Current Research in Microbiology

Chapter 2

Microbial Production of Nanoparticles and their Applications

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Abstract

Nanoparticles are particles that have size of 100 nm or less with one or more dimensions has gained larger attention due to their characteristic and unique properties apart from wide range of applications over their other counterparts. The physical, chemical, biological and hybrid ways of synthesis of nanoparticles is dependent on the requirement and type of nanoparticles however for clinical and biological application the chemical methods have proven to be toxic to the living system therefore better and safer alternatives are chosen like biological methods of production of nanoparticles. In biological methods the use of microorganism for production of nanoparticles is gaining lots of attention for being economical, rapid and safer alternative to physical and chemical methods. The wide range of microorganism and their potential to adapt in different environment gives them an edge over other ways. The microbial production of nanoparticles is the part of microbial growth that involves two processes: reduction process and precipitation process. The latter is further achieved by either nucleation or crystal growth. The entire production is controlled by controlling the growth parameter of the microbes. Thus the process is simpler and economical but is slow and time consuming as compared to chemical ways, however the quality and quantity of the nanoparticle is far better in biological methods than in chemical methods.

1. Introduction

Nanoparticles are those entities of matter that have one or more dimensions ranging from 100 nm or even less. These particles have higher surface area but smaller size making them a better alternative for application than their bulk counterparts [1,2]. The physical, chemical, biological and hybrid ways of synthesis of nanoparticles is dependent on the requirement and type of nanoparticles [3-6].

Since physical and chemical methods are more fastidious and give high yield of nanoparticles, they are most popular ways for nanoparticle synthesis, however the toxicity in the living system due to use of chemicals greatly limits their biomedical applications, particularly in clinical use. Secondly it was found that biogenic nanoparticles had greater potentials to include wider varieties and different shapes, compositions, coatings and structures of nanoparticles with special properties as compared to their chemical counterparts [7]. Thirdly it was reported that even if synthetic nanoparticles are not used directly to the living system yet their accumulation was found because of use of certain daily products like consumer products which contains trace amount of nanoparticles that can lead to their accumulation into the living system which is harmful for both prokaryotic and eukaryotic system [8-9]. By using microorganisms for synthesis of nanoparticles, a reliable, nontoxic and eco-friendly methods is designed that is of utmost importance to expand the biomedical applications of nanoparticles and also keeping in mind the environmental hazard the accumulation of synthetic nanoparticles can lead to.

Biological entities of matter have tremendous property to produce variety of potential nanoparticles. If fully understood and deciphered, these entities can be used for large scale production of almost all types of nanoparticles at industrial level manufacturing. The biologically aided synthesis not only decreases the consumption of energy and toxic chemicals but also opens the path for environmentally friendly green manufacturing [10].

The use of bacteria among all biological systems for production of metal and metal oxide nanoparticles of various sizes, compositions and properties are well documented. For example the use of Bacillus sp. for reduction of Tellurium to Rosette- aggregated rod shape nanoparticles of size approximately 30x200 nm and Selenium to 200 nm spherical nanoparticles [11,12]. Another example is of *Shewanella oneidensis*, a specialized bacterium with a property of reducing metals like Tellerium to spherical nanoparticles of size 50-80 nm [13] and *Magnetospirillum magneticum* that produces magnetic nanoparticles of 30-120 nm [14].

Despite of the fact that there are sufficient examples of different types of biological entities that can produce variety of nanoparticles of varying properties, yet there is a huge knowledge gap in understanding the mechanism behind the formation of those nanoparticles and the mechanism to control the final product is still unclear. There is still not sufficient information

that can lead to standardization of the process of formation of nanoparticles with specific desired properties, concentration and size. Similarly there is no possible information that can provide the ways to standardize the final product when the nanoparticles are used in a process. This knowledge gap refrains the use of biological agents for manufacturing of nanoparticles at industrial level. The bacterial based nano-manufacturing for mass production is precluded because of insufficient knowledge.

This chapter is an attempt to explore the available information about microbial production of nanoparticles as a salient need to develop a mechanistic understanding of the processes that lead to the formation of solid state nanoparticles by bacteria. The fundamentals that are derived from the natural microbial process are used in production of these nanoparticles, will be explored and studied to strengthen the further knowledge. Similarly the content will also cover the examples with their mechanism of production of nanoparticles so that the fundamentals can be well explained and can be used as a document for further research studies (**Figure 1**)

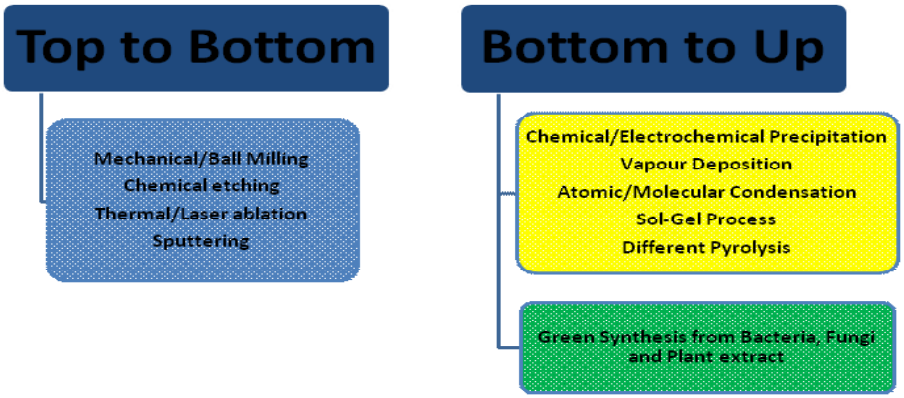

Figure 1: Synthesis of Nanoparticles

2. Mechanism of Production of Nanoparticles

The nanoparticles from microbes are produced by microbial enzymatic reactions that are far superior to the chemical reactions and far more rapid in production of reactant free specie for nanoparticle production. The green approach is far more suitable as the production of nanoparticles of different size and type can be calibrated by changes in pH, temperature and pressure, the conditions that defines the growth of majority of bacteria that are used to produce nanoparticles. The biochemistry that lies behind the phenomena is simple enzymatic actions that are involved in production of nanoparticles. By calibrating the ambient conditions the catalytic sites of interactions are changed. This produces highly reactive species that leads to higher catalytic action, strong binding between the enzyme and metal precursor and increased binding potential because of increase in specific surface area. The nanoparticles are hence formed when the microbial enzymes feed on the metal precursor as substrate from the environment and reduce it to reactive metal specie that acts as precursor for formation of nanoparticles. The synthesis of nanoparticles can be intercellular or extracellular depending on the location of formation of nanoparticles [17,18]. In the intercellular synthesis the metal

substrate from the environment is transported into the cell where microbial enzymes acts on it and reduce it to reactive specie leading to formation of nanoparticle inside the cell whereas in extracellular the metal substrate is trapped on the cell surface and the enzyme is transported or excreted out of the cell where it reacts with the metal ion on the cell surface to reduce it to reactive species thereby the nanoparticle is formed on the cell surface [19]. In general the use of microorganisms leads to the nanoparticle formation by two distinct approaches. First is: Bottom up approach in which the supersaturated solution is made to saturate more till it settles down in some phase and nanoparticles of particular size is produced. Second is: Top down approach in which the organic polymer produced by microorganism leads to the nucleation of first reactive specie also called as nanoparticle seed. These organic polymers modify the nucleation process of the nanoparticle seed by either favouring it or by inhibiting it, either way it can stabilize the molecule to produce the nanoparticle of particular size.

The bacterial production of metallic nanoparticles is by two processes:

a. Reduction Process

b. Precipitation Process

The precipitation process is further achieved by two ways

a. Nucleation

b. Crystal growth

From the above two processes, the reduction process is most studied and documented than precipitation process by nucleation or by crystal growth.

