

Qualitative and Quantitative Evaluation of Biosynthesized Nanoparticles in Textile Composites for Practical Applications

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ABSTRACT

Particulate dispersions or solid particles with a size below 100nm are categorised as nanoparticles. They inherently possess superior properties than bulk material in terms of mechanical strength, thermal stability catalytic activity, electrical conductivity, magnetic properties, optical properties, surface properties, etc. which make their end use application area much wide. Popularly used metal nanoparticles in various fields, such as textile, biomedical, health care, food agriculture, industrial, electronics, renewable energy, environment, etc. are silver, gold, copper, alumina, titanium dioxide, zinc oxide. They can be synthesised either of chemical, physical, mechanical or biological method. However, the chemical, physical and mechanical methods have major drawbacks of toxicity, high cost and high energy. Against that biological method is safe, cheaper and consumes less energy. The source material used in this synthesis process is normally plants, fungi, yeast, actinomycetes, bacteria, etc. and system is also classified accordingly. Hence nanoparticles are usually reinforced in base material to enhance its functional characteristics required, and such nanocomposites can be used more preferentially by end users. Cosmetic, medical, photo thermal therapy, drug delivery, biosensors, cancer therapy, imaging, antimicrobial agent, etc. are some of the identified domains of end users.

Keywords: Base material, Biomedical, Composite, Health care, Nanoparticles, Synthesis.

I. INTRODUCTION

A composite material is defined as a combination of two or more materials that result in better properties than the original elements. The two constituents of composite always consist of reinforcement and a matrix. Reinforced matter acts as a functional group whereas matrix forms base ground for the composite. A type of composite is usually defined on the basis of matrix. The major groups identified in this area are:

- Metal matrix composite
- Ceramic matrix composite
- Polymer matrix composite

Metal matrix composite (MMC) are made by dispersing a reinforcing material into a metal matrix. Ceramic matrix composite (CMC) consist of ceramic fibres embedded in a ceramic matrix. Polymer matrix composite is also known as fibre reinforced plastics (FRP). The plastic (matrix) is reinforced with fibres (reinforcement) in FRP [1]. The application areas of FRP are very wide but transitorily summarized in Table 1.

Nano composite textile is one of the popularly raising categories of FRP observed in recent times. Its application is fostered up mainly due to its excellence in performance availed at considerably reduced price rate.

TABLE 1 Brief summary of FRP Applications:

Application area	Uses	Function
Conveyor Belts [2]	Narrow, Fast Belts	<ul style="list-style-type: none"> • High modulus but Light weight Belt, • High Strength & flexibility, • Low extension in Service. • Resistance to Abrasion, Impact & Tearing resistance, • Resistance to moisture, oils & chemicals
Aerospace [3]	Antenna Ribs & Struts, Fairing, Composite propulsion shafts, Engine Cowls, Rudder, Fin Boxes, Doors, Floor Boards & many other Interior gadgets, Landing gears,	<ul style="list-style-type: none"> • Weight savings of 20-30%, • High specific modulus, Strength and Dimensional stability, • High specific stiffness, • Lower ablation rate,

	Graphite/Epoxy Honeycomb Payload Bay doors, Rocket Motor Cases & Linters	<ul style="list-style-type: none"> Higher bulk density and superior mechanical strength
Pipes, Tanks & Vessels [4]	Adhesively bonded joints, Butt and Wrap joints, Glass reinforced Epoxy tubes, Pipework for aqueous liquids, Rubber seal joints	<ul style="list-style-type: none"> Chemical resistance, Largely immune to the effect of Hydrogen Sulphide and Carbon dioxide, Highly defect tolerant.

Nano composite textiles:

Nano composite textiles have composite fibre materials containing one or more nanostructured or nanoscale components. This nanostructured component can be nanoparticle, nanotube, nanofiber and so forth. The most prevalently used nanoparticle in this area can be prepared either by nanoparticle synthesis or by processing nanomaterial into nanostructured particles. Synthesis of nanoparticle can be carried out by either of chemical, physical, mechanical or biological method [3].

