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BIOLOGICAL SYNTHESIS OF METAL BASED NANOPARTICLES INCLUDING BACTERIA AND FUNGI

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ABSTRACT

Nanoparticles have taken attention in recent years and it is essential to produce different types because they have interdisciplinary working areas. Microbial synthesis of nanoparticles is a favourable approach as it is unrealised environmentally friendly, fast, non-toxic, economical, and biocompatible. Biological synthesis pathways use a different microorganism, that is bacteria and fungi, for the purpose of synthesis of different nanoparticles such as Ag, Au, Se, Fe, Zn, Cu, Si, Mg, Pt, and Pd. Microorganisms has the capacity to produce nanoparticles by intracellular or extracellular synthesis pathways. This article highlights the types of biological production or green synthesis of nanoparticle and bio-based production by fungus and bacteria with experimental condition, enzymes that are present within the organism that influence the reduction procedure, along with factor affecting the process with comparison of biological and non-biological production.

KEYWORDS

Biological synthesis, Metal based nanoparticles, Bacteria and Fungus factor affecting.

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INTRODUCTION

NPs can arise naturally, or be synthesised intentionally by physical, chemical, and biological methods as shown in the (Figure No.1). The two methods mentioned as physical, and chemical have disadvantages that is the use of toxic and dangerous chemicals, less efficiency, and greater temperature-energy consumption[1].

Considering the disadvantage, research has focused on the synthesis of NPs using biocompatible, cost-effective, non-toxic, and eco- friendly biological components[3]. This green synthesis gives another advantage as it is generally carried out at room temperature or with less heating. Therefore, the utilisation of NPs synthesized by physicochemical methods in biomedical use has been stopped, especially due to biocompatibility and toxicity[4].

TYPES OF BIOLOGICALSYNTHESIS

Synthesis using plant extracts

The benefit of plants in the synthesis of nanoparticles is quite a few studied areas as compared to the utilisation of microorganisms to give nanoparticles. There are some examples that suggest that plant extracts can be utilised as the synthesis of nanoparticles. To attain gold nanoparticles by geranium plant extract is stated here. Finely crushed leaves are placed into an Erlenmeyer flask and boiled with water just for one minute. Leaves ruptures and cells releases intracellular material.

The solution is cooled down and decanted. This solution is added with HAuCl4 aqueous solution, and nanoparticles of gold start forming within one minute[5].

Bio-based methods

Various reports prevailed within the literature shows that the synthesis of nanoparticles through chemical approaches is expensive and eco unfriendly. Thus, the need for environmentally and economically friendly processes has developed, that do not use toxic chemicals in the synthesis protocols. This has forced researchers to look at the organisms. The capacity of organisms in nanoparticle synthesis is from simple prokaryotic bacterial cells to eukaryotic fungi and plants. Some examples of nanoparticle production have used bacteria for gold, silver, zinc, cadmium, magnetite, and iron NPS; yeasts for lead, silver, and cadmium NPS; fungi for gold, cadmium, and silver NPS; algae for gold and silver NPs; plants for silver, gold, palladium, platinum, zinc oxide, and magnetite NPs[6]. Bio-based protocols can use for the synthesis of well stable and well-characterized NPs when critical aspects, such as inheritable, types of organisms, and genetical properties of organisms, optimal conditions for enzyme activity and cell growth, optimal reaction conditions, and selection of the biocatalyst state must be stated. Sizes and morphologies of the NPS can be controlled by changing some critical conditions, with light, temperature, substrate concentration, pH, buffer strength, electron donor (e.g., glucose or fructose), biomass and substrate concentration, mixing speed, and exposure time[7].

Tollens's method

Aneasy1-step process, Tollens's method, is used for the synthesis of silver NPs along with controlled size. This green synthesis technique includes the reduction of Ag (NH3)2 + (as Tollens‘s reagent) with an aldehyde. In the modified Tollens procedure, silver ions are reduced with the help of saccharides with the presence of ammonia, yielding silver hydrosols (20-50nm), silver nanoparticle films (50-200nm) and silver NPs of different shapes. In this method, the nature of the reducing agent and ammonia concentration plays a vital role in controlling the size and morphology of silver NPs. It was showed that the smallest particles were formed at the less ammonia concentration[8]. Glucose and the less ammonia concentration (5mM) resulted in the minute average particle size of 57nm with an intensity increased of surface plasmon absorbance at 420nm. Moreover, more in NH3 from 0.005M to 0.2M showed in a simultaneous rise in particle size and polydispersity. Silver NPs with controllable sizes were synthesized by reduction of [Ag (NH3)2] + along with glucose, galactose, maltose, and lactose[9].

