is appealing for prospective usage in electronics, optoelectronics, and laser technology because of its broad energy band (3.37 eV), high bond energy (60 meV), and strong thermal and mechanical stability at ambient temperature. [19][20][21]. Because of its piezoelectric and pyroelectric capabilities, ZnO can be employed as a hydrogen sensor, converter, energy generator, and photocatalyst [22][23]. The

chemical, mechanical, electrical, structural, morphological, and optical properties of NPs materials can be altered by decreasing them to the nanoscale. These changed properties allow them to interact with cell biomolecules uniquely, making the physical translocation of NPs into inner cellular structures easier. [24]. Many organic contaminants have been degraded with the use of zinc oxide. Several methods have been used to make ZnO nanoparticles, including sol-gel, solvothermal, direct precipitation, solution combustion and hydrothermal. Most of these methods necessitate time-consuming procedures, costly substrates, specialized equipment, strict testing conditions and hazardous impact on the environment [25]. Biological methods that use plant extracts or microorganisms are preferred as an alternative. Plant extract contains phytochemicals that function as both a reducing and stabilizing agent. Plant parts such as leaves, seeds, stems, fruits, and stems have also been employed as a fuel source for the production of ZnO nanoparticles [25]. With the help of enzymes and other biomolecule components secreted or synthesized by the microorganisms, the bacteria work as a miniature nano-factory, reducing metal ions into metal NPs [3]. At low concentrations, metal oxide nanoparticles such as zinc (Zn), calcium (Ca), and magnesium (Mg) oxide nanoparticles strongly limit microbial development. [26] ZnO is known to suppress the growth of a wide range of bacteria, according to prior research. [27] [28] [29] Biogenic synthesis with bacteria has an advantage over plant synthesis since microorganisms are easily procured [3].

### **Biosynthesis of ZnO nanoparticles**

Many microorganisms, including bacteria, fungi, actinomycetes, and yeast, are capable of generating nanoparticles, mineral crystals, and metallic nanoparticles both intracellularly and extracellularly [30]. Nanoparticle synthesis with bacteria and fungi has sparked more interest than synthesis with actinomycetes and yeast, because of established technology [1]. The production of different sizes and shapes with mono- and poly dispersed NPs was attributed to the multiple organic components secreted in the suspension or growing medium [31]. Furthermore, the protein released by bacteria may serve as a capping agent, ensuring the stability of NP synthesis. (Figure 3) Microorganisms biosynthesize nanoparticles by grabbing target ions from their surroundings and then converting the metal ions into the element metal using enzymes produced by cell activities. According to where nanoparticles are generated, they can be divided into intracellular and extracellular synthesis [32] [33][34].

**Figure 3-Subsequent formation of nanoparticle corona: (a) biosynthesis of naked particle, (b) stabilization of nanoparticle by capping agents in algal extract solution, (c) naked nanoparticle in biological environment, and (d) proteins and enzymes firmly attached to nanoparticle surface to form an equilibrium layer with the surrounding environment. (Derek Fawcett *et al*)**

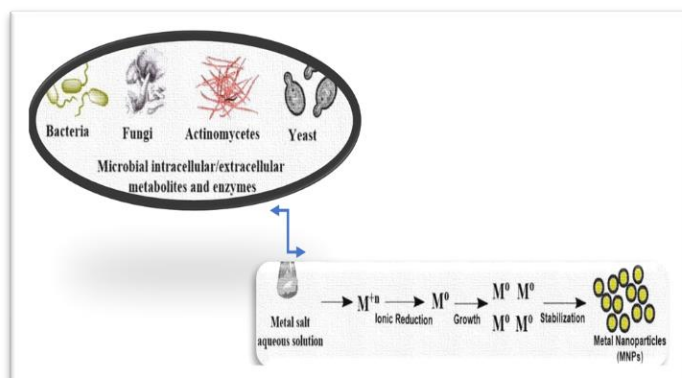


The biological synthesis of metal and metal oxide NPs necessitates the use of metal precursors, which are typically given as soluble salts that precipitate in a suspension containing microbial cells or biological component extracts from microbes. Depending on the culture conditions, the synthesis reaction is usually finished in minutes or hours, resulting in white deposition in the bottom flasks or changes in the color of the suspensions indicating good transformation. Temperature, pH, metal precursor concentration, and reaction time are all key factors in determining the rate of synthesis, yield, and morphologies of NPs [35]. Microbes produce oxide nanoparticles, which are a common type of compound nanoparticle. There are two types of biosynthesized oxide nanoparticles: Nonmagnetic oxide nanoparticles and magnetic oxide nanoparticles [34].

