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LIVING SYSTEMS: ECO-FRIENDLY NANOFACTORIES

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Living organisms possess various Nature's secrets that are not completely understood by the human beings. They have inherent capacity to cope up with several types of stresses. In the present review, we emphasized on to the potential of living organisms like microbes and plants to synthesize nanoparticles not even in lab but also in their natural environment. We are highlighting the fact that biological methods of nanoparticles synthesis are more eco-friendly and safe as compared to other methods. So these can be used as factories for the production of nanoparticles and other future nanodevices.

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1. Introduction

Nanotechnology is the most fascinating area of research in the field of material science. Currently, it involves various physical and chemical methods for nanoparticles synthesis. But the main problem with these methods is the production of toxic byproducts, shows that these are not environmentally safe methods. Thus there is a growing need for 'green chemistry' that includes a clean, nontoxic and environmental friendly methods of nanoparticles synthesis. For environmental safety concerns, researchers in the field of nanoparticles synthesis and assembly have turned to the biological systems [63]. This is really surprising that from simpler microbes to higher plants, have the capacity to produce nanoparticles. These nanoparticles are environmental friendly and completely safe. Moreover, this biosynthesis approach is close to principles of 'Nature'. Biological synthesis of nanoparticles involves natural phenomenons that take place in the biological systems. At present, there are several evidences of nanoparticles formation in Algae, Fungi, Yeast and higher plants. It has been shown that several types of inactivated biomasses and living organisms have the ability to remove high concentration of gold from solution and reduce gold from higher oxidation state to zero oxidation state or formation of gold nanoparticles [1,3,4]. However, the elucidation of the exact mechanism of nanoparticles production using living organisms needs much more experimentations. This area enlightens the future potentialities of taking a new look at old systems that retain some of the most fascinating Nature's secrets. Moreover these studies provide the possibility of an environmental friendly method to remediate mining wastes[41].

BACTERIA

Bacteria are considered as the most potent eco-friendly nanofactories. Beveridge and co-workers [74] have demonstrated that gold particles of nanoscale dimensions may be readily precipitated within the bacterial cells by incubation of the cells with Au^{3+} ions. Likewise, Tanja Klaus and co-workers [75,76] have also shown that the bacteria *Pseudomonas stutzeri* AG259 isolated from the silver mine, form silver nanoparticles when placed in silver nitrate solution. They have observed the formation of silver nanoparticles of well defined morphology and size within the periplasmic space of the bacteria. Moreover the magnetotactic bacteria such as *Magnetospirillum magneticum* have been reported to produce magnetic nanoparticles [77].

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Similarly, Nair and Pradeep [78] reported that *Lactobaccillus* strains present in butter milk, form metal nanoparticles when challenged with silver and gold ions. Likewise sulphate reducing bacterium *Desulfovibrio desulfuricans* NCIMB 8307 has been shown to synthesize palladium nanoparticles [79]. Recently, extracellular gold nanoparticles are observed in the *Pseudomonas aeruginosa* by the reduction of gold ions [80]. Holmes and co-workers81 have shown that the bacterium *Klebsiella aerogens* when exposed to Cd2+ ions resulted in the intracellular formation of CdS nanoparticles in the size range 20-200 nm. Likewise Mandal and co-workers have observed the spherical aggregates of 2-5 nm sphalerite ZnS nanoparticles, formed by the sulphate reducing bacteria under the anaerobic conditions and *Clostridium thermoaceticum* and *Klebsiella aerogens* were used to form CdS nanoparticles [10]. Moreover it has also been published that gold nanowires can be synthesized from the extract of *Rhodopseudomonas* capsulate [47]. This procedure offers control over the shape of gold nanoparticles with the change of HAuCl4 concentration. It has been also published that a major biomolecule probably a protein involved in the bioreduction and synthesis of gold nanoparticles [47].

ALGAE

Unicellular organisms such as Diatoms (brown algae) are able to carefully design and control natural nanostructures formation. They have a silica exoskeleton[5] named Frustules, consists of SiO_2 nanoparticles (50-100 nm) assembled in a highly organized structure. This is formed within a few hours, from naturally occurring precursors at low concentration and near or below the room temperature[6]. These nanostructures have several futuristic applications. Also the possibility to control the formation and assembly of silica nanoparticles in biological conditions opens wide opportunities for the design of nanodevices such as membranes, controlled-release systems or bioencaptulation hosts [7-8].

