Nanobiosynthesis

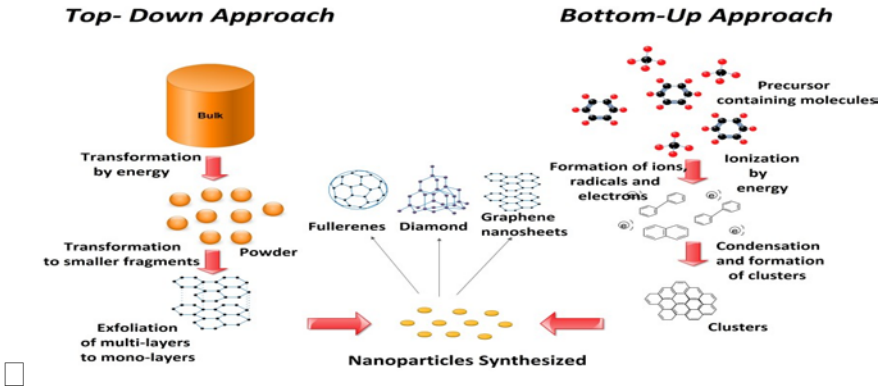
 To date, there are numerous techniques for synthesizing nanoparticles. However, these techniques fall into two broad approaches and can be defined as either a top down approach or a bottom up approach.

 The top down approach starts with a material of interest, which then undergoes size reduction via physical and chemical processes to produce nanoparticles.

 Importantly, nanoparticles are highly dependent on their size, shape, and surface structure and processing tends to introduce surface imperfections.

 In **the bottom up approach** nanoparticles are built from atoms, molecules and smaller monomers.

In either approach, the resulting nanoparticles are characterized using various techniques to determine properties such as particle size, size distribution, shape, and surface area.



PHYSICAL AND CHEMICAL METHODS

 Various physical and chemical processes have been exploited in the synthesis of several inorganic metal nanoparticles by wet and dry approaches.

 The high energy requirement in physical methods of nanoparticle synthesis and the waste disposal problems in chemical synthesis, so both methods are costly and generate toxic by product are major demerits of the conventional nanoparticle synthesis.

 However, these methods are burdened with various problems including:

 -use of harmful chemical agents.

 -production of hazardous property.

 -expensive chemicals.

 -and high energy consumption.

 Biosynthesis of nanoparticles

 Accordingly, there is a necessary need to extend for environmentally benign procedures for synthesis of nanoparticles.

 A promising move towards to reach this objective is to develop the array of biological resources in nature.

 Such drawbacks demand the development of clean, nonhazardous, inexpensive, energy-efficient, and eco-friendly methods for nanoparticles synthesis.

 Biosynthesis is the phenomena which take place by means of biological or enzymatic reaction.

 The most adaptable location of biosynthesis of nanoparticles is biological cellular entitles and their cell membrane.

 Over the past several years, the biological method for the synthesis of nanoparticles employs use of biological agents like:

 plants, algae, fungi, actinomycetes, yeast, bacteria, and viruses have been used for production of nanoparticles.

 The main interest is production of nanoparticles using a biological method from a cheap resource and uniform production of nanoparticles.

 Utilizing a biological source gives an easy approach, easy multiplication, and easy increase of biomass.

  A great deal of study has been carried out on synthesis of nanoparticles by prokaryotic bacteria since they are the easiest organisms to handle and can be manipulated most easily.

 Nanoparticle synthesis by Bacteria

 Nanoparticles are synthesized by microbes and have biological applications in the fields of bioremediation, bio-mineralization, bioleaching, and bio-corrosion.

 Bacteria are able to form nanoparticles both intracellularly via bioaccumulation and extarcellularly on the cell wall using its enzymes.

 Hence, extracellular production has more commercial applications in various fields.

  Experiments show that the growth phase of the cells affect the formation rate of nanoparticles and is 20 times more in stationary phase than logarithmic phase.

 To obtain intracellular particles from bacteria requires further processing steps like ultrasound treatment or reaction with suitable detergents.

 This property can be exploited for extraction of precious metals from mine wastes.

 When cell wall reductive enzymes or secreted enzymes are involved in the reduction of metal ions then it is logical to find the metal nanoparticles outside the cell.

 The mechanisms which are considered for the biosynthesis of nanoparticles included:

 -efflux system.

 -alteration of solubility and toxicity via reduction or oxidation.

 -bio-absorption.

 -Bioaccumulation.

 -and precipitation of metals.

 A prokaryotic bacterium *Rhodopseudomonas capsulata, was found to deposit gold nanoparticles* of 10-20 nanometers at 7 pH and room temperature extarcellularly.

 Similarly, silver nanoparticles can be produced extarcellularly using *Enterobacter culture supernatant.*

 These bacteria secrete enzymes in their culture solutions which are able to reduce silver and assist in the formation silver nanoparticles.

 When *Klebsiella aerogenes is exposed cadmium ions* in the growth medium it forms cadmium sulfide nanoparticles of 20-200 nanometers deposited on the cell surface.

 Escherichia coli when incubated with cadmium chloride and sodium sulfide forms intracellular cadmium sulfide nanoparticles in crystal phase.

 In the case of biological synthesis of nanoparticles, the aqueous metal ion precursors from metal salts are reduced and as a result a color change occurs in the reaction mixture.

 This is the first qualitative indication that nanoparticles are being formed.

