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### **Strategies of Biosynthesis of Nanoparticles and Their Potential Applications**

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#### **ABSTRACT**

Synthesis of nanoparticles by using biological systems such as plants, fungi, bacteria has been emerged as a substantial research interest since past few years. Nanotechnology deals with the study and use of nano sized materials in wide ranging applications. There are many physical and chemical methods of synthesis of nanoparticles but the advantage of using biological synthesis over physical and chemical synthesis is that biological methods are ecofriendly, low cost, low energy requirement and free from toxic chemicals which make these techniques a promising approach in the field of Green Nanotechnology. The aim of this review is to give an overview of general introduction and classification of nanoparticles, various biological methods of nanoparticles synthesis and their potential applications in wide spectrum areas.

**KEYWORDS:** Nanoparticles, Green synthesis, Classification, Applications

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## INTRODUCTION

Nanoparticles are defined as particles with a size in the range of 1-100 nm (1 nm =1 billionth of a meter) and Nanotechnology is the study and use of these nano-sized materials with their unique physical, chemical, and biological properties. It is an interdisciplinary science involving physics, chemistry, biology, engineering, materials science, computer science etc. The term “Nanotechnology” has been coined by Norino Taniguchi (1974), a researcher at the University of Tokyo, Japan. Nanoparticles create many new materials and devices with a wide range of applications such as in environmental remediation, medicines, medical devices, food processing, electronics, molecular biology, energy production consumer products etc.

Nanoparticles have structural features that lie in between atoms and bulk materials. Thus, they have significant properties that are dissimilar from those of atoms and bulk materials. Two of the major properties that make nanomaterials unique are- First, they have a huge surface area to volume ratio which makes the large proportion of the atoms of the material to be the surface or interfacial atoms that result in the increase of the surface-dependent properties. For example, metallic nanoparticles can be a very active catalyst. Second, Nanomaterials have spatial confinement that leads to quantum effects that bring profound effects on the properties of nanomaterials. The energy band structures and charge carrier density in the materials can be changed quite differently from their bulk count part and in turn, will modify the electronic, optical and magnetic properties of the materials.

Nanoparticles can be made of elements, organic/inorganic compounds, metallic/semiconductor particles<sup>1</sup>. Nanoparticles have been used for a very long time and exist widely in the natural form in the world: for example as the products of volcanic activity, sea spray, erosion and created by biological systems like plants and microbes. The human body contains natural nano-sized materials such as proteins and other molecules to regulate the body’s many systems and processes. Nanoparticles have also been produced by combustion, food cooking, and vehicle exhausts. Nanoparticles can be manufactured, such as metal oxides.

Although its potential applications, nanoparticles have many adverse effects as well. Toxic effects of nano-residues on the environment and the health of human beings are commonly known as Nanotoxicology. It is also very important to remember that not all nanoparticles are toxic and harmful. Toxic nature of nanoparticles depends on their chemical composition, shape, size, aggregation, concentration and particle aging. Many types of nanoparticles appear to be non-toxic while others seem to have beneficial health effects<sup>2</sup>.

This review tries to explain the classification of nanoparticles, various methods of synthesis of nanoparticles with a special purpose to highlight the biological synthesis and potential applications of nanoparticles in various fields.

## CLASSIFICATION OF NANOPARTICLES

### 1) On the basis of Dimensions –

- a) **One Dimensional nanoparticles** – 1D nanoparticles have one dimension in nanometres such as thin films or surface coatings. These are used in various fields such as electronics, chemistry, and engineering. These thin films are used in various applications such as chemicals & biological sensor, fibre optic system, magneto- optic and optical devices.
- b) **Two Dimensional nanoparticles**– 2D nanoparticles have two dimensions in nm such as carbon nanotubes, nanowires, and biopolymers. Carbon nanotubes have a great capacity for molecular absorption and offering a 3D configuration.
- c) **Three Dimensional nanoparticles**– 3D nanoparticles have three dimensions in nm such as natural nanomaterials and combustion products, metallic oxides. Dendrimers, fullerenes and quantum dots represent the greatest challenges in terms of production and understanding of properties<sup>3</sup>.

### 2) On the basis of morphology –

- a) **High aspect ratio Nanoparticles**. It includes a high length to width ratio. E.g. Nanotubes & nanowires with various shapes such as helices, zigzag, belts etc.
- b) **Small aspect ratio Nanoparticles**- It includes spherical, oval, cubic, prism, helical or pillar.