The chemical method has always scored advantage over rest of all the methods due to its shortest synthesis time period. Conversely chemical reagents used are expensive and highly toxic, which lead to generation of non-eco-friendly by-products. Against that longer routed physical and mechanical methods have not caused much harm to the environment. No doubt they consume higher energy and occupy large space, so usually results in more expense and time. Biological method differs from them; as it does not generate any toxic by-products or say environment friendly although follows shorter production route and also having greater product durability. Nanoparticles are availed from natural resources exist in abundant varieties and quantities; thus biological method on an aggregate a low cost, eco-friendly as well as reliable technique [4].

II. BIOLOGICAL NANO SYNTHESIS METHOD

The biggest constrain of nanoparticles synthesis process is environment pollution. Eco-friendly or non-toxic biological method has thereby earned a highest credit amongst all in recent time [3].

Numerous pathways of biosynthesis were casted by various researchers and most of them met commercial success. Classification of the synthesis methods is usually based on the resource used in the process [4]. One such classification has been shown diagrammatically in figure 1.

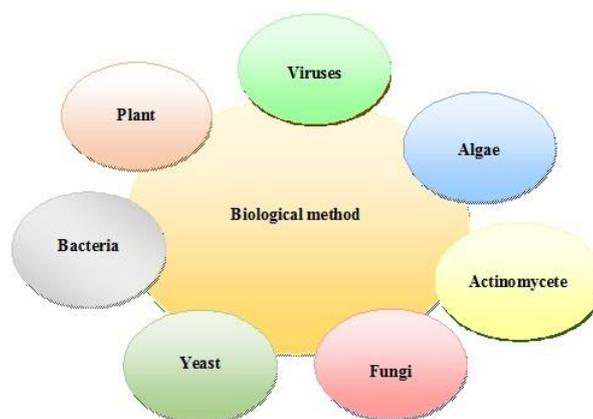


Figure 1: Classification of Biological Nano synthesis methods

Plant based resources

Medicinal importance of plant is very well-known since the days of grandma treatment recipes, e.g. turmeric and tulsii are used for curing cough – cold, ginger for digestion etc. The ready availability of capping agent from the resource plant itself is an additional advantage of this system. Lot of work has been reported in this area and an attempt has been made here to summarise them in brief [3].

Sastry *et al* [4]. have worked on the synthesis of gold and silver nanoparticles using geranium extracts. Gold nanotriangles and silver nanotriangles were also synthesised using Aloe Vera plant extracts [5]. Synthesis of silver and gold nanoparticles using the sundried cinnamomum camphora leaf extract is reported by Huang *et al.* [6]. The

marked difference of shape control between gold and silver nanoparticles was observed due to the comparative advantage of protective biomolecules and reductive biomolecules during this study. Lin *et al* [4]. have reported a simple green synthesis method for production of well-defined silver nanowires. The method involves without using any additives, reduction of silver nitrate with broth of sundried cassia fistula leaf at room temperature.

Quantum dots have huge application in nano biotechnology and plants have been observed to be a good source for the synthesis of quantum dots. Alfalfa roots have capability for absorbing Ag (0) from agar medium and transferring them to shoot of the plant in the same oxidation state. In the shoot, these Ag atoms arranged themselves to form nanoparticles by joining themselves to form larger arrangements [3].

Extracellular synthesis of nanoparticles was also become possible using plants [4]. Using Geranium (*Pelargonium graeolens*) leaf extract silver ions were reduced to silver nanoparticles which were highly stable and crystalline in solution by Shankar *et al.* [8]. In addition to individual pure metallic Ag and Au, bimetallic Ag/Au nanoparticles (50–100 nm) using *Azadirachta indica* leaf broths have also been synthesized extracellularly Shankar *et al.* [9]. Synthesis of bimetallic Au core–Ag shell nanoparticles in solution was accomplished due to competitive reduction of Au³⁺ and Ag⁺ ions present simultaneously in the solution during the exposure to Neem leaf extract.