The nanoparticle synthesis was carried out at different ammonia concentrations (0.005-0.20M) and pH conditions of 11.5-13.0 showing in average particle sizes of 25-450nm. The particle size was increased by increasing (NH3), and the variation in the structure of the pH (particles obtained at pH 11.5 were smaller than those at pH 12.5) and reducing agent (monosaccharides and disaccharides) influenced the particle size. Polydispersity also reduced by lowering the ph. Produced silver NPs were stabilized and protected with sodium dodecyl sulphate (SDS), polyoxymethylene sorbinatemonooleate (Tween 80) and polyvinylpyrrolidone (PVP 360)[10].

Irradiation methods

Silver NPs can be prepared by using a different irradiation method. Laser irradiation by an aqueous solution of surfactant and silver salt can produce silver NPs with proper defined shape and size distribution. Furthermore, the laser was employed in a photo-sensitization synthetic method of synthesizing silver NPs using benzophenone. At lower irradiation times, less laser powers gave silver NPs of about 20nm, while araised irradiation power gave NPs of about 5 nm. Mercury lamps and laser can be used as light sources for the generation of silver NPs. Within visible light irradiation studies, photo-sensitized growth of silver NPs by thiophene (sensitizing dye) and silver nanoparticle generation by illumination of Ag (NH3) + in ethanol have been done[11].

ELECTROCHEMICAL SYNTHETIC METHOD

The electrochemical synthetic method can be employed for synthesis of silver NPs. It is possible to alter particle size by adjusting electrolysis parameters and to make it better the homogeneity for silver NPs with alteration of composition of electrolytic solutions. Polyphenolpy rrole coated silver nano spheroids (3-20nm) can be synthesized by electrochemical reduction within liquid/liquid interface. This nano-compound was prepared by the silver metal ion with aqueous phase and then to the organic phase, where it gives reaction with the pyrrole monomer. In another study, monodisperse silver nano spheroids (1-18nm) was synthesized with the help of electrochemical reduction outside or inside zeolite crystals according to the silver exchange degree of compact zeolite film-modified electrodes[12]. Furthermore, spherical silver NPs (10-20 nm) have narrow size distributions was conveniently synthesized with in aqueous solution by an electrochemical method. Poly N-vinylpyrrolidone was taken as the stabilizer for the silver clusters for the study. Poly N-vinylpyrrolidone guards NPs from agglomeration, potentially reduces silver deposition rate, and enhance silver particle formation rate and silver nucleation. Use of rotating platinum cathode potentially solves the technological difficulty of frequently transferring metallic NPs from cathode vicinity to the bulk solution, reducing the flocculates formation in the vicinity of the cathode, and increase monodisperses of particles. The addition of sodium dodecyl benzene sulfonate to the electrolyte enhances particle size distribution and particle size of silver NPs[13].

Bio-based synthesis

Among other production methods, the green synthesis of NPs with microorganisms has gained a special place due to theireasy cultivation, rapid growth, and ability to grow under ambient pH, temperature, and pressure conditions[14]. As biological agents, viruses[15], bacteria[16], fungi[17], algae[18], protozoa[19] and plant[20] are used in the formation of NPs[19]. Today, the biosynthesis of NPs using microorganisms is studied widely because it is a simple, cost-effective, as well as environmentally friendly method. This approach is also effective for large-scale production of NPs[17].

Microorganisms are crucial nano factories that can accumulate, reduce, and detoxify heavy metals with their various reductase enzymes[21]. Microorganisms act as vital nano factories for synthesis for NPs such as silver, gold, iron, copper, zinc, nickel, palladium, and titanium. These nanoscale substances can be found in various forms and shapes that has application on treatment as mentioned in (Table No.2)[22].