2.1. Reduction process of nanoparticle synthesis by microbes

The microbes use their reducing agents in formation of nanoparticles from its precursor molecule. These reducing equivalents can be taken by inorganic compounds as in lithotrophs or by organic compound as in organotrophs thus these precursors of nanoparticles act as substrates for reducing agents. The reduction of metals to their corresponding sulphides by metal reducing bacteria is an example of such reduction mechanism. The variety of metal nanoparticles produced by microorganisms are deposited in the cytoplasm, periplasm, and extracellular area or on the cell surface. These nanoparticles are produced either by energy conserving metal reduction dissimilation process or by cell building assimilation or by both as in co- metabolism. Such nanoparticles generally helped in remediation process as most of them lowered the concentration of toxic compounds. Usually such reduction processes occur in growth phase of microbial culture but some of them were reported in stationary phase too or can be produced extracellular by isolated microbial enzyme from the growing culture [15]. With the biological

chemical reduction method the M^+ stage is converted to M0 active stage or formation of free radicle is there that initiates the further production of large amount of nanoparticles in low cost and less time. Secondly it is easier to tune the formation of nanoparticles of varying size and tune by just changing the reducing agent, the dispersing agent, temperature and time. However in case of microbial production of nanoparticles no reducing agent is added from outside as the biological entity itself has reducing agent in large amount in growing microbial culture, that are highly reactive and capable of producing the nanoparticles in less time. However the dispersing agent can be added to give the desired size to the nanoparticles. Since no chemical agent like reducing agent is added from outside the amount of impurity is lesser than in chemical production of nanoparticles. So this process is used in biological manufacturing of nanoparticles. Once the nanoparticles are formed they are then precipitated by crystal growth or by nucleation (**Figure 2**).

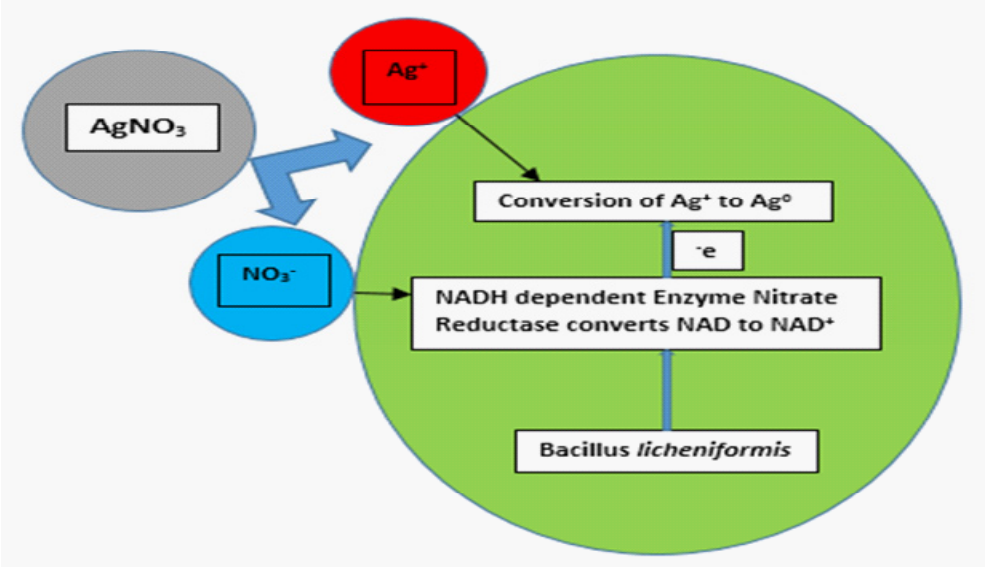

Figure 2: Example of Microbial Synthesis of Nanoparticles

2.2. Precipitation of nanoparticles

There are many ways of separation of Nanoparticles like poly condensation of sol to gel or gel to sol state sedimentation as seen in metallic nanoparticles. The aggregation of crystals can be by simple drying after the liquid phase sedimentation. In order to form mono disperse nanoparticle of particular size it is important that crystal grows at very slow and steady rate from the rapidly generating seed particles. Once the desired nanoparticles is formed then dispersing agent is added to avoid further aggregation like addition of citrate as dispersing agent in formation of gold nanoparticles. Once the desired size nanoparticles is achieved then it is separated by various methods like the sedimentation method of nanoparticles by co-precipitation or alkaline precipitation, by gel filtration, by gel electrophoresis and by centrifugal separation.

On the basis of fact that precipitation of nanoparticles is most fruitful method for sedimentation of desired nanoparticles from colloid with varying sizes of nanoparticles, new tech

niques are being used in this process. When the precipitation is done under high gravity conditions, it produces larger amount of nanoparticles and that too in low cost. This technique is finding its application for industrial production of nanoparticles because of its low cost and environment friendly green approach. This technique is called HIGH GRAVITY REACTIVE PRECIPITATION (HGRP) [16].

3. Production of Nanoparticles by Microbes

The microbes are those biological entities that are well studied for their constant environment interactions. Therefore they have wide range of energy producing processes ranging from organotrophy to lithotrophy in presence of oxygen or in absence of it, thus using different bio-mechanism as per the environmental need. This basic fact led to the use of microbes for production of nanoparticles by lithotrophy in presence of air or in absence of it. The commonest nanoparticles produced by microbes are discussed here in details to give an insight of the biosynthesis of nanoparticles by the microbes (**Table 1**). The nanoparticles are divided under four categories: Metallic nanoparticles (Au, Ag, Alloy, and metal nanoparticles), Oxide nanoparticles (metallic and non-metallic oxide nanoparticles), Sulphide nanoparticles and other miscellaneous nanoparticles. Each type when studied in details helped in understanding the mechanism behind the production of nanoparticles by microbes.

3.1. Metallic nanoparticles

The metallic nanoparticles show different optical property that is of the fundamental attraction and characteristics of nanoparticle. In general these properties are size dependent ranging from 1-100 nm. The metallic nanoparticles have different physical and chemical properties than bulk metals and so they exhibit optical characteristics for example 20 nm Au-Np exhibit wine red colour, Ag-Np exhibit yellowish grey and Pd-Np; Pt-Np exhibit black colour.

The metallic nanoparticles are produced by microbes as a result of reduction of metal to free radicle specie that aggregates to form nanoparticles. Thus M^+ is converted to M^0 in presence of reducing agent of microbial origin or system.

The environmental toxicity is also largely due to accumulation of heavy metals that are toxic to microbes as well, however there are certain microbes that are resistant to these heavy metals and can use them as substrate in one or the other biochemical reaction for generation of energy as ion efflux from the cell by membrane proteins that function either as ATPase or as chemiosmotic cation or proton anti transporters that causes chemical detoxification. This led to the research behind production of nanoparticles from heavy metals as well for example production of Palladium, Mercury, Platinum etc. nanoparticles apart from gold and silver or their alloy nanoparticles.

Microorganism	Nanoparticle	Culture Temperature (oC)	Size (nm)	Shape and Location
Shewanella oneidensis	Fe_3O_4	28	40–50	**Rectangular and Extracellular**
Saccharomyces cerevisiae	Sb_2O_3	25–60	2–10	**Spherical and Intracellular**
Lactobacillus sp.	TiO_2	25	8–35	**Spherical and Extracellular**
Fusarium oxysporum	$BaTiO_3$	25	4-5	**Spherical and Extracellular**
Brevibacterium casei	PHB (Poly hydroxybutyrate)	37	100–125	**Intracellular**
E. coli	CdS	25	2–5	**Wurtzite crystal and Intracellular**
Rhodobacter sphaeroides	ZnS	Unknown	10.5+/-0.15	**Spherical and Extracellular**
Desulfobacteraceae	FeS	Unknown	2	**Spherical and Extracellular**
Brevibacterium casei	Au, Ag	37	10–50	**Spherical and Intracellular**

Table 1: Common examples of Nanoparticles produced from microbes

The extracellular synthesis of gold nanoparticles by fungus *Fusarium oxysporum* and actinomycete *Thermomonospora* sp. and the intracellular synthesis of gold nanoparticles by fungus *Verticillium* sp. has been reported by Mukherjee, Sastry and co-workers [20,21,22]. Similarly it has been demonstrated that gold nanoparticles can be produced intercellularly inside the bacterial cell when it is incubated in media with Au^{3+} ions [23]. The study was also done in microbial synthesis of monodisperse Au nanoparticles from alkali tolerant *Rhodococcus* sp. in alkaline environment under bit high temperature [24]. The synthesis of Au nanoparticles in different structure was reported by Lengke et al. They claimed to produce Au nanoparticles of different structures like spherical, cubical and octahedral from filamentous cyanobacteria by using Au(I)- thiosulfate and Au(III) chloride complexes [25,26]. Similarly Lactate degrading bacteria, Lactobacillus was reported to produce nanocrystals and nanoalloys at the time of microbial log phase of growth by Nair and Pradeep [27].