#### Microbial mediated synthesis of ZnO NPs

It's difficult to make uniform, ultrafine, well-dispersed functional nanoparticles in a regulated way under normal conditions [36] [37]. Biological resources have been extensively investigated in recent years for the biosynthesis of metal or metal-based nanoparticles. These biological resources are usually a versatile, cost-effective, and environmentally benign way to make metal nanoparticles[37] [38].The use of plant extracts and microorganisms such as bacteria, yeasts, and fungi in bio-based metal nanoparticle synthesis methods is based on the systematic use of plant extracts and microorganisms such as bacteria, yeasts, and fungi.(Figure 4) Because biomolecules can accomplish this job on their own, no capping or stabilizing chemicals are used in biological synthesis[39].[40] [41]

**Figure 4-Microbial synthesis of Metal Nanoparticles**-Mechanisms of microbes mediated synthesis of NPs



There are two basic types of microbial synthesis: intracellular and extracellular nanoparticle formation. Transporting ions inside the bacteria cell

to create NPs in the presence of enzymes, coenzymes, ions, and other substances are known as intracellular production. Extracellular nanoparticle

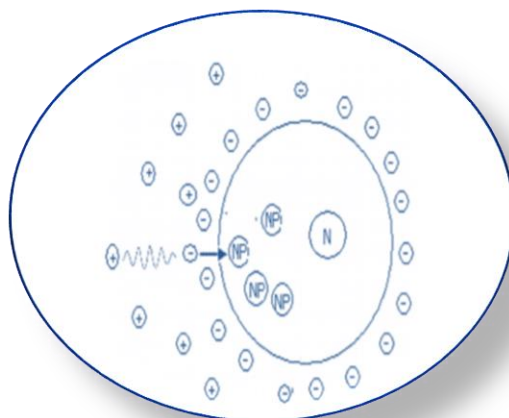


creation involves trapping metal ions on the surface of cells and interacting zinc with bioactive components generated by microbial cells (e.g. reductase, polysaccharides, glycoproteins), resulting in Zn/ZnO nanoparticles [1]. In comparison to the intracellular pathway, extracellular synthesis is more favorable and has been frequently used. This is primarily because it can be used to synthesize huge quantities and requires simple downstream processing that removes numerous synthesis processes, as well as facile separation and industrialization. pH has been shown to affect the intracellular, extracellular, and surface generation of NPs.

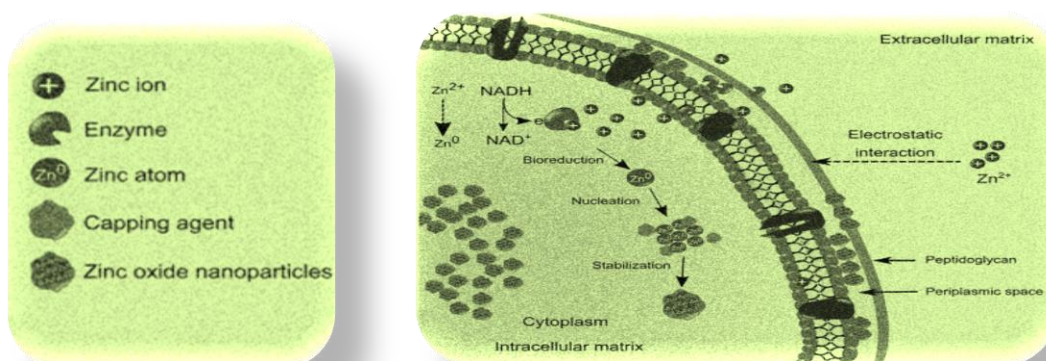
### Intracellular mechanisms of microbial synthesis

With different biological agents, the mechanism for intracellular and extracellular nanoparticle differs. In the intracellular technique, the microbial cell uses a unique ion transport system. The cell wall of microorganisms plays a significant role in the intracellular creation of nanoparticles. The mechanism involves the positive charge of metal ions interacting with the negative charge of the cell wall via electrostatic contact. The ions are reduced to nanoparticles by enzymes found within the cell wall, and these nanoparticles are dispersed out through the cell wall [42]. (Figure 5, 6)