Microorganisms are employed in the synthesis of NPs by intracellular and extracellular synthesis[23]. Extracellular synthesis process is simple, easier, and cost-effective to purify.

Intracellular biosynthesis

The cell walls are negatively charged, positively charged metal ions get deposited on them by electrostatic interactions. NPs are produced by reducing ions by enzymes that is nitrate reductase[24]. Microorganisms that are used in intracellular NP synthesis are grown in a suitable medium, their biomass is collected by centrifugation at the end of incubation and is washed by sterile distilled water, after that the biomass is collected again by centrifugation, and NPs are produced by mixing along with metal salt solution[25].

EXTRACELLULAR BIOSYNTHESIS OF NPS

Nitrate reductase is present in the cell wall or secreted from the cell to the growth medium provides the synthesis of NPs[31]. It is the use of cell-free culture supernatant for the extracellular synthesis of NPs. After incubation of the microorganisms in a suitable liquid culture medium, the mixture containing the culture medium and biomass is centrifuged and the supernatant is collected. To synthesize the NPs, the supernatant is mixed with the metal salt solution[25]. In addition to the culture medium compounds, the supernatant contains suitable enzymes and other extracellular secretion components produced by microorganisms as reducing agents in NPs synthesis.

The Synthesis of Biological NPs by *bacteria*in Extracellular mode of synthesis described in the above given (Table No.4) with suitable species and suitable class of nanoparticles simultaneously. As similar Synthesis of Biological NPs by *fungi* in Extracellular mode of synthesis discussed in the below (Table No.5).

The presence of different components within microorganisms i.e., enzymes, biosurfactants, pigments, proteins, polysaccharides, and other biological molecules also plays a vital role in the synthesis of NPs [Figure No.2]. It has been determined that main reductase enzyme is NADH-dependent nitrate are responsible for NP synthesis[44]. Exopolysaccharides (EPS) can be produced by different bacteria, yeast, and fungi. Different functional groups within EPS act as stabilizing and reducing agents to synthesize NPs[45]. Biosurfactants (such as rhamnolipid and emulsan) are one of the best capping agents, providing surface stabilization of NPs and avoids aggregation during biosynthesis and is a helpful for production of smaller NPs[46]. Extracellular or cell membrane-bound proteins helps in the metallic NPs formation via carboxylate and/or amino groups[47]. It also takes place when the metal salts are reduced and then converted to NPs with the help of reducing molecules such as phenolic compounds and organic acids produced by microorganisms[48]. Different NPs synthesized by various reducing agents are summarized in [Figure No.2].

FACTORS AFFECTING THE BIOSYNTHESIS OF NPS

Different physiological parameters that are reaction temperature, pressure, incubation time, pH, media composition, and metal salt concentration/type affect the formation of different NPs by microbial biosynthesis. Optimization of these parameters is vital for the biosynthesis of NPs to get desired shape, structure, size, and chemical composition. The yield of NPs under suitable conditions changes according to the microbial species. With this the composition of the growth medium for microorganisms is also essential. The reaction rate and production of NPs become faster as the temperature rises, furthermore the average particle size reduces by rising the temperature[49]. For optimal growth within microbial nanoparticle synthesis, it is guided to grow microorganisms at the maximum temperature that is possible, since the enzyme responsible for nanoparticle synthesis is highly active at increased temperatures[20].

In the biosynthesis of NPs, reaction time is vital in size and shape control. Excess reaction time decreases the stability of the synthesised NPs and rises the aggregation by size increase[50]. Metal salt concentration changes the shape and size of NP synthesis. Generally, 1-5mM of these salts are placed to the reaction medium. In addition, the formation of NPs is affected by type of salt. Metal salts can be acetate (OAc-), nitrate (NO3 -), sulfate (SO4 2-), and chloride (Cl-) are mostly used in the green synthesis of NPs[51]. Silver nitrate (AgNO3) is one of the vital source of Ag used experimentally for microbial AgNP synthesis.