The rate of reduction of metal ions by Neem leaf extract has been found to be much faster compared to that of microorganisms use. While, treatment of aqueous solutions of silver nitrate and chloroauric acid with Neem leaf extract has been observed for rapid formation of stable Ag and Au nanoparticles at higher concentration. It was observed that flavanone and terpenoid constituents of leaf broth were the surface active molecules stabilizing the formation of nanoparticles, in contrast to high molecular weight proteins in case of fungi.

With the use of *Emblica Officinalis* fruit extract as reducing agent, the extracellular synthesis of highly stable Ag and Au nanoparticles has been achieved by Ankamwar *et al.* [10]. In their sequential study [11], the rapid synthesis of stable gold nanotriangles was became possible at high concentration using tamarind leaf extract as reducing agent. Similarly, experimentation was carried out by using Aloe Vera leaf extract as a reducing agent the synthesis of gold nanotriangles as well as silver nanoparticles [3]. Pretty good results were obtained in single crystalline triangular form while using aqueous chloroaurate ions with the Aloe Vera plant extracts. Further effects were studied by varying the amount of extract in reaction medium, percentage of gold nanotriangles to that of spherical particles as well as the size of nanotriangles (50–350 nm) have been modulated.

Bacteria based resource:

Bacteria have been most extensively researched for the synthesis of nanoparticles because of their fast growth and relative ease of genetic manipulation. Joerger *et al.* [4] and Klaus *et al.* [4] have isolated Silver producing bacteria from the silver mines *Pseudomonas stutzeri* AG259 and found accumulation of the silver nanoparticles in the periplasmic space but the particles size was ranging from 35 to 46 nm.

Magnetotactic bacteria such as *Magnetospirillum magneticum* produces two types of particles; some produce magnetic (Fe_3O_4) nanoparticles in chains and some produce greigite (Fe_3S_4) nanoparticles, while some other produce both types of nanoparticles [4]. Roh *et al.* [12] have synthesised magnetic nanoparticles by using magnetotactic bacteria.

Similarly Yong *et al.* [4] have synthesized palladium nanoparticles in the presence of exogenous electron donor, sulphate- reducing bacterium *Desulfovibrio desulfuricans* NCIMB 8307. The *Lactobacillus* strains present in the milk were exposed to larger concentration of nanoparticles to produced silver, gold, and alloy crystals of defined morphology, this work was reported by Nair and Pradeep [13].

Husseiny *et al.* [14] have used bacteria to synthesize gold nanoparticles. Recently, bacterial cell supernatant of *Pseudomonas aeruginosa* was used for the reduction of gold ions resulting in extracellular biosynthesis of gold nanoparticles [14].

Morphological control over the shape of gold nanoparticles has been achieved by using *Plectonema boryanum* UTEX 485, a filamentous cyanobacterium. When it was reacted with aqueous $Au(S_2O_3)_2^-$ and $AuCl_4^-$ solutions at 25–100°C for up to 1 month and at 200°C for 1 day resulted in the precipitation of cubic gold nanoparticles and octahedral gold platelets, respectively [4].

Lengke *et al.* [4] have documented the mechanisms of gold bioaccumulation by cyanobacteria (*Plectonema boryanum* UTEX 485) from gold (III) - chloride solutions. Accordingly interaction of cyanobacteria with aqueous gold (III)-chloride was initially promoted the precipitation of nanoparticles of amorphous gold (I)-sulfide at the

cell walls, and finally deposited metallic gold in the form of octahedral (III) platelets near cell surfaces and in solutions. During extension work to the mechanism, a sulfate-reducing bacterial enrichment was used to destabilize gold (I)-thiosulfate complex to elemental gold and proposed that this could occur by three possible mechanisms involving iron sulfide, localized reducing conditions, and metabolism [4].