The other metallic nanoparticles that are being produced rapidly by microbes are silver nanoparticles that has more importance in biomedical sector as they have antimicrobial activities that led to the development of biomimetic approach for their production. Since Vedic ages, silver is known for its antimicrobial activities and so the use of silver utensils for eating was very common and application of silver vark on sweets to prevent bacterial and fungal growth on them were common practices. It has been proved that they not only show antimicrobial activity against Gram negative and Gram positive bacteria but also against highly tolerant and multi resistant strains like methicillin resistant *Staphylococcus aureus* [28]. Various mi-

crobes are known to reduce the Ag^+ ions to form silver nanoparticles mostly spherical in shape [29–31]. The research group has also reported to produce nanoparticles from a specific strain isolated from a silver mineof Pseudomon *asstutzeri* which they tagged as AG259. This strain produced silver nanoparticles within periplasmic space when placed in concentrated aqueous solution of Silver Nitrate. The bacterium produced free reactive Ag^0 species from silver nitrate solution by reduction of Ag^+. It was found that the Ag nanoparticles so formed were deposited in periplasmic space in bacterial cell [32]. On other hand when fungi, *Verticillium, Fusarium oxysporum, or Aspergillus flavus,* were employed, the synthesis of Silver nanoparticles were in the form of afilm or they were released in solution or they aggregated on the cell surface [33].

After the production of silver and gold nanoparticles, nanoparticles in form of alloy hold numerous application in the field of electronics, alloy coatings, as catalyst in reactions and as optical material for communication etc. [34].

Moving ahead with alloy nanoparticles, Senapati *et al.* reported that fungi *F.oxysporum* can synthesise hybrid alloy of Ag-Au in presence of Co-factor NADH secreted indigenously by the microbe that even decides the composition of the alloy [35]. Similar hybrid alloy of Ag-Au is also reported to be synthesised by yeast cells by *Zheng et al.* that after the synthesis of alloy, did the characterisation by fluorescence microscope and transmission electron microscope that indicated that the alloy is produced extracellularly in form of polygons. Similarly by the same group the electrochemical study stated that the vanillin sensor was a modified glass carbon electrode with Ag-Au alloy coatings that enhanced the electrochemical activity of vanillin by five folds [36]. After the report of synthesis of polygonal hybrid alloy of Ag-Au, there were reports of synthesis of core shell alloy nanoparticles of Ag-Au that were synthesised by fungus Fusarium *semitectum* and these nanoparticles were found to be highly stable in suspension for many weeks. This study was done by *Sawle et al.* [37].

In the genre of metallic nanoparticles is the new edition of heavy metal nanoparticles synthesised by metal resistant microbes. The use of metal ion-reducing bacterium Shewanella *algae* for production of Platinum nanoparticles in periplasm of 5nm size is a microbial biochemistry of reduction of Platinum chloride to Platinum free radicles at room temperature and p^H within an hour in presence of Lactate as electron donor [38]. Similarly Mercury nanoparticles of size 2-5 nm were prepared by Enterobactersp. at slightly alkaline p^H of 8 and lower concentration of mercury lead to increase in chemical detoxification [39]. The palladium nanoparticles could be synthesized by the sulphate reducing bacterium, Desulfovibrio *desulfuricans*, and metal ion-reducing bacterium, *S. Oneidensis* mentioned earlier [40]. Similarly the metal resistant bacteria that use Hydrogen as electron donor are capable of reducing large amount of heavy metals like Chromium, Uranium, and Cobalt etc. [41].

3.2. Magnetic and Non- magnetic Oxide Nanoparticles

Magnetic oxide nanoparticles and nonmagnetic oxide nanoparticles are important type of compound nanoparticles that are synthesized by microbes. The magnetic Nanoparticles have gained so much importance because of their unique micro configuration and super paramagnetic properties. Biocompatible magnetic nanoparticles like Fe_3O_4 (magnetite) and Fe_2O_3 (Maghemite) are found to be clinically safe. Since they are biocompatible, they can be used for clinical application as in targeted cancer treatment, sorting and manipulation of stem cell, site directed drug delivery, targeted gene therapy, targeted DNA analysis, and identification by magnetic resonance imaging (MRI).

The microbes used for production of such nanoparticles are Magnetotactic bacteria. These bacteria are capable of synthesizing intracellular magnetic particles that comprises of iron oxide, iron sulfides, or even both [42,43]. These magnetic particles being of microbial origin are enveloped by phospholipids and proteins organic membranes that can easily disperse them in aqueous solutions. Furthermore, an individual nanoparticle or magnetite is a mini magnet that contains a single magnetic domain that yields higher magnetic properties [44]. The members of the family Magnetospirillaceae are the bacteria that are found to produce the maximum number of magnetic nanoparticles or to say it this way that to date the maximum number of magnetotactic bacteria belong to this family. These bacteria are found in fresh water sediments and they were segregated from other fresh water bacteria by differential growth medium and magnetic isolation techniques. The bacteria can be chemoorganotroph or chemolithotroph. The first isolated bacteria of this family was *Magnetospirillum magnetotacticum*, identified as strain MS-1 [45]. Mostly cultured magnetotactic bacteria are mesophilic and tend not to grow much above 30^{o}C however uncultured magnetotactic bacteria were mostly at or below 30^{o}C with only few reports describing thermophilic magnetotactic bacteria. These bacteria tend to form magnet aggregates lined in form of chain along the geometric north of the earth and often cluster in periplasm or intercellular spaces that help the bacteria to move in oxygen gradient under the influence of Earth's magnetic field. It was reported that magnetic Fe_3O_4 nanomaterials with mesoporous structure were synthesized by co-precipitation method using yeast cells as a template [46,47] that led to precipitate out the magnetic oxide nanoparticle from the growing bacteria without its lysis.

Beside magnetic nanoparticles, large number of nanoparticles were produced from nonmagnetic elements too. The mechanism of production remains the same. It was reported by Jha and co-workers that biosynthesis of Sb_2O_3 nanoparticles can be mediated by Saccharomycescerevisiae and this green process is economical and reproducible [48]. Similarly Bansal et al. used *F. oxysporum* (Fungus) to produce SiO_2 and TiO_2 nanoparticles from aqueous anionic complexes $SiF_6^{2-}$and TiF_6^{2-}, respectively [49].

3.3. Sulphide and other Nanoparticles

The next in the generation of nanoparticles used extensively in biomedical fields as cell labelling agents, for protein targeting and for developing quantum dots as they exhibit novel electronic and optical properties [50]. These are Sulphide nanoparticles of CdS nanoparticle is the commonest example that act as quantum dots in technical applications apart from labelling agent. These quantum dots were formed by the reaction of Cd^{2+} ions with sulphide ions which were produced by the enzymatic reduction of sulphate ions to sulphide ions (SO_4^{2-} to S^{2-}, Cd^{2+} $S^{2-} \rightarrow CdS$). The sulphate reducing bacteria use sulphur as electron donor that act as reducing agents to reduce metal sulphates to their corresponding metal sulphides.