### 3) On the basis of uniformity & agglomeration-

- a) **Dispersed isometric** i.e. nanoparticles are of equal size and dispersed.
- b) **Dispersed inhomogenous** i.e. nanoparticles are of unequal size and dispersed.
- c) **Agglomerated isometric** i.e. nanoparticles are of equal size and assembled.
- d) **Agglomerated inhomogenous** i.e. nanoparticles are of unequal size and assembled.

### 4) On the basis of composition-

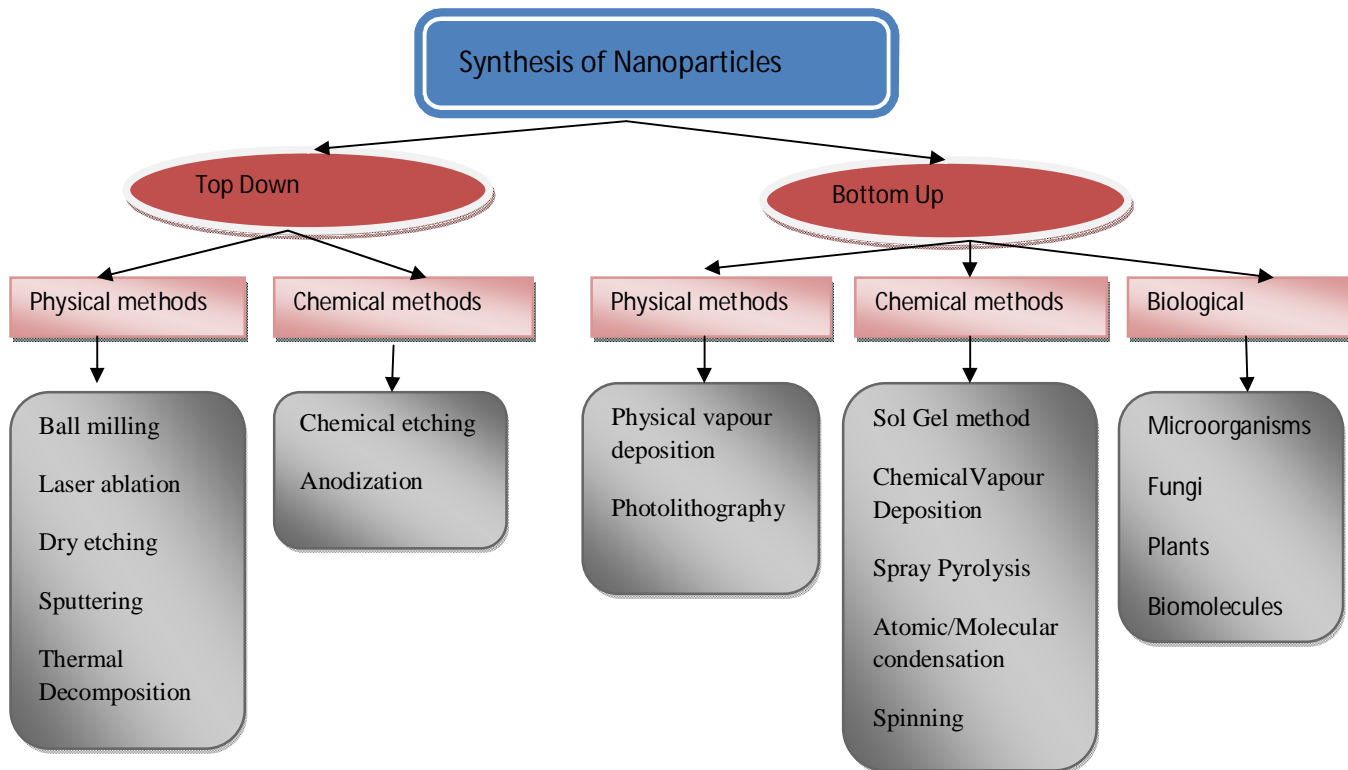
- a) **Organic Nanoparticles**- E.g. Carbon nanoparticles
- b) **Inorganic nanoparticles**- E.g. Magnetic nanoparticles, noble nanoparticles (like gold and silver) and semiconductor nanoparticles (like TiO<sub>2</sub>, ZnO).

## NANOPARTICLE SYNTHESIS

There are two main methods for the synthesis of nanoparticles:

1. **‘Bottom up’ approach**– It is a build up method that produce nanoparticles from smaller component i.e. atom by atom or molecule by molecule which assemble themselves by physical or chemical forces.