2. Hypothetical mechanism

Silicic acid $Si(OH)_4$ is a naturally occurring precursor of silica in aqueous environment[9]. It is thought to be transported into the cell through specific trans-membrane protein named SIT (Silicic acid Transporters) [82] where it can accumulate at concentrations that may be thousand fold higher than the environmental amount. These silicon precursors also have to be transported into a specific vesicle, named Silica deposition vesicle (SDV), located at the vicinity of cytoplasmic membrane[11]. The first isolated Silicic acid transporter (SIT) was from diatom species *Cylindrotheca fusiformis* [82]. According to a hypothetical model, SIT protein should be localized in the cytoplasmic membrane (Fig. 1) but it may also be localized in specific intracellular vesicles i.e. SDV (Silica deposition vesicles). Inside these vesicles, Si polymerization occurs during cell division of diatoms, where Si is transported through SIT proteins [[82]. SIT proteins are encoded by some transporter genes that seems to be highly expressed during silicic acid replenishment experiments of silicate starved cells [[83]. Detailed chemical studies shows that SiO_2 is not a salt formed through ionic associations but built up via. inorganic polymerization of molecular precursors[12]. First molecular explanation about the frustule formation came only recently by the isolation of some ‘active compounds’ [84]. It has been shown that some proteins may involve in the Si polymerization process. A novel family of protein was named silaffins, for peptide that have affinity for silica, and members of this family have now been isolated from two different diatom species. The main important characteristics of this family of proteins is that they are enriched in charged amino acids and frequently undergoes phosphorylation, glycosylation etc.[85,86,87] A silaffins protein from *C. fusiformis* (Sil 1) gives a zwitterionic character, and strongly influences its ability to form Si nanoparticles invitro from silicic acid precursors [88]. Another class of proteins are long chain polyamines (LCPAs) present the ability to form Si nanoparticles invitro

[89,90,91]. Diatoms shells when treated with magnesium vapors at high temperature, leading to mixed Mg-Si oxides replica of the frustule13. This process was suggested to be compatible with other metals, thus representing a significant step towards direct application of diatoms in nanotechnology. So, several biomimetic approaches has led to the identification of several natural or synthetic molecule that are able to activate silica formation in conditions that closely resemble those found in the living organism intracellular compartment [6,101]

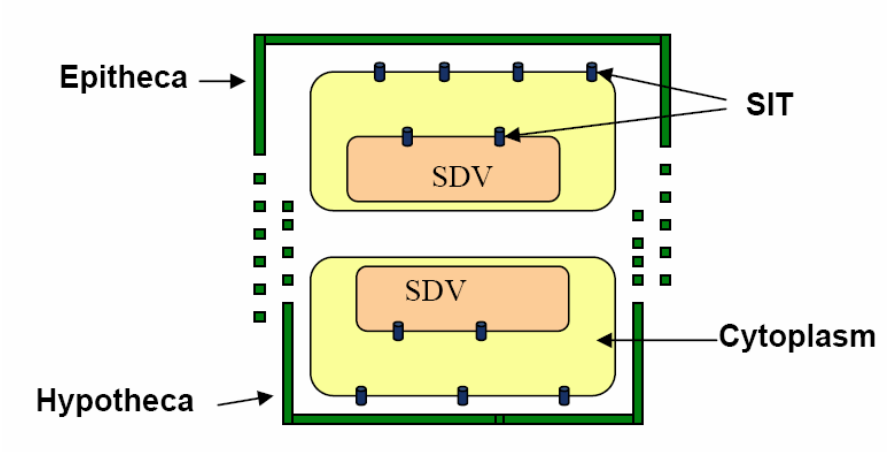

Fig. 1. A hypothetical model showing location of transporters in diatom

YEAST

Yeast has been used with success in the synthesis of CdS and PbS nanoparticles. Kowshik and co-workers[92] have identified the yeast, *Torulopsis* sp. as being capable of synthesizing nanoscale PbS crystalline intracellularly, when exposed to aqueous Pb^{2+} ions. Later on these scientists [93] observed the CdS Quantum dots are synthesized intracellularly in *Schizosaccharomyces pombe* yeast cells. They have also been suggested that the formation of CdS nanocrystals was dependent on the growth phase of the yeast. Likewise, large quantity of the silver nanoparticles by using silver tolerant yeast strains MKY3 have also been reported [94]. Recently, yeast strains have been identified for their ability to produce gold nanoparticles, whereby controlling the growth and other cellular activities, controlled size and shape of the nanoparticles was achieved [95].