Yeast based resources:

Yeast is indirectly associated with bacterial group as per its method of cultivation. Dameron *et al.* [4]. have used the *Candida glabrata* and *Schizosaccharomyces pombe* for the first time in the biosynthesis of cadmium sulphide (CdS) nanocrystals. These nanocrystals were produced using cadmium salts and are now used in quantum semiconductor crystallites. Further experiments were also carried out to improve the quantity of semiconductor CdS nanocrystals production that was achieved by using *Schizosaccharomyces pombe* cells. When these cells were incubated with 1mM Cd during their mid-log phase of growth, maximal nanocrystals were obtained by Kowshik *et al.* [15]. This study has suggested that the formation of CdS nanocrystals was dependent on the growth phase of yeast. When Cd was added during stationary phase, its uptake as well as production of CdS nanocrystals was decreased or resulted in no CdS formation. Upon adding Cd during early exponential phase of yeast growth, CdS nanocrystals were formed but this time it was affecting the cellular metabolism of the yeast and resulted in efflux of Cd from the cells [4].

The possible mechanism of decrease in CdS nanocrystals formation could be like this: upon exposure to Cd as a stress, a series of biochemical reactions were triggered to overcome the toxic effects of this metal. Firstly, an enzyme phytochelatin synthase was activated to synthesize phytochelatins (PC) that chelated the cytoplasmic Cd to form a low molecular weight PC-Cd complex and ultimately transport them across the vacuolar membrane by an ATP-binding cassette-type vacuolar membrane protein (HMT1).

Ortiz *et al.* have suggested that in addition to Cd, sulphide could also be added to this complex in the membrane and that results in formation of high molecular weight $PC - Cd S^{2-}$ complex that also allow them to sequester into vacuole. Conditions have also been standardized for the synthesis of large quantities of silver nanoparticles by using silver-tolerant yeast strain MKY3. The procedure for separation of these silver particles has also been documented by Kowshik *et al.* [16] and that was based on differential thawing of the samples.

Recently, Gericke and Pinches have identified yeast strains for their ability to produce gold nanoparticles. Accordingly with growth control and other cellular activities; controlled size and shape of the nanoparticles was achieved [4].

Fungi based resource:

The fungi taking the centre stage of studies on biological generation of nanoparticles because of the tolerance and bioaccumulation [4]. Fungi are efficient secretor of extra cellular enzymes which can easily obtain for large scale production of enzymes. Thus the biggest advantage of using fungi lies in their scale up process. Further advantages of using fungal mediated green approach for synthesis of metallic nanoparticles include economic viability and ease in handling biomass. However, the main drawback of biosynthesis of nanoparticles in eukaryotic organisms lies in the problem of genetic manipulation of the organism as a mean to over express the enzymes which is relatively much more difficult in eukaryotes than that in prokaryotes.

Mukherjee *et al.* [17] have demonstrated “green synthesis” of highly stabilized nanocrystalline silver particles by a nonpathogenic and agriculturally important fungus, *Trichoderma asperellum*. During their experimentation bioreduction of aqueous $AuCl_4^-$ ions was carried out using the fungus *Verticillium sp.* that led to the formation of gold nanoparticles with fairly well-defined dimensions and good monodispersity. Accordingly trapping of $AuCl_4^-$ ions on the surface of fungal cells could occur by electrostatic interaction with positively charged groups such as, lysine residues in enzymes that are present in the cell wall of the mycelia. Even gold ions were reduced by enzymes within the cell wall leading to aggregation of metal atoms and formation of gold nanoparticles. However, they could not find the exact mechanism for the formation of gold nanoparticles. Hence fungi are known to secrete much higher amounts of proteins, so they have significantly higher productivity of nanoparticles in biosynthetic approach. This was substantiated by the conclusion drawn for the entire study of Mukherjee *et al.* [17], accordingly compared to bacteria, fungi could be a source for large amount production of nanoparticles.

Towards elucidating mechanism of nanoparticles formation, an *in vitro* approach was followed where species specific NADH dependent reductase, released by the *Fusarium oxysporum*, were successfully used to carry out the reduction of $AuCl_4^-$ ions to gold nanoparticles. This has first time opened up a novel fungal/enzyme-based in

vitro approach for nanomaterials synthesis by Mukherjee *et al* [18]. Based on properties of *Fusarium oxysporum*, it was also used in the formation of extremely stable silver hydrosol by Ahmad *et al.* [3]. The acidophilic fungus *Verticillium sp.* has capability of producing gold as well as silver nanoparticles upon their incubation with Ag^+ and $AuCl_4^-$ ions Sastry *et al.* [3]. However, a novel biological method for the intra- and extra-cellular synthesis of silver nanoparticles using the fungi, *Verticillium* and *Fusarium oxysporum* respectively has been documented. This has opened up an exciting possibility wherein the nanoparticles may be entrapped in the biomass in the form of a film or produced in solution, both having interesting commercial potential [3].