2. **‘Top down’ approach**- It is a break down method by which nanoparticles are synthesized by breaking up a large solid material until nanometric dimensions are reached.



*Fig.2: Different methods of synthesis of nanoparticles*

### **Biological synthesis of nanoparticles**

It is also called as Green synthesis and it is a bottom up approach. It falls into three categories-

- Synthesis using plant extract
- Synthesis using microorganisms
- Synthesis using biological particles

#### **Synthesis using plant extract**

A number of plants are being explored for the synthesis of nanoparticles (*Fig:3*). The advantages of using plants are that they provide single step process, easily available, safe to handle as the process is free from toxicant and they have many metabolites and enzymes for biosynthesis that act as both reducing and capping agent.

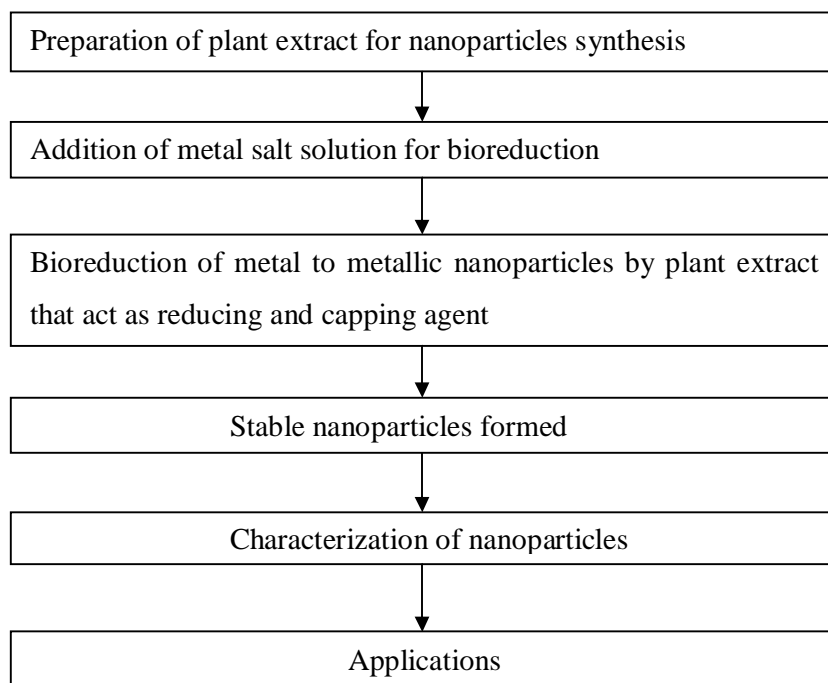


Fig. 3: General method of nanoparticle synthesis by plants

Table 2: Some examples of metal and oxide nanoparticles synthesized using plants

Plant used	Nanoparticles	References
<i>Acalypha indica</i>	Ag, Au	<sup>4</sup> Krishnaraj et al. (2010)
<i>Oscimum sanctum</i>	Ag	<sup>5</sup> Jain et al. (2017)
<i>Aloe vera</i>	In <sub>2</sub> O <sub>3</sub>	<sup>6</sup> Maensiri et al. (2008)
<i>Azadirachta indica</i>	Ag, Au, Ag/Au bimetallic	<sup>7</sup> Shankar et al. (2004); <sup>8</sup> Tripathy et al. (2009)
<i>Jatropha curcas</i>	Ag	<sup>9</sup> Pala et al. (2010)
<i>Cassia occidentalis</i>	Ag & Cu	<sup>10</sup> Gondwal et al.(2018)
<i>Hibiscus rosa sinensis</i>	Ag	<sup>11</sup> Daizy (2010)
<i>Citrus sinensis</i>	Ag	<sup>12</sup> Kaviya et al. (2011)
<i>Eucalyptus</i>	Fe oxides	<sup>13</sup> Wang et al. (2014)
<i>Ziziphus ziziphus</i>	Au	<sup>14</sup> Aljabali et al. (2018)
<i>Calotropis Gigantea</i>	Zn	<sup>15</sup> Vidya et al. (2013)
<i>Mirabilis jalapa</i>	Au	<sup>16</sup> Vankar et al. (2010)
<i>Prunus persica</i>	Ag	<sup>17</sup> Kumar et al. (2017)
<i>Psidium guajava</i>	TiO <sub>2</sub>	<sup>18</sup> Santoshkumar et al. (2014)
<i>Glycosmis mauritiana</i>	Fe oxides	<sup>19</sup> Amutha et al. (2018)
<i>Solanum lycopersicum</i>	Cu	<sup>20</sup> Batool et al.(2017)
<i>Coriandrum sativum</i>	Ni	<sup>21</sup> Vasudeo et al. (2016)