FUNGI

Fungi are the extremely good candidates in the synthesis of metal and metal sulphide nanoparticles [14]. Two different genera of fungi, *Verticillium sp.* and *Fusarium oxysporum*, when exposed to aqueous gold and silver ions, reduced the metal ions fairly rapidly. In the case of *Verticillium sp,* reduction of the metal ions occurred intracellularly leading to the formation of gold [15] and silver [16] nanoparticles in the size range 2-20 nm. However, *Fusarium oxysporum* behaved considerably differently, the reduction of metal ions occurring extracellularly resulting in the rapid formation of highly stable gold [17] and silver [18] nanoparticles of 2-50 nm dimensions. Moreover, extremely stable quantum dots of CdS may be formed extracellularly by challenging the fungus *F. oxysporum* with aqueous CdSO4 solution [19]. Gold nanocrystals can be produced from extremophillic actinomycete *Thermomonospora sp.* Extracellularly [20]. There are also observations of production of bioactive nanoparticles from lichen fungi (*Usnea longissima*) in the culture conditions [21]. Moreover, the fungus *Aspergillus flavus* when challenged with Silver nitrate solution accumulates Ag nanoparticles on the surface of its wall in 72 hrs [73]. In *Aspergillus fumigatus* also extracellular synthesis of silver nanoparticles was observed [70].

Likewise, extracelluar synthesis of silver nanoparticles in the fungus *Fusarium semitectum* was also reported and possible medicinal applications of these silver nanoparticles are also envisaged [71]. In white rot fungus *(Phaenerochaete chrysosporium)*, extracellular synthesis of silver nanoparticles was observed. It has also been published that incubation of *P. Chrysosporium* with silver nitrate solution produced silver nanoparticles in 24 h [72]. These nanopaticles on were characterized by means of UV-VIS spectroscopy, X-ray diffraction analysis, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Photoluminescence spectroscopy. The SEM characterization of the fungus reacted on the Ag^{+} indicated that the protein might be responsible for stabilization of silver nanoparticles [72].