This is mainly due to its greater solubility in water as compared to other Ag salts[52]. Many studies have been carried out at various AgNO3 concentrations. Light affects the synthesis of NPs and it has been studied that the culture supernatant of Klebsiella pneumonia can significantly accelerate the production of silver NPs when placed in visible light[53].

The capacity of Lactobacilli to grow even when the oxygen is presence makes them highly metabolically interesting. To have aerobic conditions in an anaerobic environment, particular conditions can be created by raising the pH of the environment. Altering the culture medium conditions is a way that plays a vital role in the biosynthesis of NPs. Lactobacilli has less metal detoxification system, minute acidic pH activates membrane bound oxidoreductases and the metabolic pathway are employed in NP synthesis[54]. The components of the media within the microorganism's growth affect the production of NPs. The production of smaller NPs has been reported with high yeast extract concentration[55].

Advantages and disadvantages of biological NPS

Biological Synthesis has a physical advantage of high speed, no use of toxic chemical, purity, uniform size and shape with disadvantage of productivity, high cost, exposure to radiation, require high energy, temperature and pressure, large amount of waste generation, high dilution, different size and shape tunability, lower stability, altered surface chemistry and physicochemical properties of nanoparticles.

Its chemical advantage is cost effective, high versatility in surface chemistry, easy functionalization, high yield, size controllability, thermal stability, reduced dispersity with disadvantage of low purity, use of toxic chemicals and organic solvents, hazardous to human beings and environment. It's bacteriogenic synthesis has an advantage that it is simple, facile eco-friendly, nontoxic, and biocompatible with disadvantage of hard to control size, shape, crystal growth, stability and aggregation possible presence of endotoxin, time consuming purification processes.

Biological synthesis and non-biological synthesis

Conventional physical and chemical processes involve the use of expensive chemicals, and these methods are non-eco-friendly, while biological process is inexpensive and eco-friendly as mentioned in [Figure No.4].

Table No.1: Reducing agents present within the micro-organism[21]

S.No	Micro organism	Reductase Enzymes
1	Bacteria	NADH – dependant Reductase or nitrate dependent reductase
2	Algae	Hydroxyl group involve in the reduction of particle
3	Fungi	Specific reductase Enzyme and biometric mineralization
4	Yeast	Membrane bound and cystosolic oxido reductase and Quinones

Table No.2: Application of metal-based NPS in pharmaceuticals[22]

S.No	Metal NPs	Specification
1	Gold NPs	Hyperthermia cancer treatment
2	Silver NPs	Antibacterial, antifungal, antiviral and anti-inflammatory agents
3	Titanium and Palladium NPs	Catalysis, chemical sensors optoelectronics
4	Gold-Silver alloys NPs	Vanillin content in vanilla beans

Table No.3: Synthesis of biological NPs by bacteria and fungi in Intracellular mode of synthesis

S.No	Organism	Species	NPs	Experimental condition	Reference
1	Bacteria	*Serratia marcescens*	Bi	30^{o}C, 48h	26
2	Bacteria	*Pseudomonas aeruginosa*	Co, Li	25°C, 24 h	27
3	Fungi	*Saccharomyces cerevisiae*	Se	32°C, 14d	28
4	Fungi	*Rhodotorula mucilaginosa*	Se	25^{o}C, 48 h	29

Table No.4: Synthesis of biological NPs by bacteria in extracellular mode of synthesis

S.No	Organism	Species	NPs	Experimental condition	Reference
1	Bacteria	*Bacillus cereus*	FeO	40°C, pH 5.5	31
2	Bacteria	*Pseudomonas aeruginosa*	Ag	37°C, 24 h	32
3	Bacteria	*Shewanella loihica*	Cu	30°C, 120 h	33
4	Bacteria	*Streptomyces griseoplanus*	Ag	28°C, 96 h	34
5	Bacteria	*Bacillus subtilis*	ZnO	33°C, 72 h, pH 7.5	35
6	Bacteria	*Bacillus thuringiensis*	Au	37°C, 12 h	36
7	Bacteria	*Pseudomonas aeruginosa*	Ag, Pd, Fe, Rh, Ni, Ru,	25°C, 24 h	27
8	Bacteria	*Bacillus licheniformis*	Au	25°C, 24 h	37
9	Bacteria	*Pseudomonas fluorescens*	Ag	80°C, pH 8	38