It was found that *Clostridium thermoaceticum* could precipitate CdS from $CdCl_2$ on the cell surface as well as in the medium in the presence of Cysteine Hydrochloride in the growth medium as Sulphide source [51]. Similarly *Klebsiella pneumonia* and *E. Coli* were reported to form CdS on cell surface when grown in media with Cd^{2+} ions [52]. The production of CdS and other commonly produced nanoparticles of ZnS and PbS were synthesised from *Rhodobacter sphaeroides* and *Desulfobacteraceae* and the diameter of the nanoparticles were controlled by the culture time [53-55]. Production of magnetic nanoparticles like Fe_3S_4 or FeS nanoparticle from uncultured magneto tactic sulphate reducing bacteria was also reported [56,57]. The sulphide nanoparticles can also be generated extracellular by the fungus Fusarium oxysporum when exposed to aqueous solution of metal sulphate [58].

Other Nanoparticles: In nature the compounds are never in free form they are always bound to one another for stability and better interaction, such compounds are called biopolymers that can be synthesised by other biopolymers like proteins or by using microbes for example $PbCO_3$, $CdCO_3$, $SrCO_3$, PHB, $Zn_3(PO_4)_2$, and CdSe nanoparticles were reported to be synthesized by microbes like *Fusarium oxysporum* [59,60] and Yeast [61] that can form nanoparticles in form of crystals or in form of powder.

4. Microbial Biochemistry of Production of Important Nanoparticles

The microorganism are the biological entities that have more than one mechanism for living and they can use many different ways to produce nanoparticles. The metal ions are first trapped metal ions are first trapped on the surface or inside of the microbial cells that are then reduced to nanoparticles in the presence of enzymes. The exact mechanism of intracellular formation of nanoparticles is not well understood, however the presence of silver and gold nanoparticles on the surface of the algal mycelia supports the theory. The precursor ions of these nanoparticles are found to be trapped in surface of microbial cell via electrostatic interaction between positive charge on ions and negative charge on microbial cell surface where the enzymes reduces the metal ions to form gold and silver radicles that further forms nuclei and grow through further reduction and accumulation. Some workers speculated that the syn-

thesis of silver nanoparticles in *B. licheniformis* is mediated by nitrate reductase enzyme. The possible mechanism involving this enzyme could be reduction of silver ions to reactive silver specie because of electron activity due to reduction of nitrate ions. This generates Co factor NADH, a powerful reducing agent that further reduce silver ions [62]. It has to be noted that the synthesis of metal nanoparticles in presence of enzyme reductase is directly dependent on NADH and if not in presence of enzyme then only NADH in system acts as an important factor.

The formation of heavy metallic nanoparticles from heavy metal ions like Hg^{2+}, Cd^{2+}, Ag^{+}, Co^{2+},Cu^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} as discussed earlier is due to the metallophilic microbes that have potential to synthesise heavy metal nanoparticles in presence of toxic heavy metals. These bacteria develop heavy metal resistance in order to survive in the heavy metal toxicity. The microbes were thus well adapted in heavy metal environment and developed metal homeostasis gradually. This generated a unique genetic and proteomic responses in this bacteria to toxic environment like mines, waste rock piles, metal processing plants drains or natural mineralized zone of earth. Such responses were uncommon in bacteria inhabiting the normal surrounding [63,64,65].

The formation or bio mineralization of bacterial Magnetic Nanoparticles and its molecular mechanism is hypothesized to be a multistep process as following:

1. The mechanism of vesicle formation: It is proposed that the process vesicle formation resembles the process of formation of mesosomes in eukaryotes that is an energy dependent process and utilises GTPase enzymes at time of invagination. Similarly the invagination of cytoplasmic membrane forms vesicle in presence of GTPase enzyme. These vesicles are seeds to bacterial magnetic nanoparticles that are surrounded by phospholipids and protein organic membranes because of invaginations of cytoplasmic membranes.

2. The linear arrangement of vesicle: the arrangement of formed vesicles is in the linear form along with cytoskeletal filaments. This form linear chains of small magnets surrounded by organic membranes called as magnetosomes.

3. Accumulation of iron ions: The accumulation of ferrous ions occurs into the vesicles with the help of iron transporters that are transmembrane proteins or by siderophores, maintaining the external ion concentration by the process called biomineralization, however the internal ion concentration is maintained by simple cellular oxidation-reduction system.

4. Nucleation: This process is the final stage. The magnetosomes bounded by organic membranes form magnetite crystals because of the process of nucleation. There are various proteins associated with the bacterial magnetic particle membrane that play functional roles involved in magnetite generation. The last step involves accumulation of supersaturating iron

concentrations inside the cell. The high concentration causes partial reduction and dehydration in case ferrohydrite is used for production of magnetite crystalsor else it is the maintenance of reductive conditions and the oxidation of iron to induce mineralization to magnetite.

The formation of magnetic nanoparticles from bacteria like *Shewanella oneidensis* has already been discussed earlier, however the mechanism that involves the production of magnetites consists of both passive and active mechanisms. It involves following two steps:

1. Production of Fe^{2+}: The utilization of ferrohydrite by bacteria as a terminal electron acceptor for active production of Fe^{2+} and the pH value surrounding the cells rises probably due to the bacterial metabolism of amino acids.

2. Localization of Iron ions: The localized concentration of Fe^{2+} and Fe^{3+} at the net negatively charged cell wall, cell structures, and/or cell debris is through a passive mechanism that induces a local rise of supersaturation of the system with respect to magnetite, causing the magnetite phase to precipitate. Thus the precipitation of nanoparticle crystals by simple low cost and efficient method is only feasible because of microbial interaction.

Next in the line are quantum dots or CdS nanoparticles produced from sulphur reducing bacteria that follows the following steps:

1. Breakage of Cysteine bridges on Cell surface of bacteria: The proposed mechanism can be because of disulphide (cysteine) bridges in cell structure of the bacteria and may be because of cleavage of S–H bond and formation of a new bond, that is, –S–Cd bond where Cd is from Cd-thiolate ($Cd–S–CH_2COOH$) causing the nanoparticle production on the surface.

2. Interaction of Cd-thiolate group: It has to be noted that the –COOH groups from the cadmium-thiolate complexes do not react with the $–NH_2$ groups of protein on the cell surface of the bacteria because of electronegative potentials but interact with hydrogen bond.

3. Capping of CdS Nanoparticles: The capping the CdS nanoparticles is because they are bonded to $–NH_2$ groups by hydrogen bond [66] and one of the oxygen atoms of the carboxylic group (–COOH) forms the coordinate bond between the oxygen atomand Cd^{2+} ions [67], thus on grounds of electric potentials it competes with the thiol group of cysteine bridges to assemble onto the surfaces of the CdS nanoparticles causing its capping. This leads to accumulation of CdS nanoparticles on the surface of bacteria.

4. Application of Nanoparticles Produced from Microbes and Future Prospects

There are many applications of nanoparticles but when it comes to biomedical application the nanoparticles risk analysis needs to be done. To overcome this problem the green production of nanoparticles was done. As a result the use of nanoparticles from such biologi-

cal producers makes them a safer option in nanomedicines and nanotherapeutics involving safe delivery of drugs, proteins or targeting of oncogenes or immune systems etc. The field of nanomedicines in diagnosis and treatment, primarily of human diseases is an upcoming avenue in the field of research thriving for continuous improvement and standardization. The biosynthesis of nanoparticles by microbes makes it a safer option in this field of nanomedicines as the green chemistry procedure is found to be clean, biocompatible, nontoxic and environmentally safe. The production of desired nanoparticle can be manipulated as to its intracellular and extracellular synthesis that employs the use of microbes, right from bacteria to actinomycetes depending upon the location where the nanoparticles to be formed.