**Figure 5**-Diagrammatic representation of intracellular synthesis of nanoparticles. N – nucleus, NP – nanoparticles, +ve = ions, -ve = charges on the cell wall.[12]



**Figure 6**- Schematic representation of intracellular synthesis mechanisms of ZnO NPs. [3]

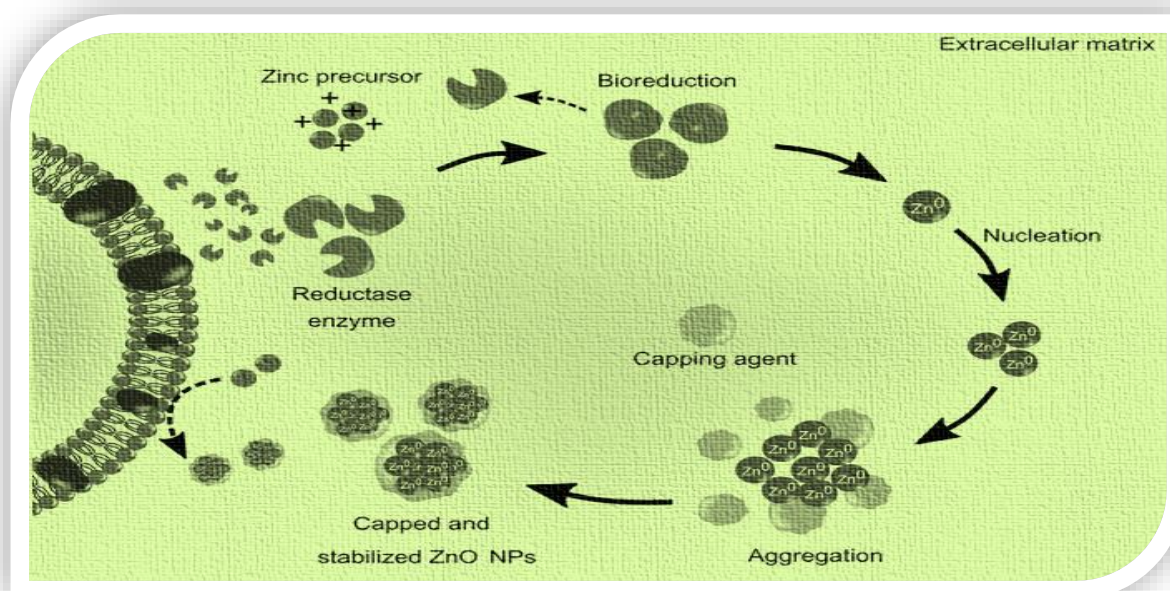


The formation of nanoparticles involves trapping, bioreduction, and capping, according to a stepwise mechanism for intracellular synthesis of nanoparticles were seen in *Verticillium spp.* When metal ions come into touch with the surface of a fungal cell, electrostatic interactions occur, trapping the ions. Metal ions are converted to metal nanoparticles by enzymes present in the cell wall [43]. The earliest phase in the bacteria *Lactobacillus sp.* is the nucleation of metal ion clusters were seen. As a result of this electrostatic interaction between the bacterial cell and the metal clusters, nanoclusters are formed. The nanoclusters of various sizes are then transported through the bacterial cell wall [44]. Metal ions are reduced on the surface of mycelia and the cytoplasmic membrane in actinomycetes, leading to the creation of nanoparticles.[45][46]

### Extracellular mechanisms of microbial synthesis

It is a nitrate reductase enzyme mediated synthesis. The extracellular synthesis pathway entails either enzyme-mediated synthesis on the cell membrane or the release of the enzyme as an extracellular enzyme into the growth medium. The enzyme nitrate reductase catalyzes the conversion of nitrate to nitrite in the nitrogen cycle. For example, the electron transfer from NADH through NADH-dependent reductase, which acts as an electron transporter, started the bio reduction of  $Zn^{2+}$ . As a result,  $Zn^{2+}$  received an electron and was reduced to  $Zn^0$ .  $ZnO$  NPs were formed as a result of this process. The extracellular synthesis pathways are depicted in a diagram (Figure 7).[47] [42]

**Figure 7 Schematic representation of extracellular**  
**8- Microbe-mediated synthesis of metal and metal oxide NP[3]**