Table No.5: Synthesis of biological NPs by fungi in Extracellular mode of synthesis

S.No	Organism	Species	NPs	Experimental condition	Reference
1	Fungi	*Fusarium solani*	Ag	25°C, 24 h	39
2	Fungi	*Penicillium oxalicum*	Ag	60°C, pH 7	39
3	Fungi	*Saccharomyces cerevisiae*	TiO2	60°C, 10-20 min	40
4	Fungi	*Aspergillus brunneoviolaceus*	Ag	60°C, pH 9, 36 h	41
5	Fungi	*Trichoderma viride*	Ag	27°C, 48 h, pH 7.2	41
6	Fungi	*Aspergillus terreus*	CuO	36-48 h, pH 7.4	42
7	Fungi	*Candida glabrata*	Ag	25°C, 12 h	43
8	Fungi	*Rhodotorula mucilaginosa*	Se	25°C, 48 h	29

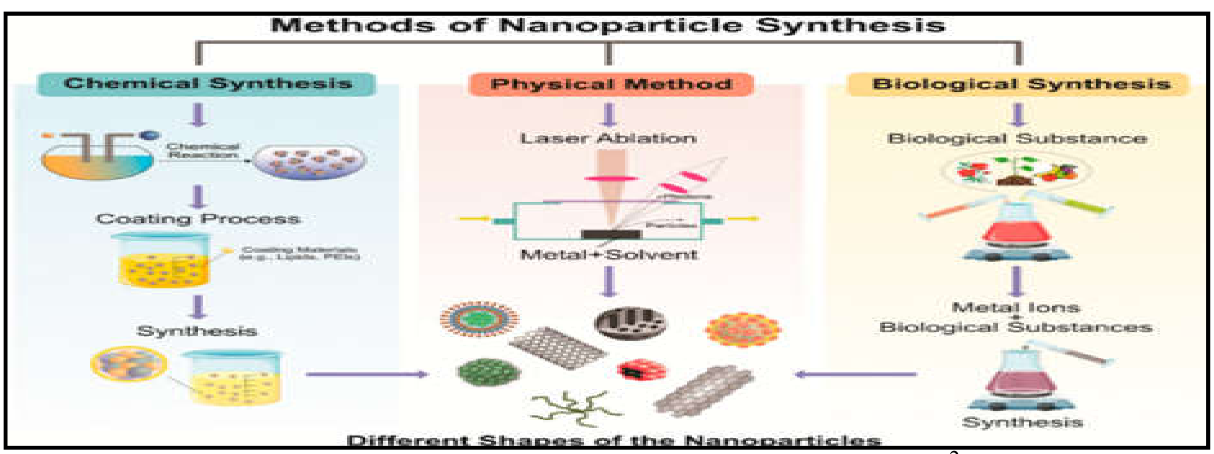

Figure No.1: Method of synthesis for nanoparticles[2]