 Some of the spectroscopy and microscopy techniques routinely used include:

 -Atomic force microscopy (AFM),

 -Transmission electron microscopy (TEM),

 -Scanning electron microscopy (SEM),

 -X-ray diffraction (XRD),

 -Fourier transform infrared spectroscopy (FT-IR).

 -UV-visible spectroscopy (UV-vis)

 Microscopy based techniques such as AFM, SEM and TEM are considered direct methods of obtaining data from images taken of the nanoparticles.

 In particular, both SEM and TEM have been extensively used to determine size and morphological features of nanoparticles.

 Spectroscopy based techniques such as UV-vis, XRD, and FT-IR are considered indirect methods of determining data related to composition, structure, crystal phase, and properties of nanoparticles.

  The UV-visible spectroscopy covers the UV range between 190 and 380 nm and the visible range between 380 and 800 nm.

 Absorption measurements for silver (Ag) nanoparticles are usually between 400 and 450 nm, while gold (Au) nanoparticles are generally detected by the presence of peaks between 500 and 550 nm.

 Synthesis of nanoparticles by fungi

 With the beginning of modern nanotechnology in the 1980s, fungi have remained important by providing a greener alternative to chemically synthesized nanoparticle.

 The most common nanoparticles synthesized by fungi are silver and gold, however fungi have been utilized in the synthesis other types of nanoparticles including zinc oxide, platinum, magnetite, zirconia, silica, titanium, and cadmium sulfide and cadmium selenide quantum dots.

 Silver nanoparticle production

 Extracellular systhesis has been demonstrated by *Trichoderma virde*, *Aspergillus niger*, *Penicillium brevicompactum*,

  while intracellular synthesis was shown to occur in a *Verticillium* species, and in *Neurospora crassa*.

 Gold nanoparticle production

 Extracellular gold nanoparticle synthesis was demonstrated by *Fusarium oxysporum*, *Aspergillus niger*, and *Candida albican*.

  Intracellular gold nanoparticle synthesis has been demonstrated by a *Verticillum* species.

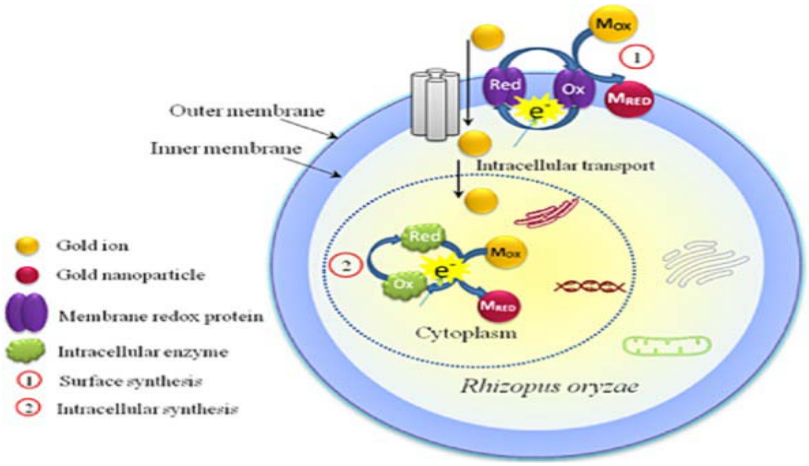
 Miscellaneous nanoparticle production

 In addition to gold and silver, *Fusarium oxysporum* has been used to synthesize zirconia, titanium, cadmium sulfide and cadmium selenide nanosize particles.

 Cadmium sulfide nanoparticles synthesized by *Trametes versicolor*, and *Candida glabrata*.

 Gold and silver Formation mechanisms
Nitrate reductase was suggested to initiate nanoparticle formation by many fungi including *Penicillium* species, while several enzymes, α-NADPH-dependent reductases, nitrate-dependent reductases were implicated in silver nanoparticle synthesis for *Fusarium oxysporum*.

 External gold nanoparticle synthesis was attributed to laccase, while intracellular gold nanoparticle synthesis was attributed to ligninase.



Intracellular silver and gold nanoparticle synthesis are not fully understood, but similar fungal cell wall surface electrostatic attraction, reduction, and accumulation has been proposed.

Fungi		Bacteria
High/Very high (Rajapaksha *et al.*, 2004)	Metal toxicity resistance	Medium/High (Rajapaksha *et al.*, 2004)
Commonly extracellular (Durán *et al.*, 2011)	AuNP location	Both intracellular and extracellular (Lengke and Southam, 2006)
Very fast in cell-free filtrate (<1 h) (Du *et al.*, 2011)	Biosynthesis rate	Relatively slow (>24 h) (Du *et al.*, 2011)
Shape and size depend on biomass/Au ratio (Pimprikar *et al.*, 2009)	AuNP shape and size	Prevalently spherical, small AuNPs (Wen *et al.*, 2009)
NADH-reductases, phytochelatins, melanin (Mukherjee *et al.*, 2001)	Bioreducing agent(s)	Microbially produced redox mediators, membrane proteins and cytochromes, inorganic redox compounds (von Canstein *et al.*, 2008; (Marshall *et al.*, 2008; Mukherjee *et al.*, 2002)
Unidentified surface-bound proteins (Shankar *et al.*, 2003; Das *et al.*, 2009)	Bio-capping agent(s)	Not yet identified
More likely	Scalability to industrial process	Less likely