3. Mechanism

The general mechanism of the nanoparticle formation in fungus is based on the enzymatic reduction process. The quantum dots are formed by the reaction of Cd2+ ions with sulphide ions that are produced by the enzymatic reduction of sulphate ions to sulphide ions19. Fungus *F. oxysporum* consists of enzymes like sulphate reductase [19] that are responsible for such reduction. Likewise, *Verticillium* fungus when challenged with the Ag+ and AuCl4- ions lead to their reduction and accumulation as silver and gold nanoparticles within the fungal biomass [14]. The reaction was carried out for a period of 72 h and when thin sections of *Verticillium* cells were prepared then TEM results indicate the presence of gold and silver nanoparticles. The appearance of a dark brown color in the fungal biomass after reaction with the Ag+ ions is a clear indicator of the reduction of metal ions and formation of silver nanoparticles in the fungal biomass14. Gold and silver nanoparticles exhibit striking colors (pink to blue for gold and light yellow to brown for silver) due to excitation of surface plasmon vibrations in the particles22. These nanoparticles get uniformly distributed over the surface of fungal cells. Moreover, *Verticillium sp*. doesnot die on either exposure to Ag+ ions and their ability to multiply also was not compromised14. The exact mechanism leading to intracellular formation of gold and silver nanoparticles by challenging the fungus *Verticillium sp.* is not fully understood [14]. The nanoparticles are formed on the surface of mycelia not in the solution. So the possible mechanism (Fig. 2) is suggested that in the first step, trapping of the Ag+ ions on the surface of the fungal cells possibly via. electrostatic interactions between the Ag+ and negatively charged carboxylate groups in enzymes present in the cell wall of mycelia. Then in the next step, silver ions are reduced by enzymes present in the cell wall leading to the formation of the silver nuclei, which subsequently grow by further reduction of Ag+ ions and accumulation on these nuclei [14]. The TEM analysis shows the presence of some silver nanoparticles both in the cytoplasmic membrane and in the cytoplasm [14]. Whereas in case of the fungus *F. oxysporum*, when exposed to metal ions, yield highly stable nanoparticles in solution [17,18]. Likewise, Nanoparticles are also reported in Actinomycetes. These are the microorganisms that share important characterstics of fungi and prokaryotes such as bacteria [23]. The extremophillic actinomycete *Thermomonospora sp.* when exposed to gold ions reduced the metal ions extracellularly, yielding gold nanoparticles with a much improved polydispersity20. It has been published that when gold and silver nanoparticles formed then they are stabilized by the proteins. Proteins can bind to gold nanoparticles either through free amine groups or cysteine residues in the proteins [14, 24]. One or more these proteins may be enzymes that reduce chloroaurate ions and cap the gold nanoparticles formed by the reduction process. Thus capping and stabilization of the gold nanoparticles is affected by a different protein14. Moreover, bioactive nanoparticles are observed in Lichen fungi (*Usnea longissima*) in culture condition21. Lichen fungi have diverse range of chemicals and produced some characteristic metabolites during artificial culture in synthetic medium. Many lichen substances have been detected in cultured lichens like Usnic acid [25-27], depsides and depsidones [27-29], vulpinic acid [30], anthraquinones[31] and salazinic acid [32]. in culture medium, light yellow-green substances are observed [21]. Later these nanoparticles were aggregated to form a uniform crystalline layer (nanoparticle) below the medium [21].

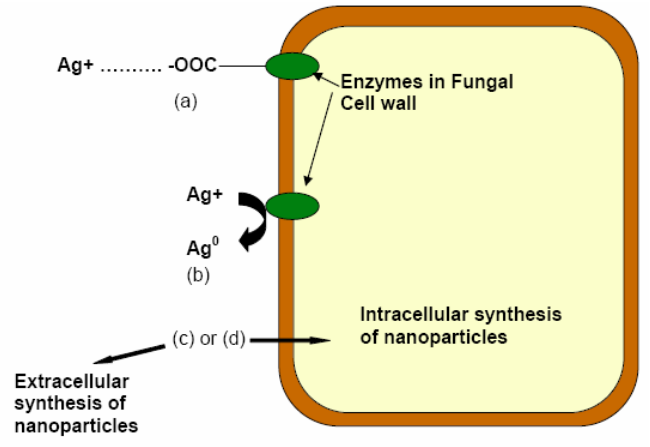

Fig. 2. Hypothetical mechanism of Nanoparticles formation in the Fungus. a. Electrostatic interaction between the metal ion and the enzyme present of the Fungal cell wall. b. Reduction of Ag+ to Ag0 by the enzyme; c. and d. The Ag0 nanoparticles may be synthesized intracellularly or extracellularly

4. Plants

Some plants can accumulate metals from the soil in which they are grown. The plants which can accumulate a metal to 1000 times the highest concentration found in normal accumulator plants are termed as 'hyperaccumulators' [33]. There are several researches to elucidate nature of the metal accumulated on plants. Some researchers show that gold accumulated by the plants and stored in leaf and stem biomass can be present as discrete nanoparticles of pure metal34. This discovery was made after alfalfa sprouts germinated on gold-chloride enriched agar (320 mg/kg) were analyzed using X-ray absorption spectroscopy (XAS) and Transmission electron microscopy (TEM) [41]. Discrete nanoparticles of 2-20 nm in diameter as well as coalesced particles of 20-40 nm scale were observed distributed through certain zones of plant tissue41. These observations further illustrated that alfalfa roots are capable of absorbing silver as Ag(0) from the agar medium and then transferring it to shoot of the plant in the same oxidation state. Silver atoms arrange themselves to form nanoparticles and these nanoparticles join to form larger arrangements, suggesting different organization levels [35]. The concentration of gold from ores by plants, or phytomining has also been proposed as an environmental friendly and economic method of recovering gold [36,37]. This property of accumulation of gold in the form of nanoparticles by the plants lead to new area called Gold phytomining, the extraction of metals from soils or ores for recovery and sale [38]. Gold and silver nanoparticles are also observed in *Cinnamomum camphora*2, *Brassica juncea [*38,102] and *Capsicum annum [*49] extracts during experimentation.