The second property of these green nanoparticles is that by simple manipulations of p^H, temperature and other growth conditions like substrate concentration and exposure time, the rate of intercellular production of nanoparticles and their sizes can be calibrated. Some changes occur in exponential phase and some occur in lag and few in stationary phase of microbial growth (**Figure 4**).

Thus these green particles have some chief applications as mentioned below;

1. In Cancer targeted treatment: The use of Iron nanoparticles like Magnetite and Maghemite for targeted cancer treatment as already been reported as they are biocompatible and has role gene therapy and DNA analysis. They also hold application in MRI imaging and stem cell sorting that further helps in tracking the oncogenesity. It was also found that when these magnetosomes were used on mammalian immune system then they showed neutral behaviour without altering the immunology of the host [68]. In another experiment directly the drug duboroxin, an anti-tumour drug was loaded on magnetosomes and it was found to effectively target and kill the tumour cells without effecting the normal cells [69]. Thus proving that these magnetosomes can be effective carriers of drug, gene or any other therapeutic for cancer treatment. Similarly Silver nanoparticles were found to be anti angiogenic and exhibited caspase dependent apoptosis of the tumour cell line. Thus it can be seen that these green nanoparticles are capable of acting in more than one way.

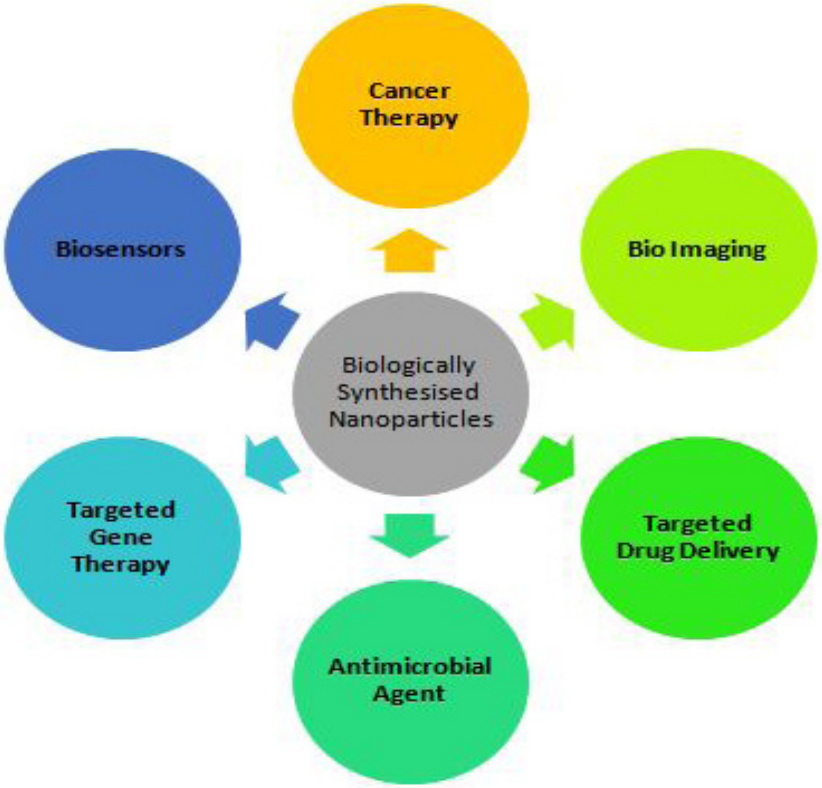

Figure 4: Application of biologically synthesised nanoparticles

2. The targeted drug delivery: The sulphide nanoparticles, the iron nanoparticles were reported to be the best drug delivery carriers as they bear all the properties of a good carrier, being smallest in size, bear large surface area, are biocompatible and inert and most importantly can cross the blood brain barrier and surface epithelial junctions without being rejected. These carriers had potential to distribute the drug at the targeted site without causing its accumulation elsewhere and the probability of drug toxicity is reduced. They have improved pharmacokinetics and biodistribution of therapeutic agents. The use of magnetosomes and the bacterial cell as whole with magnetosomes as carriers for drug delivery has been used extensively. The bacterial cells with magnetosomes can be derived to the targeted area under the influence of magnetic field, however for this MRI imaging is very important that can show the movement of bacteria to the targeted site. Once the bacteria reaches the targeted site then the magnetosomes on its surface deliver the drug to the target and the treatment of the disease is there [70]. Similarly the use of gold as therapeutic agent has been since a very long time and their nanoparticles are far more effective than the compound because of smaller size, high surface to volume ratio, unique optical and electronic properties and are tuneable. These gold nanoparticles can be easily modified by binding ligands that exhibit gold affinity like thiols, amines and phosphines that increase the reactivity of these particles. This has made them more promising for drug and gene delivery. Thus the nanoparticle-mediated targeted delivery of drugs modifies chemotherapy by reducing the dosage of less specific and highly toxic anticancer drugs and by using chemo drugs with better specificity that enhances the efficacy of therapy and causes low toxicity in the system. The process will be less expensive and fastidious. Secondly it will be biocompatible so chances of rejection is also ruled out. Thus the upcoming trends is of

nanomedicines to solve the problems in cancer therapy that arises due to heterogeneity, non-targeted therapy and development of drug resistance in cancer patients.

3. Antimicrobial agent: The use of silver nanoparticle as antimicrobial agent is already know. However the only concern of use of these silver nanoparticles were toxicity that can result in their accumulation so the biomedical application of these chemically synthesised silver nanoparticles was restricted. But when the green chemistry approach of synthesis of silver nanoparticles from fungi like *F. oxysporum* was studied, it was found that these nanoparticles are highly reactive but biocompatible so the risk of toxicity was reduced. The silver nanoparticles also acted as carriers for major antibiotics like ampicillin, kanamycin, chloramphenicol and erythromycin highly reactive to Gram positive and Gram negative bacteria. When these antibiotics were loaded with Silver nanoparticles the antimicrobial activity enhanced without bringing any change in media. Recently a new type of work was done by researchers where they incorporated these green nanoparticles in a textile to prevent it from *Staphylococcus aureus* [71] infection. Similarly the beauty products also have these biocompatible silver nanoparticles

4. Biosensors: The optical and electronic properties of nanoparticles make them an efficient biosensors. The single ion reactivity can be detected making these biosensors highly sensitive. It was reported that when conventional glucose biosensor was compared with gold nanoparticle based biosensor then the activity of glucose oxidase for smaller amount of sample was increased by folds, making the sensor highly sensitive to even a drop of the sample. Thus the use of such glucose sensor is now common in biomedical applications [72]. Similarly the use of Gold Silver alloy nanoparticles in modified glassy carbon electrode whose commercial application is as Vanillin biosensor in testing purity and amount of vanilla extract or vanillin from vanilla beans or vanilla tea [73]. The modifications were also done in conventional first enzyme based biosensor with enzyme Horseradish Peroxidase, making it more sensitive and highly specific. The modified Horseradish Peroxidase biosensor contains Selenium nanoparticles produced from Bacillus species. These H_2O_2 biosensors had high sensitivity and affinity for H_2O_2. The highly reactive Se- NP, with large surface to volume ratio, is stable at room temperature and has good adhesive ability, and biocompatibility that led to enhancement of the HRP- biosensor. These sensors exhibited good electrocatalytic activity towards the reduction of H_2O_2 due to the good adhesive ability, and biocompatibility of Se-NP [74]. Another effective biosensor is that of Gold nanoparticles being largely used in cancer targeting [75] because of its surface plasmon resonance properties of light scattering.