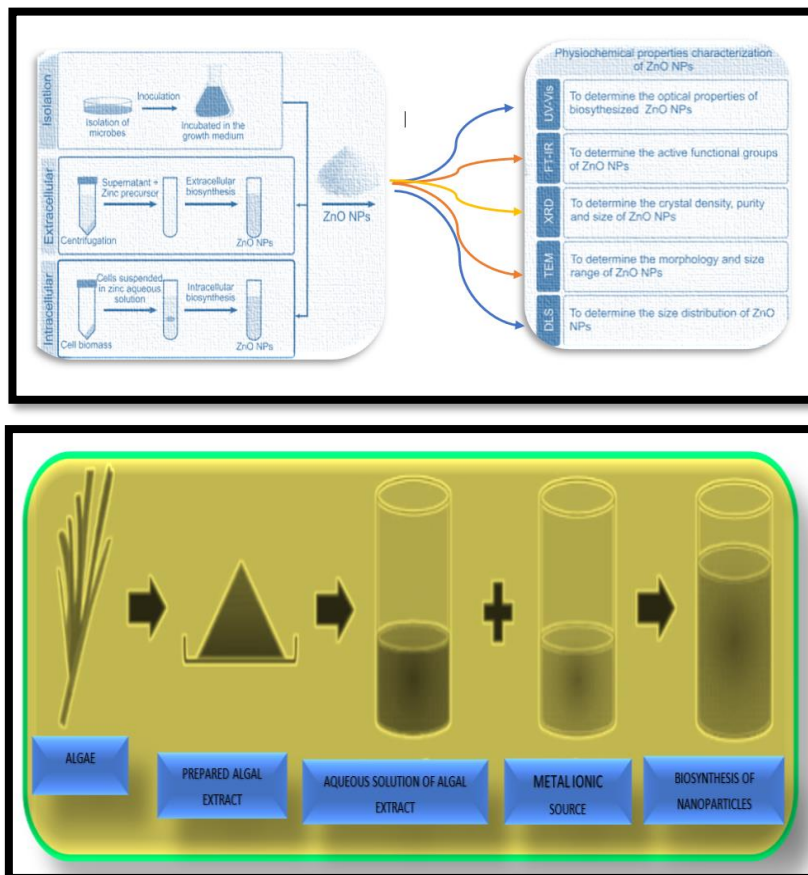
### Bacterial biosynthesis of Zinc Oxide Nanoparticle

Bacteria is thought to be better for producing nanoparticles due to their ease of genetic manipulation and modification, as well as the ability to extrapolate findings from one bacterium to others [48]. It has been proven that both extracellular and intracellular techniques can be used. Extracellular biosynthesis occurs outside the bacterial cell utilizing a variety of approaches, including (a) the use of bacterial biomass, (b) the use of bacterial culture supernatant, and (c) the use of cell free extracts.[49][50]. Probiotic bacteria such as *Lactobacillus sporogens* or *Lactobacillus plantarum* are utilized to make zinc oxide NPs. Lactic acid bacteria are non-toxic and have a negative

electrokinetic potential in synthesis pH, which draws cations easily and aids in the biosynthesis of  $ZnO$  nanoparticles [51]. Biosynthesis has also been reported with other bacteria such as *Aeromonas hydrophila* and *Bacillus cereus* [52]. Selvarajan and Mohanasrinivasan employed  $ZnSO_4 \cdot H_2O$  as a precursor to create pure and crystalline spherical nanoparticles with diameters ranging from 7 to 19 nm [53] [54]. *Lactobacillus sporogens* and zinc chloride were employed as a precursor for the production of  $ZnO$  nanoparticles by Prasad and Anal Jha [55] *Aeromonas hydrophila* bacteria are used in a low-cost and easy biosynthesis of  $ZnO$  NPs [52]. Microbial synthesis of  $ZnO$  NPs necessitates the selection of bacteria, appropriate cell growth

conditions, and biosynthesis pathway (intra- or extracellular).[3] (Figure 8).