There are several evidences that support the formation of gold and silver nanoparticles in living plants [37,41]. Some researchers directly attained the biosynthesis of metal nanoparticles by plant leaf extracts and their potential applications [42-48]. They studied bioreduction of chloroaurate ions or silver ions by a broth of Geranium leaf *(Pelargonium graveolens)* [42,43] or Neem leaf *(Azadirachta indica* [44]. Aggregated forms of nanoparticles like Gold nanotriangles are observed in Lemon grass extracts [64] *(Cymbopogon flexuous)* as well as in the tamarind leaf extract [62]. Very recently, scientists have demonstrated the synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extracts48. It was explained that only the biomolecules less than 3 kDa molecular weight caused reduction of chloroaurate ions, leading to the formation of gold nanotriangles. Most of these experiments utilize the plant extracts employed broth

resulting from boiling fresh plant leaves [42-48] but some used sundried mass from *C. camphora* leaf [2]. Silver nanoparticles can be rapidly synthesized by treating silver ions with a *Capsicum annuum* extract49. Even dead biomass of hops *(Humulus lupulus)* also can produce gold nanoparticles [65]. Moreover, extracellular synthesis of gold and silver nanoparticles is observed by using *Emblica officinalis* fruit extract as a reducing agent [66]. It is also observed that stress tolerant plants have more capacity to reduce metal ions in to the metal nanoparticles. One such finding observed in Desert willow plant *(Chilopsis linearis)* [67]. This plant (desert willow) has the capacity to produce a large amount of biomass with low water consumption. This plant takes gold from gold enriched medium and average size of the Au nanoparticle formed by the plant is related to the total Au concentration in tissues and their location in plants [67]. The morphology and crystalline phase of the NPs were determined with the Transmission Electron Microscopy (TEM), Selected Area Electron Diffraction (SAED) and X-ray Diffraction (XRD) spectra. The crystalline phase of nanoparticles changed from Polycrystalline to single crystalline and increased in size with increasing reaction time [49]. There are several proteins that are responsible for the NPs formation in the plants. Moreover, Biosilcification also results in nanoparticles in some higher plants [6]. Whole list of microbes and plants that produce nanoparticles are shown in table 1.

Table 1. A list of living organisms synthesizing nanoparticles.

Living system	**Nanoparticles produced**	**Size of nanoparticle produced**
Bacteria		
• Magnetotactic Bacteria [77]	Magnetic (Fe_3O_4) and Greigite (Fe_3S_4)	-
• *Pseudomonas aeruginosa*80	Au Nanoparticles	5- 15 nm
• *P. stutzeri* [75,76]	Ag Nanoparticles	100-200 nm
• *Desulfovibrio* desulfuricans [79]	Palladium	-
• *Shewanella algae*[97]	Au Nanoparticles	10-20 nm intracellularly and 50-500 nm extracellularly
• *Acidithiobacillus thiooxidans* [96]	Au Nanoparticles	-
• *Klebsiella aerogens* [81]	CdS Nanoparticles	20-200 nm
• *Clostridium thermoaceticum*[10]	CdS Nanoparticles	-
• *Rhodopseudomonas* capsulate [47]	Gold Nanowires	-
Algae		
• Diatoms6	SiO_2 Nanoparticles	50-100 nm
• *Sargassum* alga [39]		
Yeast		
• *Candida glabrata* [55]	CdS Nanoparticles	20 Å
• MKY3 [94]	Ag Nanoparticles	2-5 nm
• *Schizosaccharomyces pombe* [93]	CdS Nanoparticles	1-1.5 nm
• *P. jadini* [98]	Au Nanoparticles	few to 100 nm