The fungus, *Aspergillus flavus* also resulted in the accumulation of silver nanoparticles on the surface of its cell wall when incubated with silver nitrate solution Vigneshwaran *et al.* [19]. Extracellularly produced nanoparticles were stabilized by the proteins and reducing agents secreted by the fungus. A minimum of four high molecular weight proteins released by the fungal biomass have been found in association with nanoparticles. One of these was strain specific NADH-dependent reductase. However, emission band produced by fluorescence spectra indicated the native form of these proteins present in the solution as well as bound to the surfaces of nanoparticles [3].

Reduction of metal ions and surface binding of the proteins to the nanoparticles did not compromise the tertiary structure of the proteins [3]. Endophytic fungus *collitotrichum sp.* growing in the leaves of geranium was used for the synthesis of stable and various shaped gold nanoparticles. Reducing agent in this fungus were also polypeptides/enzymes Shankar *et al.* [9]. Instead of fungi culture, isolated proteins from them have been used successfully in nanoparticles production. Nanocrystalline zirconia was produced at room temperature by the cationic proteins. These proteins were similar in nature to silicatein, secreted by the *Fusarium oxysporum*, and were capable of hydrolyzing aqueous ZrF_6^{2-} ions extracellularly Bansal *et al.* [20].

Growth conditions play an important role during the production of nanoparticles while using the fungi cultures. When gold ions were incubated with the *Trichothecium sp.* biomass under stationary conditions led to the formation of extracellular nanoparticles. While under shaking conditions, this was resulted in the formation of intracellular gold nanoparticles. The possible reason for this could be the enzymes and proteins responsible for the synthesis of nanoparticles. These proteins were released into the medium under stationary conditions and did not release under shaking conditions Ahmad *et al.* [3].

Bharde *et al.* [3] reported the synthesis of magnetic nanoparticles by using *Fusarium oxysporum* and *Verticillium sp.* at room temperature. Both fungi secreted proteins which were capable of hydrolyzing iron precursors extracellularly to form iron oxides predominantly in the magnetite (Fe_3O_4) phase. Also a nitrate-dependent reductase and a shuttle quinone from several *Fusarium oxysporum* strains were found to be involved in extracellular production of silver nanoparticles or silver hydrosol. However, it was not true with all *Fusarium sp.* *Fusarium moniliforme* produces reductase enzyme but could not form silver nanoparticles upon their incubation with silver ions Duran *et al.* [21]. However, by controlling the amount of cofactor NADH, synthesis of quite stable Au–Ag alloy nanoparticles of various compositions have been made possible. This approach was further employed for producing various other composite nanoparticles by Senapati *et al.* [22]. During elucidating the mechanism of synthesis of nanoparticles, a- NADPH-dependent nitrate reductase and phytochelatin isolated from *Fusarium oxysporum* was used for in vitro silver nanoparticle production by Kumar *et al.* [3].

Minimum time, miniaturization and non-hazardous processes are key parameters for any kind of technology acceptance. In this effort, Bhainsa and D'Souza [3] could get fairly monodispersed silver nanoparticles within 10 min by using *Aspergillus fumigatus*. This was the first report of such rapid synthesis of nanoparticles using fungus. The production was even faster compared to the physical and chemical processes of nanoparticles synthesis. Hence, this process could be suitable for developing a biological process for mass scale production of nanoparticles. Tetragonal barium titanate ($BaTiO_3$) nanoparticles of sub-10 nm dimensions produced by *Fusarium oxysporum* under ambient conditions have been observed an ecofriendly and economically viable methods for the synthesis of complex oxide nanomaterials of technological interest Bansal *et al.* [23].