#### a) Synthesis using microorganism

- **By fungi** - Fungi are eukaryotes which are characterized by the production of large amounts of enzymes. Fungi are reported to produce both intracellular and extracellular metal nanoparticles<sup>22</sup> as shown in Table 3. Extracellular biosynthesis of silver nanoparticles by *Aspergillus niger*<sup>23</sup>, *Fusarium solani*<sup>24</sup> are reported. Gold nanoparticles were formed by *Verticillium luteoalbum*<sup>25</sup>. The use of specific enzymes secreted by fungi in the synthesis of nanoparticles becomes promising.
- **By bacteria** - The biosynthesis of metal nanoparticles by prokaryotic bacteria gained large concern among other microorganisms. Gold nanoparticles were first produced using *Bacillus subtilis* by reduction of Au<sup>3+</sup> ions when it was incubated with gold chloride<sup>26</sup>. Use of *Lactobacillus* strains to synthesize the titanium nanoparticles has been reported<sup>27</sup>. Recently silver nanoparticles synthesis by holoarchaea *Halococcus salifodinae* BK3 has been reported<sup>28</sup>.

Some microbes can sustain and grow even at high metal ion concentration due to their resistance to the metal. The mechanisms include alteration of solubility and toxicity via oxidation or reduction process, biosorption, bioaccumulation, efflux systems, extracellular precipitation of metals and lack of specific metal transport systems<sup>29</sup>. For instance, *Pseudomonas stutzeri* AG 259 has been shown to produce silver nanoparticles when isolated from silver mines<sup>30</sup>.

A remarkable advantage of biologically produced nanoparticles from microorganisms is that they are proteinaceous in nature which facilitates the functionalization of these nanoparticles with other biomolecules leading to improvement of their antimicrobial properties by enhancing the interactions with the microorganisms<sup>31</sup>. Furthermore, silver nanoparticles produced by microorganisms showed high stability over a long time period. These findings were confirmed when extracellular nanoparticles produced by *Aspergillus fumigates* showed high stability for four months<sup>32</sup>. Furthermore, *Bacillus subtilis* nanoparticles were stable for six months<sup>33</sup>. Finally, nanoparticles produced by microorganisms are characterized by their easy separation from the medium by centrifugation<sup>34</sup>.

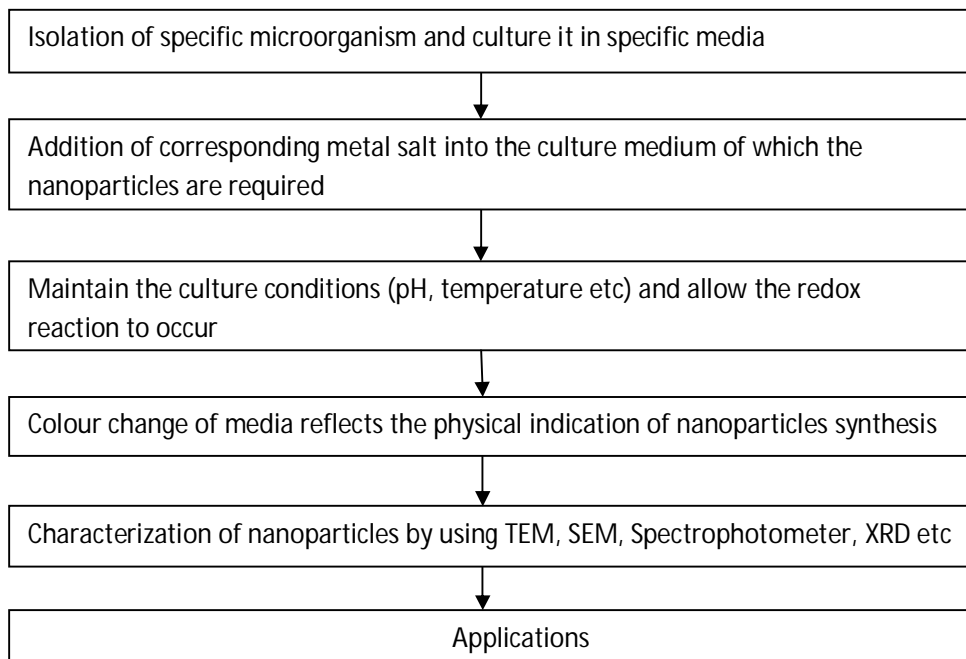


Fig. 4: General mechanism of nanoparticles synthesis using microorganisms.