Living system	Nanoparticles produced	Size of nanoparticle produced
Fungi		
• *Aspergillus* fumigatus [70]	Ag Nanoparticles	5-25 nm
• *Colletotrichum sp.*[42]	Au Nanoparticles	20-40 nm
• *Fusarium oxysporum* [17,18]	Au and Ag Nanoparticles	20-40 nm & 5-15 nm respectively
• *Trichothecium sp.*100	Au Nanoparticles	-
• *Rhodococcus sp.* (Actinomycete) [99]	Au Nanoparticles	5-15 nm
• *Thermonospora sp.* (Actinomycete)[20]	Au Nanoparticles	8 nm
• *Verticillum sp.* [15,16]	Au and Ag	20-25 nm
• *Fusarium semitectum*[71]	Ag Nanoparticles	-
• *Phaenerochaete chrysosporium* [72]	Ag Nanoparticles	-
Plants		
• Alfalfa plant *(Medicago sativa)* [41]	Au and Ag	20-40 nm
• *Avena sativa* [69]	Au	25-85 nm
• *Azadirachta indica* [44]	Ag, Au and Ag/Au bimetallic	50-100 nm
• *Aloe vera* [48]	Ag	15-15.6 nm
• *Emblica officinalis* [66]	Ag & Au	10-20 nm and 15-25 nm respectively
• *Cinnamomum camphora*2	Au & Ag	55-80 nm
• *Tamarind* leaf extract [62]	Au nanotriangles	20-40 nm
• *Pelargonium graveolens* [42,43]	Ag Nanoparticles	16-40 nm
• *Capsicum annum* [49]	Ag	-
• *Chilopsis linearis* [67]	Au	-
• *Humulus lupulus* [65]	Au	-
• *Cymbopogon flexuous* [64]	Au Nanotriangles	-
• *Brassica juncea* [38,54,102]	Au Nanoparticles	-

5. Mechanism of nanoparticles formation in plants

The formation of nanoparticles is based on the principle of mineral uptake from the soil. The soil or medium rich in minerals like Au, Ag etc. is used as a source for nanoparticles accumulation in the plants. As far as mechanism is concerned, there are still several doubts. The debatable thing is that nanoparticles synthesized both inside the living plants [54] and within the sundried biomass [2]. The basic mechanism in all cases involves the accumulation of nanoparticles after the reduction of metal ions. Definitely this reduction process is mediated by some reducing

agents or may involve some enzymes that are bound to the cell wall. Many experiments suggest that different biomolecules like proteins are involved in this process [49].

In some higher plants, Biosilcification process is also very common6. In these systems, it was suggested that silicic acid is taken up through the plant roots and transported as a silicon complex through the xylem. When this complex reaches the stems or leaves, where mineral deposition should occur, its breakdown is triggered by change in pH, inducing the release of silicic acid that will further condense to form the silica [40]. The protein plays an important role in the formation of nanoparticles in plants [2,49]. The effect of *Capsicum annuum* proteins on the formation of the silver nanoparticles was investigated by using X-ray Photoemission spectroscopy (XPS), Electrochemical measurements, Fourier-transform infrared spectroscopy (FTIR) and differential spectrum techniques [49]. The results indicated that the proteins, which have amine groups, played a reducing and controlling role during the formation of silver nanoparticles in the solution, and that the secondary structure of the protein changed after reaction with the silver ions [49].

When sundried mass of *C. camphora* leaf [2] was treated with the aqueous silver or gold precursors at ambient temperature, then silver nanoparticles ranging from 55-80 nm in size can be produced. There is formation of triangular or spherical shaped gold nanoparticles. Further investigations suggests at the biomolecules play an important role in the formation of gold and silver nanotriangles. The polyol components and the water soluble heterocyclic components were mainly responsible for the reduction of silver ions or chloroaurate ions and stabilization of the nanoparticles respectively [2]. Gold nanotriangles might grow by a process involving rapid reduction, assembly and room temperature sintering of spherical gold nanoparticles [50]. However till now mechanistic aspects of nanoparticles formation in plants are not clear but it may be somewhat associated with the phytoremediation concept in plants [52-54].