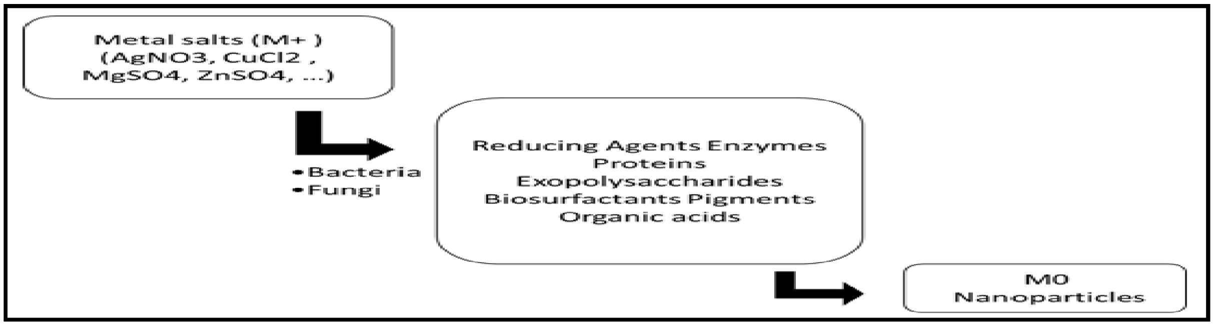

Figure No.2: Process of synthesizing the NPs

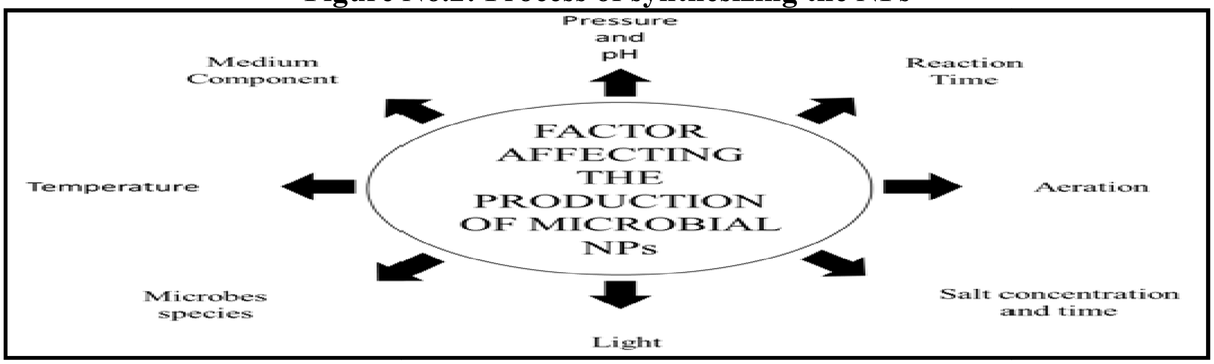

Figure No.3: Factor affecting synthesis of biological NPs

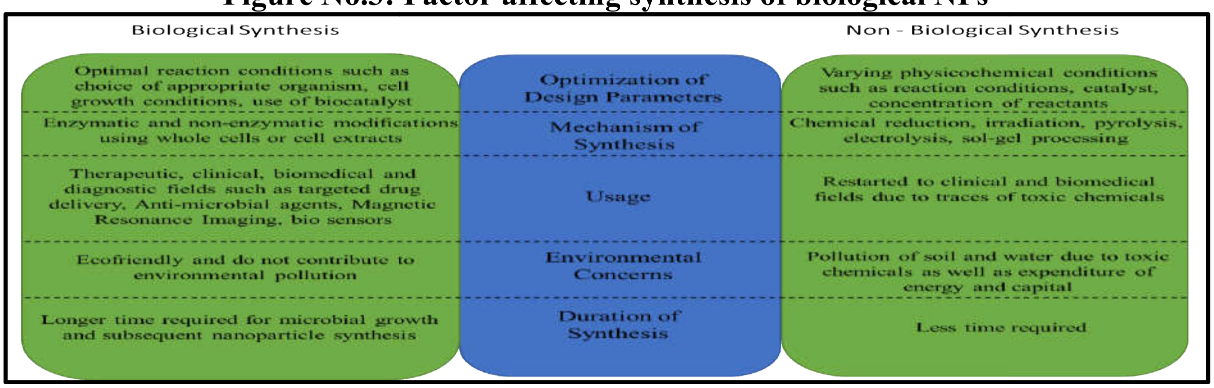

Figure No.4: Comparison of biological and non- biological synthesis

CONCLUSION

Microbially synthesized NPs are non-toxic, environmentally friendly, and biocompatible, however, the formation of NPs using microorganisms is still at the research and laboratory level is essential for large-scale production. In recent years, microorganisms such as bacteria and fungi have been employed in biotechnology and nanotechnology as a trusted source for the formation of NPs and the development of environmentally friendly methods for their biomedical and biological applications. The formation of NPs is classified as extracellular and intracellular. The shape and size of metallic NPs can be controlled by optimizing the reaction parameters such as reaction temperature, time, medium composition, pH, and reactant/salt ratio. In the microbial synthesis method, various biomolecules are used in the formation of NPs. Therefore, the mechanisms behind microbial formation of NPs need to be demonstrated. In addition, different application areas of NPs should be considered by microbially producing new and various commercial NPs.

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CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

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