5. As reducing and catalytic agents: The nanoparticles being a highly reactive species act as effective reductants and catalyst in many chemical reactions. Their high surface to volume ratio and electronic properties facilitate the chemical process. It has been reported earlier the use of silver nanoparticles with antibiotics to enhance the antimicrobial activity. Similarly the magnetosomes capping on bacteria or their formation enhance the microbial activity like

enhancement of desulphurisation of complex polymer by *Pseudomonas* sp. when coated with magnetite [76] or enhancement in detoxification of heavy metals by the magnetotactic bacteria with magnetosomes. The magnetic nanoparticles bearing high surface energy caused their strong adsorption on the cells where they behave as catalyst and just like enzymes can be procured back similarly in presence of an external magnetic field these particles were always in suspended form in the solution and can be collected back thus the cells with nanoparticles can be used several times making the use of nanoparticles as reductants or catalyst for any chemical reactions more economical affair.

6. As a tracer and imaging particle: The optical and electronic properties of nanoparticles make them and efficient tracer molecule in detection of complex biochemical pathways. These particles exhibit different light scattering patterns at different sizes like gold nanoparticles exhibit optical activity at different sizes and it is this property that was exploited for biomolecular recognition with help of single gold nanoparticle functionalised with biotin to which when streptavidin binds. The reactivity of gold nanoparticles produces high light scattering wherever the binding of biotin with streptavidin will take place and the biomolecule can be recognised [77]. Similarly as discussed earlier that iron nanoparticles since are magnetic in properties, in presence of magnetic field when tagged with a biomolecule helps in knowing the bioassay of that biomolecule and so they act as effective biological label. Competitive chemiluminescence, enzyme immunoassays using antibodies immobilized onto bacterial magnetic particles, modified biosensors, and were developed for the rapid and sensitive detection of small molecules, such as environmental pollutants, hormone, and toxic detergents [78]. Apart from magnetic particles acting in presence of magnetic field there are certain specialised nanoparticles like that of gold quantum dots of Au67 that can trace DNA directly in one step process under influence of magnetic field [79].

The MRI imaging in presence of magnetic particles has proven to be more effective than conventional imaging of cancer targeted treatment. Similarly Cadmium Sulphide nanoparticle tags are extensively used in DNA hybridisation experiments in electrochemical stripping method [80]. The nanoparticle tracers are also being used for environmental concerns. The tracers are the most direct ways of diagnosing environmental problems of groundwater contaminations or for knowledge of natural gas and oil productions by tracing the subsurface fluid flow pathway. The nanoparticle tracers are more effective as they are path sensitive and highly specific so they never diffuse out of the specified flow channel and the time taken to cover the distance between the two points is very less. The green nanoparticle tracers are tuneable and so the chances of their aggregation or sticking to the narrow porous channels is greatly reduced and far more avoidable [81].

The above mentioned applications are milestones in field of nanomedicines and biomedical treatments and lay the foundations for better prospects in the field of therapeutics by

modifying the existing processes or by producing more varieties of nanoparticles employing green technologies. The recent advances focus around manipulations at microbial molecular level involving alterations at genomic and proteomic levels to produce highly efficient nanoparticle that can be used extensively for biomedical application. Secondly the manipulations at molecular level can help in standardizing the process so that the large and commercial scale production of nanomedicines can be facilitated as boon in health care sector.

Apart from the prospective applications of the nanoparticles from microbes still there are certain consequences that need to be overcome so that the microbial production of nanoparticles becomes the best commercial process that can be used in large scale. The microbial production of nanoparticles is still less rapid and slow process as compared to physical or chemical ways of production of nanoparticles. Secondly lot of effort is required to improve the synthesis efficiency and effort to control the particle size and morphology. The reduction of synthesis time and making the process tuneable will make this biosynthesis route much more attractive. The desired particlesize and the nature of nanoparticles are two important issues in the evaluation of monodisperse nanoparticle synthesis. This requires an effective dispersing agent along with microbial reductants. To identify more and more dispersing agents that are eco-friendly is the area of study. Thirdly it was seen that the shelf life of the nanoparticles produced by microbes was very intangible as such the decomposition rate was nearly rapid and after certain time, they decomposed. Thus, the tangibility of nanoparticles production by biological means needs extensive study and standardization. It has already been seen that the control of particle size is in physical and chemical ways is easily feasible however with biological ways the control of particle size can be by varying parameters like the type of microbes, their stage of microbial growth, p^H, substrate concentration, temperature, the concentration of source of target nanoparticles, the reaction time and the capping or coatings with different nanoparticles or by adding an untargeted ion that can act as dispersing agents, can lead to control of particle size and monodispersity. Sometimes the coating with lipids and proteins also confer the physiological stability of the nanoparticles making them more biocompatible and with longer shelf life, that is important for biomedical applications

The research is currently revolving around manipulating cells at the genomic and proteomic levels, because that will help in creating microbes that can produce stable and biocompatible nanoparticles with longer shelf life. With a better understanding of the mechanism at the cellular and molecular level, the isolation and identification of compounds, better reductants and production conditions could be explored. This further helps in reducing the reaction time and increasing the efficacy of the process and the product that is nanoparticle, important for biomedical applications. The microbial approach to production of biocompatible nanoparticles that are economical, nontoxic and safer to the environmentfurther strengthen the nanomedicines mediated therapeutics.

As it is said what you give to the nature comes back to you so if we give a healthier green approach to the environment then environment will also keep us healthy. The latest technologies and research should mow focus more on green approaches like use of flora and fauna for innovations rather than using the consumables for research that will exhaust one day and lead to the accumulation of toxic substance. Therefore to conclude it can be said that the tiny factories (microbes) are harbours of most skilled technicians (nanoparticles) for Dynamos (energy efficient process/products) that is by employing microbes the nanoparticles can be produced and these nanoparticles can be used for different applications that are necessary for the environment wellbeing.

5. References

1. Daniel MC, Astruc D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chemical reviews. 2004; 104(1): 293-346.

2. Kato H. In vitro assays: tracking nanoparticles inside cells. Nature Nanotechnology. 2011; 6(3): 139-140.

3. Liu J, Qiao SZ, Hu QH. Magnetic nanocomposites with mesoporous structures: synthesis and applications small. 2011; 7(4): 425-443.

4. Luechinger NA, Grass RN, Athanassiou EK, Stark WJ. Bottom-up fabrication of metal/metal nanocomposites from nanoparticles of immiscible metals. Chemistry of Materials. 2009; 22(1): 155-160.

5. Tiwari DK, Behari J, Sen P. Time and dose-dependent antimicrobial potential of Ag nanoparticles synthesized by top-down approach. Current Science. 2008; 647-655.

6. Mohanpuria P, Rana NK, Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications. Journal of Nanoparticle Research. 2008; 10(3): 507-517.

7. Rai M, Gade A, Yadav A. Biogenic nanoparticles: an introduction to what they are, how they are synthesized and their applications. InMetal nanoparticles in microbiology 2011 (pp. 1-14). Springer Berlin Heidelberg.

8. Gottschalk F, Nowack B. The release of engineered nanomaterials to the environment. Journal of Environmental Monitoring. 2011; 13(5): 1145-1155.

9. Wiesner MR, Lowry GV, Jones KL, Hochella, Jr MF, Di Giulio RT, Casman E, Bernhardt ES. Decreasing uncertainties in assessing environmental exposure, risk, and ecological implications of nanomaterials.

10. Pearce CI, Coker VS, Charnock JM, Pattrick RA, Mosselmans JF, Law N, Beveridge TJ, Lloyd JR. Microbial manufacture of chalcogenide-based nanoparticles via the reduction of selenite using Veillonella atypica: an in situ EXAFS study. Nanotechnology. 2008; 19(15): 155603.

11. Oremland RS, Herbel MJ, Blum JS, Langley S, Beveridge TJ, Ajayan PM, Sutto T, Ellis AV, Curran S. Structural and spectral features of selenium nanospheres produced by Se-respiring bacteria. Applied and environmental microbiology. 2004; 70(1): 52-60.