The presence of ferroelectric properties in these nanoparticles would revolutionize the electronic industries by making ultra-small capacitors and ultrahigh density nonvolatile ferromagnetic memories. Also, the synthesis of highly luminescent CdSe quantum dots at room temperature, reported recently by the fungus, *Fusarium oxysporum* when incubated with a mixture of $CdCl_2$ and $SeCl_2$ would be of great importance [3]. Recently, *Fusarium oxysporum* fungus has also been used for the production of silica and titania nanoparticles from aqueous anionic complexes SiF_6^{2-} and TiF_6^{2-} respectively. Extra-cellular protein-mediated hydrolysis of the anionic complexes results in the facile room temperature synthesis of crystalline titania particles while calcination at 300 °C is required for crystallization of silica [3].

Actinomycetes based resource:

The monodispersity of the silver and gold nanoparticles produced either intracellular or extracellular is of interest to scientists. But it was not very high and was far inferior to that obtained by conventional methods [4].

Most of the actinomycetes especially the thermophilic actinomycetes, *Thermomonospora* sp. when exposed to gold ions reduced the metal ions extracellular, yielding gold nanoparticles with a much improved polydispersity [4]. However, in an effort towards elucidating mechanism or conditions favouring the formation of nanoparticles with desired features, Ahmad *et al.* [24] carried out the reduction of $AuCl_4^-$ ions by using an extremophilic *Thermomonospora* sp. biomass that has resulted in efficient synthesis of monodisperse gold nanoparticles.

The reduction of metal ions and stabilization of the gold nanoparticles were believed to occur by an enzymatic process [3]. They compared their earlier work of synthesis of polydisperse gold nanoparticles by using *Fusarium oxysporum* to this study and concluded that the monodisperse gold nanoparticles synthesis could be due to extreme biological conditions such as alkaline and slightly elevated temperature conditions used for the synthesis of nanoparticles.

Based on the hypothesis alkalotolerant *Rhodococcus* sp. has been used for intracellular synthesis of good quality monodisperse gold nanoparticles. They observed that the concentration of nanoparticles were more on the cytoplasmic membrane than on the cell wall. This could be due to reduction of the metal ions by enzymes present in the cell wall and on the cytoplasmic membrane but not in the cytosol. These metal ions were not toxic to the cells, which are producing them, and were continued to multiply even after the biosynthesis of gold nanoparticles [3]. These micro-organisms have developed numerous special adaptations to survive in such extreme habitats which include new mechanism of enzyme transduction, regulating metabolism, maintaining the structure and function of the membrane. Even though the Actinomycetes have close relation with the mycobacterium and coryneforms, they were originally designated with 'ray fungi' (Strahlenpilze). These actinomycetes are able to produced secondary metabolites. The nanoparticles were also found to be non-toxic to the cells which continued to multiply even after the formation of the nanoparticles [4].

Algae based resource:

Algae are similar to yeast for biosynthesis of nanoparticle, still very few reports used algae as a "Bio factory" for the nanoparticles synthesis. The marine algae used for the biosynthesizing of highly stable extracellular gold nanoparticles in a relatively short time period compare to other biosynthesizing process. Palladium and platinum nanoparticles starting with their corresponding metallic chloride- containing salts have been investigated [4].

Viruses based resource:

Biological synthesis of nanoparticles has been extended to biological particles like viruses, proteins, peptides and enzymes. Cowpea chlorotic mottle virus and cowpea mosaic virus have been used for the mineralization of inorganic materials [4]. Tobacco mosaic virus has shown to direct successfully the mineralization of sulphide and crystalline nanowires. One step further, peptides capable of nucleating Nano crystal growth have been identified from combinatorial screens and displayed on the surface of M13 bacteriophage [4].

III. SUMMARY

Nanotechnology has found commercial success in short duration due to its outright performance at an economical rate. Hence conventional techniques like chemical, mechanical and physical for the synthesis of nano particle were not able to score well due to shortfalls ground in terms of toxicity level, higher energy conservation or fear of material damage respectively. Biosynthesis technique developed two decades back has provided superior option in all such regards and becomes technology of modern world

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