**Figure 8-Bottom-up assembly of atoms via biosynthesis using marine algae and metallic ionic sources to form metal or metal oxide nanoparticles.**



Using zinc-tolerant probiotic *L. plantarum* TA4 generated from CFS and CB, a more ecologically friendlier and less expensive technique for the production of ZnO NPs was discovered. The existence of a peak in the UV–Vis-spectrum confirmed the production of ZnO NPs. The reduction process was aided by the presence of hydroxyl, amine, and carboxyl groups from proteins produced from CFS and CB that were discovered on the surface of ZnO NPs using FTIR. HR-TEM micrographs of ZnO NPs-CFS and ZnO NPs-CB displayed flower- and irregular-shaped patterns, respectively, based on DLS analysis of both biosynthesized ZnO NPs. [3]

#### Fungal biosynthesis of Zinc Oxide Nanoparticle

Biosynthesis of various metal nanoparticles has been successfully achieved using myco-nanotechnological techniques. Metal salts are transformed into a less hazardous form in the mycelia during intracellular synthesis, which the fungi can utilize [56]. Fungal extracts are used in extracellular biosynthesis[57]. Furthermore, the fungus was found to be capable of secreting a significant variety of extracellular

redox proteins and enzymes. As a result, the metal ions were reduced into NPs in higher quantities, which is ideal for large-scale production[31]. The fungi released a larger amount of protein in the media, which acted as a capping protein, further binding and coating the NPs surface and conferring stability. Raliya and Tarafdar exhibited the synthesized by utilising *Aspergillus fumigates* TFR-8, which led to the synthesis of NPs with an average diameter of 3.8 nm and high monodispersity (uniformly distributed) particles without agglomeration[3][58]. In addition, *Alternaria* filtrate-cell free supernatant (FCF) was used in the production of ZnO NPs. The presence of protein and other organic components on the ZnO NPs generated was also confirmed by FTIR absorption spectra investigation[3]. This finding supported a previous study that found fungi can produce a large amount of extracellular protein that binds to the surface of NPs to stabilize and prevent them from clumping[59]. ZnO nanoparticles mediated by *Aspergillus niger* showed outstanding antibacterial properties, as well



as the ability to breakdown Bismarck brown dye by up to 90% [60]. X-ray diffraction (XRD), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and energy dispersive x-ray analysis (EDX) techniques were used to describe the nanoparticles, which revealed their spherical shape and an average size of 20.29 nm [61]. Because of their great tolerance to higher metal concentrations, strong binding capacity, and ability in metal bioaccumulation over bacteria, biological production of ZnO NPs utilizing fungi is a potential strategy [62].

#### Algal biosynthesis of Zinc Oxide Nanoparticle

For the production of ZnO nanoparticles, Nagarajan and Kuppusamy looked at three varieties of seaweed: green (*Caulerpa peltata*), red (*Hypnea valentiae*), and brown (*Sargassum myriocystum*). Brown seaweed (*Sargassum myriocystum*) was found to be successful in creating ZnO nanoparticles after examining experimental parameters such as metal ion concentration, seaweed extract concentration, temperature, pH, and reaction time [63]. Spherical, triangular, hexagonal, rod, and rectangular nanoparticles diameters ranging from 76 to 186 nanometers were produced [63]. Azizi *et al.* discovered that bioactive elements such as amino, sulphate, carboxyl, and hydroxyl groups all played a key part during the biosynthesis process utilizing a brown seaweed (*Sargassum muticum*). The generated ZnO nanoparticles had a hexagonal crystal shape and were between 3 and 57 nm in size [64]. Francavilla *et al.* investigated an alternate ZnO biosynthesis process. During a reactive milling process, they used agar produced from red seaweed (*Gracilaria gracilis*) as a sacrificial template material. The zinc precursor Zn (NO<sub>3</sub>)<sub>2</sub> was milled into a highly crystalline hexagonal wurtzite structure with nanoparticles ranging from 18 to 50 nm in size. The porous ZnO nanoparticles were shown to exhibit outstanding photocatalytic capabilities after being calcined at 600°C, and may be employed to decompose aqueous phenol solutions [65].

#### CONCLUSION

In recent years, biogenic production of metal and metal oxide nanoparticles have gained a lot of attention. This fascination stems from the unique features of nanoparticles, which make them ideal for a variety of pharmaceutical and biological applications. This technique, which is based on green chemistry, has the potential to provide an alternative to traditional manufacturing procedures, which often involve harmful chemicals and solvents. However, only a small number of microbes have been examined to date, and the research field is still

unknown. As a result, there are unique prospects for exploring and developing innovative green chemistry-based biogenic techniques for metal and metal oxide nanoparticle manufacturing. Hopefully, this review will spur much-needed research in this sector, which is still relatively young and unexplored.

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