12. Baesman SM, Bullen TD, Dewald J, Zhang D, Curran S, Islam FS, Beveridge TJ, Oremland RS. Formation of tellurium nanocrystals during anaerobic growth of bacteria that use Te oxyanions as respiratory electron acceptors. Applied and environmental microbiology. 2007; 73(7): 2135-2143.

13. Klonowska A, Heulin T, Vermeglio A. Selenite and tellurite reduction by Shewanella oneidensis. Applied and environmental microbiology. 2005; 71(9): 5607-5609.

14. Lang C, Schüler D. Biogenic nanoparticles: production, characterization, and application of bacterial magnetosomes. Journal of Physics: Condensed Matter. 2006; 18(38): S2815.

15. Narayanan KB, Sakthivel N. Biological synthesis of metal nanoparticles by microbes. Advances in colloid and interface science. 2010; 156(1): 1-3.

16. Chen JF, Wang YH, Guo F, Wang XM, Zheng C. Synthesis of nanoparticles with novel technology: high-gravity reactive precipitation. Industrial & engineering chemistry research. 2000; 39(4): 948-954.

17. Simkiss K, Wilbur KM. Biomineralization. Elsevier; 2012.

18. Mann S. Biomineralization: principles and concepts in bioinorganic materials chemistry. Oxford University Press on Demand; 2001.

19. Zhang X, Yan S, Tyagi RD, Surampalli RY. Synthesis of nanoparticles by microorganisms and their application in enhancing microbiological reaction rates. Chemosphere. 2011; 82(4): 489-494.

20. Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan MI, Kumar R, Sastry M. Extracellular synthesis of gold nanoparticles by the fungus Fusarium oxysporum. Chem Bio Chem. 2002 May 3; 3(5): 461-463.

21. Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M. Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, Thermomonospora sp. Langmuir. 2003; 19(8): 3550-3553.

22. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R, Ajayakumar PV, Alam M, Sastry M. Bioreduction of AuCl4− ions by the fungus, Verticillium sp. and surface trapping of the gold nanoparticles formed. Angewandte Chemie International Edition. 2001; 40(19): 3585-3588.

23. Southam G, Beveridge TJ. The occurrence of sulfur and phosphorus within bacterially derived crystalline and pseudocrystalline octahedral gold formed in vitro. Geochimica et Cosmochimica Acta. 1996; 60(22): 4369-4376.

24. Ahmad A, Senapati S, Khan MI, Kumar R, Ramani R, Srinivas V, Sastry M. Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, Rhodococcus species. Nanotechnology. 2003; 14(7): 824.

25. Lengke MF, Fleet ME, Southam G. Morphology of gold nanoparticles synthesized by filamentous cyanobacteria from gold (I)− thiosulfate and gold (III)− chloride complexes. Langmuir. 2006; 22(6): 2780-2787.

26. Lengke MF, Ravel B, Fleet ME, Wanger G, Gordon RA, Southam G. Mechanisms of gold bioaccumulation by filamentous cyanobacteria from gold (III)− chloride complex. Environmental science & technology. 2006; 40(20): 6304-6309.

27. Nair B, Pradeep T. Coalescence of nanoclusters and formation of submicron crystallites assisted by Lactobacillus strains. Crystal Growth & Design. 2002; 2(4): 293-298.

28. Panáček A, Kvítek L, Prucek R, Kolář M, Večeřová R, Pizúrová N, Sharma VK, Nevěčná TJ, Zbořil R. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. J. Phys. Chem. B. 2006; 110(33): 16248-16253.

29. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parishcha R, Ajaykumar PV, Alam M, Kumar R, Sastry M. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. Nano Letters. 2001; 1(10): 515-519.

30. Syed A, Ahmad A. Extracellular biosynthesis of platinum nanoparticles using the fungus Fusarium oxysporum. Colloids and Surfaces B: Biointerfaces. 2012; 97: 27-31.

31. Krishna GU, Kumar SS, Pranitha VA, Alha MA, Charaya S. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against salmonella SP. International journal of pharmacy and pharmaceutical Sciences. 2015; 7(11): 84-88.

32. Klaus T, Joerger R, Olsson E, Granqvist CG. Silver-based crystalline nanoparticles, microbially fabricated. Proceedings of the National Academy of Sciences. 1999; 96(24): 13611-13614.

33. Jain N, Bhargava A, Majumdar S, Tarafdar JC, Panwar J. Extracellular biosynthesis and characterization of silver nanoparticles using Aspergillus flavus NJP08: a mechanism perspective. Nanoscale. 2011; 3(2): 635-641.

34. Babu MG, Gunasekaran P. Production and structural characterization of crystalline silver nanoparticles from Bacillus cereus isolate. Colloids and surfaces B: Biointerfaces. 2009; 74(1): 191-195.

35. Senapati S, Ahmad A, Khan MI, Sastry M, Kumar R. Extracellular biosynthesis of bimetallic Au–Ag alloy nanoparticles. Small. 2005; 1(5): 517-520.

36. Zheng D, Hu C, Gan T, Dang X, Hu S. Preparation and application of a novel vanillin sensor based on biosynthesis of Au–Ag alloy nanoparticles. Sensors and Actuators B: Chemical. 2010; 148(1): 247-252.

37. Sawle BD, Salimath B, Deshpande R, Bedre MD, Prabhakar BK, Venkataraman A. Biosynthesis and stabilization of Au and Au–Ag alloy nanoparticles by fungus, Fusarium semitectum. Science and technology of advanced materials. 2008; 9(3): 035012.

38. Konishi Y, Ohno K, Saitoh N, Nomura T, Nagamine S, Hishida H, Takahashi Y, Uruga T. Bioreductive deposition of platinum nanoparticles on the bacterium Shewanella algae. Journal of biotechnology. 2007; 128(3): 648-653.

39. Sinha A, Khare SK. Mercury bioaccumulation and simultaneous nanoparticle synthesis by Enterobacter sp. cells. Bioresource Technology. 2011; 102(5): 4281-4284.

40. Kashefi K, Lovley DR. Reduction of Fe (III), Mn (IV), and toxic metals at 100 C by Pyrobaculum islandicum. Applied and Environmental Microbiology. 2000; 66(3): 1050-1056.

41. Windt WD, Aelterman P, Verstraete W. Bioreductive deposition of palladium (0) nanoparticles on Shewanella oneidensis with catalytic activity towards reductive dechlorination of polychlorinated biphenyls. Environmental Microbiology. 2005; 7(3): 314-325.

42. Bazylinski DA, Garratt-Reed AJ, Frankel RB. Electron microscopic studies of magnetosomes in magnetotactic bacteria. Microscopy research and technique. 1994; 27(5): 389-401.

43. Bazylinski DA, Frankel RB, Heywood BR, Mann S, King JW, Donaghay PL, Hanson AK. Controlled Biomineralization of Magnetite (Fe (inf3) O (inf4)) and Greigite (Fe (inf3) S (inf4)) in a Magnetotactic Bacterium. Applied and Environmental Microbiology 1995; 61(9): 3232-3239.

44. Thornhill RH, Burgess JG, Matsunaga T. PCR for direct detection of indigenous uncultured magnetic cocci in sediment and phylogenetic analysis of amplified 16S ribosomal DNA. Applied and environmental microbiology. 1995; 61(2): 495-500.

45. Spring S, Schleifer KH. Diversity of magnetotactic bacteria. Systematic and Applied Microbiology. 1995; 18(2): 147-53.

46. Zhou W, He W, Zhong S, Wang Y, Zhao H, Li Z, Yan S. Biosynthesis and magnetic properties of mesoporous Fe 3 O 4 composites. Journal of Magnetism and Magnetic Materials. 2009; 321(8): 1025-1028.

47. Zhou W, He W, Zhang X, Yan S, Sun X, Tian X, Han X. Biosynthesis of iron phosphate nanopowders. Powder Technology. 2009; 194(1): 106-108.