6. Formation of gold and silver nanoparticles in plants – Gold Phytomining

Firstly, the hydroponic uptake of the gold by the plants was investigated in *Impatiens holsii* and *Impatiens balsamina*51. Phytomining is the use of hyperaccumulating plants to extract a metal from soil with recovery of metal from the biomass to return an economic profit [38]. Gold is a metal that is insoluble in soil solution and therefore unavailable for uptake by plants [38]. The addition of chelating agents such as thiocyanate to the soil to complex and solubilize gold has overcome this problem [52]. This strategy in the plants is known as ‘induced hyperaccumulation’.

Hyperaccumulator species have a physiological mechanism that regulates the soil solution concentration of metals. Exudates of metal chelates from root system, for example, will allow for increased flux of soluble metal complex through the root membrane. The induced hyperaccumulation is observed in a non-accumulator plant *Zea mays* that was induced to accumulate lead from the soil after treatment with EDTA53. To induce accumulation of gold in *Brassica juncea* by application of ammonium thiocynate has been reported, increasing the gold concentration to 57 μg/gm [52]. Other solubilizing agents such as cyanide, iodide, bromide and thiosulphate have been examined in gold accumulation studies [38]. So, plants could therefore accumulate gold if a soluble species were available [38]. The plants can be induced to accumulate or even hyperaccumulate gold with the addition of sodium thiocyanate solution to the substrate [36, 52]. Mixed metal nanoparticles like Au-Ag-Cu alloy were produced in *B. juncea* [54]. It was observed that *B. juncea* and *Berkheya coddii* are both capable of hyperaccumulating gold, when sodium thiocynate solution is added to artificial substrate. *B. juncea* and *chicory* are both are capable of hyperaccumulating gold when either iodide, bromide, cyanide, thiocyanide or thiosulfate solutions are added to artificial gold substrate. Addition of cyanide induces higher gold concentration in leaves and stems of *B. Juncea* and *B. coddii* than in the roots in some case. For all cases of thiocyanate addition, the root concentrations were higher. This difference in translocation

ability between cyanide and thiocyanate may be due to the effects of the plant's internal pH on the complexes formed [38,54].

7. Factors affecting the nanoparticle synthesis in biological system

Several factors influences the reduction process of metal ions into metal nanoparticles like temperature, pH etc. Temperature has a profound effect on the nanoparticles formation. It has been observed that gold nanotriangles formation is kinetically controlled and is highly favoured at the low temperature range [68]. It was demonstrated that temperature plays an important role for controlling the aspect ratio and relative amounts of gold nanotriangles and spherical nanoparticles [64]. By varying the temperature of the reaction conditions, the shape, size and optical properties of the anisotropic nanoparticles can be finely tuned [64]. Moreover pH of the medium also influences a lot the size of nanoparticles. In *Avena sativa*, it was observed that size of the gold nanoparticles can be controlled by altering the pH of the medium [69]. When the solution of Au^{3+} with the oat biomass were reacted for one hour in a pH range 2-6, the results demonstrated that Au^{3+} ions were bound to oat biomass in a pH–dependent manner [69], with highest absorption at pH 3. Moreover, it was also observed that presence of some ions like Chloride, Bromide and Iodide etc. also affects the nanoparticles formation in plants. Presence of chloride ions during synthesis promotes the growth of nanotriangles, whereas presence of Iodide ions distorts the nanotriangles morphology and induces the formation of aggregated spherical nanoparticles [64].

8. Importance of biosynthesis

There are several advantages of synthesizing nanoparticles in the biological system. Firstly, these biological approaches proved to be an environmentally friendly and economic method of recovering gold [38,52,37]. Nanoparticles of gold are catalytically active for reactions such as water gas shift reactions and selective oxidation of CO [37,56,57]. Mixed nanoparticles alloy can be made from *B. juncea* that have various nanotechnological applications [54]. Moreover, these are the most common methods for the production of catalysts of specific compositions even those are difficult to synthesize by traditional methods [35]. Gold nanoparticles also have other applications in the field of sensors [58-60] and in medicine [61].

9. Future directions

Due to enormous benefits, biosynthesis of nanoparticles is emerging as a new area of research in scientific community. As the nanoparticles synthesized in the microbes are used against the human pathogenic fungi [21], there are also hope to produce the nanoparticles in the plant cells that can be used against human pathogens or to treat human diseases.

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