Table 3: Some examples of metal and oxides nanoparticles synthesized using microorganisms

Microorganism used	Nanoparticles	References
<i>Bacillus subtilis</i>	Au	<sup>26</sup> Beveridge TJ <i>et al.</i> (1980)
<i>Lactobacillus</i> strains	Ag,Au	<sup>34</sup> Sintubin <i>et al.</i> (2009)
<i>Escherichia coli</i>	CdS	<sup>35</sup> Sweeney <i>et al.</i> (2004)
<i>Enterococcus</i> sp.	CdS	<sup>36</sup> Rajeshkumar <i>et al.</i> (2014)
<i>Pseudomonas stutzeri</i>	Ag	<sup>30</sup> Mohanpuria <i>et al.</i> (2007)
<i>Enterobacter</i> sp	Hg	<sup>37</sup> Sinha <i>et al.</i> (2011)
<i>Shwenella</i> sp	Se	<sup>38</sup> Lee <i>et al.</i> (2002)
<i>Moraxella Osloensis</i>	TiO <sub>2</sub>	<sup>39</sup> Nachiyar <i>et al.</i> (2016)
<i>Escherichia coli</i>	Pd	<sup>40</sup> Deplanche <i>et al.</i> (2010)
<i>Shewenella oneidensis</i>	Fe <sub>3</sub> O <sub>4</sub>	<sup>41</sup> Perez <i>et al.</i> (2010)
<i>Lactobacillus ap</i>	TiO <sub>2</sub>	<sup>42</sup> Jha <i>et al</i> (2009)
Sulphate reducing bacteria	FeS	<sup>43</sup> Watson <i>et al.</i> (1999)
<i>Proteus penneri</i>	Ni	<sup>44</sup> Spoorthy <i>et al.</i> (2017)
<i>Rhodopseudomonas palustris</i>	CdS	<sup>45</sup> Bai <i>et al.</i> (2009)
<i>Aspergillus niger</i>	Ag	<sup>23</sup> Gade <i>et al.</i> (2008)
<i>Trichoderma</i> sp.	Ag	<sup>46</sup> Elamawi <i>et al.</i> (2018)
<i>Neurospora crassa</i>	Au, Au/Ag	<sup>47</sup> Castro <i>et al.</i> (2011)
<i>Fusarium oxysporium</i>	TiO <sub>2</sub>	<sup>48</sup> Bansal <i>et al.</i> (2005)
<i>Trichoderma viride</i>	Ag	<sup>49</sup> Thakkar <i>et al.</i> (2010)
Extremophilic yeast .	Ag	<sup>50</sup> Mourato <i>et al.</i> (2011)
<i>Aspergillus fumigatus</i>	Zn	<sup>51</sup> Rajan <i>et al.</i> (2016)
<i>Rhodospiridium dibovatum</i>	PbS	<sup>52</sup> Seshadri <i>et al.</i> (2011)

<i>Aspergillus terreus</i>	Ag	<sup>53</sup> Singh et al.(2018)
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C) **Synthesis using biological product-** Biological products like DNA, proteins, enzymes, viruses etc are being used for the synthesis of nanoparticles.

**Table 4: Some examples of metal and oxides nanoparticles synthesized using biological particles**

Biological particle used	Nanoparticles	References
Tobacco mosaic virus (TMV)	SiO <sub>2</sub> , CdS, PbS, & Fe <sub>2</sub> O <sub>3</sub>	<sup>54</sup> Lee et al.(2002)
M <sub>13</sub> bacteriophage	ZnS	<sup>55</sup> Mao et al. (2003)
DNA mediated	Au	<sup>56</sup> Sohn et al. (2011)
Protein mediated	Au	<sup>57</sup> Leng et al.(2016)
Immunoglobulins, serum albumins	Au	<sup>58</sup> Shenton et al. (1999); <sup>59</sup> Beesley (1989)

## CHARACTERIZATION OF NANOPARTICLES

Nanoparticles are generally characterized by their size, morphology, crystallinity, surface charge, composition etc. The morphology and particle size and size distribution can be determined by AFM (Atomic Force Microscopy), TEM (Transmission Electron Microscopy), SEM (Scanning Electronic Microscopy), STM (Scanning Tunnelling Microscopy), DLS (Dynamic Light Scattering) etc. Although these all techniques have different principles and mechanisms still they all produce a highly magnified image of the sample. Furthermore, X-ray diffraction can determine the crystallinity, crystallite size, and orientation. Surface charge can be measured by Zeta Potential. Composition and purity can be determined by MS (Mass spectroscopy), NMR (Nuclear Magnetic Resonance), HPLC etc while UV-Vis spectroscopy is utilized to confirm sample formation by exhibiting the Plasmon resonance<sup>60</sup>.