48. Jha AK, Prasad K, Prasad K. A green low-cost biosynthesis of Sb 2 O 3 nanoparticles. Biochemical engineering journal. 2009; 43(3): 303-306.

49. Bansal V, Rautaray D, Bharde A, Ahire K, Sanyal A, Ahmad A, Sastry M. Fungus-mediated biosynthesis of silica and titania particles. Journal of Materials Chemistry. 2005; 15(26): 2583-2589.

50. Yang H, Sentra S, Holloway PH. Syntheses and applications of MN-doped II-VI semiconductor Nano crystals. Journal of nanosciences and nanotechnology. 2005; 5(9): 1364-1375.

51. Cunningham DP, Lundie LL. Precipitation of cadmium by Clostridium thermoaceticum. Applied and Environmental Microbiology. 1993; 59(1): 7-14.

52. Holmes JD, Richardson DJ, Saed S, Evans-Gowing R, Russell DA, Sodeau JR. Cadmium-specific formation of metal sulfide 'Q-particles' by Klebsiella pneumoniae. Microbiology. 1997; 143(8): 2521-2530.

53. Bai HJ, Zhang ZM, Gong J. Biological synthesis of semiconductor zinc sulfide nanoparticles by immobilized Rhodobacter sphaeroides. Biotechnology letters. 2006; 28(14): 1135-1139.

54. Labrenz M, Druschel GK, Thomsen-Ebert T, Gilbert B, Welch SA, Kemner KM, Logan GA, Summons RE, De Stasio G, Bond PL, Lai B. Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate-reducing bacteria. Science. 2000; 290(5497): 1744-1747.

55. Bai HJ, Zhang ZM. Microbial synthesis of semiconductor lead sulfide nanoparticles using immobilized Rhodobacter sphaeroides. Materials Letters. 2009; 63(9): 764-766.

56. Watson JH, Ellwood DC, Soper AK, Charnock J. Nanosized strongly-magnetic bacterially-produced iron sulfide materials. Journal of Magnetism and Magnetic Materials. 1999; 203(1): 69-72.

57. Bazylinski DA, Frankel RB, Heywood BR, Mann S, King JW, Donaghay PL, Hanson AK. Controlled Biomineralization of Magnetite (Fe (inf3) O (inf4)) and Greigite (Fe (inf3) S (inf4)) in a Magnetotactic Bacterium. Applied and Environmental Microbiology.1995; 61(9): 3232-3239.

58. Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan MI, Kumar R, Sastry M. Extracellular synthesis of gold nanoparticles by the fungus Fusarium oxysporum. ChemBioChem. 2002; 3(5): 461-463.

59. Rautaray D, Sanyal A, Adyanthaya SD, Ahmad A, Sastry M. Biological synthesis of strontium carbonate crystals using the fungus Fusarium oxysporum. Langmuir. 2004; 20(16): 6827-6833.

60. Kumar SA, Ansary AA, Ahmad A, Khan MI. Extracellular biosynthesis of CdSe quantum dots by the fungus, Fusarium oxysporum. Journal of Biomedical Nanotechnology. 2007; 3(2): 190-194.

61. Yan S, He W, Sun C, Zhang X, Zhao H, Li Z, Zhou W, Tian X, Sun X, Han X. The biomimetic synthesis of zinc phosphate nanoparticles. Dyes and Pigments. 2009; 80(2): 254-258.

62. Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H, Sangiliyandi G. Extracellular biosynthesis of silver nanoparticles by the culture supernatant of Bacillus licheniformis. Materials letters. 2008; 62(29): 4411-4413.

63. Reith F, Lengke MF, Falconer D, Craw D, Southam G. The geomicrobiology of gold. The ISME Journal. 2007; 1(7): 567.

64. Nies DH. Microbial heavy-metal resistance. Applied microbiology and biotechnology. 1999; 51(6): 730-50.

65. Mergeay M, Monchy S, Vallaeys T, Auquier V, Benotmane A, Bertin P, Taghavi S, Dunn J, van der Lelie D, Wattiez R. Ralstonia metallidurans, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes. FEMS microbiology reviews. 2003; 27(2-3): 385-410.

66. Tang H, Yan M, Zhang H, Xia M, Yang D. Preparation and characterization of water-soluble CdS nanocrystals by surface modification of ethylene diamine. Materials letters. 2005; 59(8): 1024-1027.

67. Løver T, Henderson W, Bowmaker GA, Seakins JM, Cooney RP. Functionalization and capping of a cds nanocluster: a study of ligand exchange by electrospray mass spectrometry. Chemistry of materials. 1997; 9(8): 1878-1886.

68. Meng C, Tian J, Li Y, Zheng S. Influence of native bacterial magnetic particles on mouse immune response. Wei sheng wu xue bao= Acta microbiologica Sinica. 2010; 50(6): 817-821.

69. Sun JB, Duan JH, Dai SL, Ren J, Zhang YD, Tian JS, Li Y. In vitro and in vivo antitumor effects of doxorubicin loaded with bacterial magnetosomes (DBMs) on H22 cells: the magnetic bio-nanoparticles as drug carriers. Cancer letters. 2007; 258(1): 109-117.

70. Felfoul O, Mohammadi M, Martel S. Magnetic resonance imaging of Fe 3 O 4 nanoparticles embedded in living magnetotactic bacteria for potential use as carriers for in vivo applications. InEngineering in Medicine and Biology Society, 2007. EMBS 2007. 29th Annual International Conference of the IEEE 2007 Aug 22 (pp. 1463-1466). IEEE.

71. Durán N, Marcato PD, De Souza GI, Alves OL, Esposito E. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Journal of biomedical nanotechnology. 2007; 3(2): 203-208.

72. Zheng B, Qian L, Yuan H, Xiao D, Yang X, Paau MC, Choi MM. Preparation of gold nanoparticles on eggshell membrane and their biosensing application. Talanta. 2010; 82(1): 177-183.

73. Zheng D, Hu C, Gan T, Dang X, Hu S. Preparation and application of a novel vanillin sensor based on biosynthesis of Au–Ag alloy nanoparticles. Sensors and Actuators B: Chemical. 2010; 148(1): 247-52.

74. Wang T, Yang L, Zhang B, Liu J. Extracellular biosynthesis and transformation of selenium nanoparticles and application in H 2 O 2 biosensor. Colloids and Surfaces B: Biointerfaces. 2010; 80(1): 94-102.

75. El-Sayed IH, Huang X, El-Sayed MA. Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. Nano letters. 2005; 5(5): 829-834.

76. Shan G, Xing J, Zhang H, Liu H. Biodesulfurization of dibenzothiophene by microbial cells coated with magnetite nanoparticles. Applied and environmental microbiology. 2005; 71(8): 4497-4502.

77. Raschke G, Kowarik S, Franzl T, Sönnichsen C, Klar TA, Feldmann J, Nichtl A, Kürzinger K. Biomolecular recognition based on single gold nanoparticle light scattering. Nano letters. 2003; 3(7): 935-938.

78. Tanaka T, Takeda H, Ueki F, Obata K, Tajima H, Takeyama H, Goda Y, Fujimoto S, Matsunaga T. Rapid and sensitive detection of 17β-estradiol in environmental water using automated immunoassay system with bacterial magnetic particles. Journal of biotechnology. 2004; 108(2): 153-159.

79. Pumera M, Castaneda MT, Pividori MI, Eritja R, Merkoçi A, Alegret S. Magnetically trigged direct electrochemical detection of DNA hybridization using Au67 quantum dot as electrical tracer. Langmuir. 2005; 21(21): 9625-9629.

80. Wang J, Liu G, Polsky R, Merkoçi A. Electrochemical stripping detection of DNA hybridization based on cadmium sulfide nanoparticle tags. Electrochemistry communications. 2002; 4(9): 722-726.

81. Li YV, Cathles LM, Archer LA. Nanoparticle tracers in calcium carbonate porous media. Journal of nanoparticle research. 2014; 16(8): 2541.