Nanobiosynthesis of bacteria

LEC.4

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- 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.

- Though numerous chemical methods prevailed for nanoparticle production, numerous problems are often experienced with:
- stability of product.
- control of the crystal growth.
- and aggregation of particles on long term exposure.

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, biocompatible, nonhazardous, inexpensive, energy-efficient, and eco-friendly methods for nanoparticles synthesis.

- Consequently, biological systems have been focused on and exploited for the synthesis of nanoparticles providing a safer alternative to physical and chemical methods.

- Indeed, 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 rate of reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions.
- It is well known that microbes such as bacteria, yeast, fungi, and alga, are capable of adsorbing and accumulating metals.
- The biological agents secrete a large amount of enzymes, which are capable of hydrolyzing metals and thus bring about enzymatic reduction of metals ions.

Why the Biological Method used for Nanoparticles Synthesis

- Biological approaches to nanoparticle and nanocrystal synthesis have been extended to intact biological particles.
- The biological process is more acceptable green route and is not energy intensive and is also ecofriendly.
- The most abundant organisms in our biosphere is bacteria.
- This biogenic approach is greatly indented with bacteria by providing ambient conditions such as temperature, pH, pressure and etc.

- 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 and size uniformity.
- 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.
- Microorganisms often produce inorganic materials of nano-size either extarcellularly or intracellularly. Microbial systems are able to detoxify heavy metals by virtue of their ability to reduce the metal ions or precipitate the soluble toxic ions into insoluble non-toxic metal nanoparticles.

- Bacteria are able to form nanoparticles both intracellularly via bioaccumulation and extarcellularly on the cell wall using its enzymes. Intracellular nanoparticles are of a fixed size with less monodispersity than extracellular particles.
- Hence, extracellular production has more commercial applications in various fields. Since monodispersity is the major factor in usefulness of nanoparticles, biological processes must be designed in such a way to ensure maximum monodispersity.

- 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 and the metal nanoparticles can also be used as catalysts.
- 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.

Mechanism Used by Bacteria

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

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

- 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.
- As the pH of the solution was changed various nanoparticles with different sizes and geometries (like triangular and spherical at 4.0 pH) were formed.
- It was found experimentally that cell free extract of *Rhodopseudomonas capsulata can also be used for production of gold nanoparticles*.

- 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.

- *Clostridium thermoaceticum was found to deposit CdS nanoparticles on the cell surface as well* as in the solution.

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

- In the case of chemical and 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:
- UV-visible spectroscopy (UV-vis),
- dynamic light scattering (DLS),
- atomic force microscopy (AFM),
- transmission electron microscopy (TEM),
- scanning electron microscopy (SEM),
- energy dispersive spectroscopy (EDS),
- X-ray diffraction (XRD),
- Fourier transform infrared spectroscopy (FT-IR),
- and Raman spectroscopy.

- 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, DLS, XRD, EDS, FT-IR, and Raman 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.

- Wavelengths between 300 and 800 nm are generally used for characterizing metallic nanoparticles ranging in size from 2 nm up to around 100 nm.

- For example, 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.