## APPLICATIONS OF NANOPARTICLES

The unique properties of nanomaterials lead to a belief that they can be applied in a wide range of fields in the fields of biology, medicine, optical, electrical, mechanical, optoelectronics, pharmaceuticals, diagnostics, food and beverages, imaging techniques, environment remediation etc. Few of them are discussed below:

### 1) In diagnostics

- **Biosensor**

There are numbers of nanoparticles that can be used as biosensor components. These work as probes that recognize an analyte of interest. In such applications, some biological molecular species are attached to the surface of the nanoparticles to recognize the target of interest through a lock-and-key mechanism.



- **Microarrays**

Nanotechnology can affect microarray technology by creating densely packed, smaller, nano-sized arrays (nanoarrays) that could enable rapid screening of a larger number of (bio)chemicals.

## 2) In imaging

Techniques such as magnetic resonance imaging (MRI), X-ray, ultrasound (US), computer tomography (CT), and nuclear medicine (NM) are well known imaging techniques. In this specific area, nanotechnologies are making their greatest contribution by developing better contrast agents for almost all imaging techniques. The physiochemical features of the nanoparticles such as particle size, surface coating, surface charge, and stability, allow the redirection and concentration of the marker at the site of interest.

## 3) Target drug delivery

A key area in drug delivery is the precise targeting of the drug to cells or tissue of choice. Target Drug delivery systems should be capable to control the fate of a drug entering the body. In this aspect, nanoparticles could be used as a great drug delivery systems, owing to their advantageous characteristics.

## 4) As a potent antimicrobial agent

The silver nanoparticles synthesized using an endophytic fungus, *Pestalotia sp.*, isolated from leaves of *Syzygium cumini* has antimicrobial potential against human pathogens, *i.e.* *S. aureus* and *S. typhi*<sup>61</sup>. Silver nanoparticles showed powerful bactericidal potential against both Gram-positive and Gram-negative bacteria. The bactericidal activity of silver nanoparticles against the MDR bacteria is also investigated<sup>62, 63</sup>.

**5) Environmental remediation:** Environmental remediation can be enhanced with nanotechnology. For example, zero-valent(Fe<sub>0</sub>) iron nanoparticles are used for the remediation of contaminated groundwater and soil.

When exposed to air, iron oxidizes to rust; however, when it oxidizes around contaminants such as trichloroethylene (TCE), carbon tetrachloride, dioxins, or PCBs, these organic molecules are broken down into simple and less toxic carbon compounds. Since iron is non-toxic and is abundant in the natural environment (rocks, soil, water, etc.), some industries have started using an 'iron powder' to clean up their new industrial wastes.

## 6) Food packaging and monitoring

Nanotechnology may have a huge impact on the way food is produced, packaged, stored and transported. Applications include improved processing and packaging, enhanced flavor and nutrition, tracking of products and ingredients from farm to shelf, and monitoring of taste, ripening, and microbiological contamination.

## 7) Nanotechnology for crop biotechnology

Nanoparticles in the form of nanocapsule can facilitate a successful invasion of herbicides, chemicals or genes through cuticles and tissues, allowing slow and regular discharge of the active substances which target precisely plant parts to liberate their substance<sup>64</sup>.

## FUTURE PROSPECTS

With the development of nanotechnology and its various applications in diverse fields, nanoparticles are being synthesized by various methods such as physical, chemical and biological methods. Biological methods have more advantages over physical and chemical methods as biological methods are nontoxic, cheap etc. As biological methods are single step process and commercially economic, biosynthesis of nanoparticles by microorganisms attracts researchers to go for future development of many areas like biosensor, bio-imaging, food packaging, detection of pathogens, antimicrobial activity, agriculture etc. Future applications of NPs show promise in advancing the fields of medical treatment (gene therapy and targeted drug delivery), semiconductors, and environmental remediation technology.

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