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Developments in green nanotechnology: biogenic synthesis, mechanisms, applications

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ABSTRACT

The conventional methods for fabrication of nanomaterials have heavily relied on the use of toxic chemicals for their reduction and stabilization as well as organic solvents, which are detrimental to the ecosystem. Thus, developing sustainable synthesis methods in line with the principles of "green" chemistry has always taken foremost precedence and at the same time, been a challenge. Over the past few decades, the synthesis of nanomaterials using environment-friendly agents has captivated the research community around the globe. A variety of plant extracts and microbes have been explored for the biogenic synthesis of different nanomaterials for myriad applications. This review aims to present the synthesis of nanomaterials using various green agents, their mechanism of formation and applications. Focus is also laid on how the morphology or property of the nanomaterials is influenced by different green agents or conditions during synthesis. Finally, the challenges and scope of using green agents for the synthesis of nanomaterials are presented.

1. Introduction

The concept of nanotechnology was introduced by Richard Feynman in his famous lecture, "There's plenty of room at the bottom" at the American Institute of Technology (Fig. 1). Today, nanotechnology offers an astounding potential for the development of next-generation materials for use in diverse sectors such as agriculture, biomedicine, catalysis, environmental remediation, electronics and packaging. Nanomaterials are known for their very high aspect ratio and a large surface area, which makes them extremely reactive. Further, they have a high degree of functionalization and morphologies that can be tailored to give size-dependent properties [1]. For example, metal particles show exceptional physical, chemical and mechanical properties in the "nano" state in comparison with the bulk state of the same material.

The conventional methods for the fabrication of nanomaterials employ either top-down or bottom-up approaches. The top-down methods commonly include ball milling, etching, lithography, laser ablation, plasma arching and sputtering; while the bottom-up approaches include chemical reduction, sol-gel process, spray pyrolysis, molecular beam epitaxy and vapor deposition [2]. Majority of these techniques make use of expensive equipment with sophisticated infrastructure and hazardous or toxic chemicals leading to serious ecological concerns. In view of this, the focus of the scientific community has shifted towards the development of ecofriendly, safe and cost-effective methods as alternatives to the physico-chemical methods, employing biogenic routes based on "green" chemistry for the synthesis of nanomaterials. A fundamental key to the approach of green synthesis is the use of renewable materials, nonhazardous chemicals and environmentally benign solvents [3]. It could be brought about by using anything of biological origin such as phytochemicals from plants, as well as biomolecules from various types of microbes such as bacteria, algae and fungi. The green biosynthesis presents a simple, ecofriendly, rapid, one-pot, sustainable and economical approach for fabrication of nanomaterials [2].

The biogenically engineered nanomaterials show promising applications ranging from biomedicine for diagnostics, therapeutics and drug delivery, to biosensing and environmental remediation [5–7]. A key advantage of the green nanomaterials is their biocompatibility with improved efficacy and safety for biomedical applications. The structure or formulation of a nanomaterial, the biological response that is desired from the nanomaterial, and the biological environments that the nanomaterial will encounter play a significant role in determining the

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biocompatibility of a nanomaterial. Biocompatibility is assessed using *in vitro* and *in vivo* assays and clinical tests that help gain valuable insights into cytotoxicity, genotoxicity, hemocompatibility, histocompatibility, and immunotoxicology. For example, the green metal nanoparticles have shown ability to cross the blood-brain barrier and more effectively deliver therapeutic agents to the brain for the treatment of neurodegenerative disorders like Alzheimer's disease [8]. Plant-synthesized metal nanomaterials have demonstrated capacity to penetrate biological barriers for the treatment of oral cancer [9]. Bioengineered silver and gold nanoparticles showed evidence in antinociceptive therapy, alleviating the nociceptive pain in mice and rats [10], as well as in anticoagulant therapy [11]. The characteristic properties of nanomaterials including, shape, size, dissolution, agglomeration state, chemical composition, specific surface area, crystal structure, surface morphology, surface energy, surface coating, and surface charge critically impact physico-chemical and biological interactions, fate, and the desired or adverse outcomes of nanomaterials.

In light of the above, this review discusses the breakthrough advancements in green nanotechnology for synthesis of nanomaterials using plant extracts and microorganisms. The biogenic route offers an ecofriendly and non-toxic route for production of nanomaterials with lower environmental impact compared to the chemical route. The mechanism of synthesis using various plant extracts and microbes has been elucidated followed by a detailed discussion on the synthesis of metal and metal oxide nanomaterials using extracts from plants and microbes including bacteria, fungi, algae, and yeast. Finally, emerging technologies in biosynthesis along with the challenges in biosynthesis have been presented. One of the major challenges in green nanotechnology is the agglomeration of nanoparticles that may alter the surface properties, and thereby impact the biological activities. The future perspectives on exploration of the biogenic nanomaterials in clinical settings with the need for further studies on evaluation of biocompatibility and cytotoxicity have been discussed.

2. Mechanism of biosynthesis

2.1. Biosynthesis using plant extracts

Plants contain a plethora of biomolecules or secondary metabolites that are responsible for formation of nanoparticles via chemical reduction and stabilization. A plant extract is a complex mixture of biomolecules that include polyphenols, flavonoids, terpenoids, carboxylic acids, polyols, carotenoids, amino acids, glycosides, etc. The main role of the biomolecules is to first reduce the metal from its ionic form to a non-ionic form followed by stabilization of the NPs. The biochemical profile of the extract along with reaction conditions of time, temperature, pH, and concentration determine the size, shape and crystallinity of the nanostructures. The phytochemicals may be characterized using spectroscopic tools- UV-VIS, FTIR, NMR or chromatographic techniques- HPLC, GC-MS.

Phytosynthesis occurs by various mechanisms that are still under exploration due to the complex nature of biomolecules involved in synthesis. Nanomaterial synthesis from plants occurs in 3 phases- activation, nucleation and growth [12]. In the activation phase, biomolecules with a reducing potential reduce the metal ions from its precursor to NPs. In the nucleation phase, the reduction proceeds from the interaction of metal ions with biomolecules as new NPs are formed. In the growth phase, metal ions aggregate to give NPs and are converted from mono- or divalent states to zero valent state on further reduction.

Plant extracts are rich in antioxidants that have hydrogen donating capability to bring about the reduction of metal ions. The antioxidants show high binding ability to metal ions that induces the chelating effect [13]. The reducing power of phytochemicals is associated with the nucleophilic character of aromatic rings, which releases electrons or hydrogen ions to aid the reduction of metal ions [14]. In this review, the phytosynthesis mechanisms have been elucidated in the corresponding sections for better understanding and clarity.

2.2. Biosynthesis using microbes

The biogenic synthesis of nanomaterials by bacteria, fungi, algae or yeast entails ease of processing, good control on morphology, better stability and inexpensive medium [14]. The microbial synthesis may be through intracellular or extracellular pathways. In the intracellular synthesis, metal ions bind on the microbial cell surface through electrostatic forces, generated by oppositely charged ions on the surface of metal ion and microbes. Subsequently, the adsorbed metal ions get reduced into metallic particles by the enzymes with positively charged groups on the microbial cell surface.

The extracellular synthesis involves the preparation of microbial extract, the preparation of metal precursor solution followed by the incubation of both extract and the solution for the synthesis reaction to occur. In this synthesis, the microbial enzymes secreted extracellularly are responsible for causing the reduction and capping of metal ions [15]. The extracellular synthesis eliminates the need for downstream processing for nanoparticle separation through cell lysis by mechanical

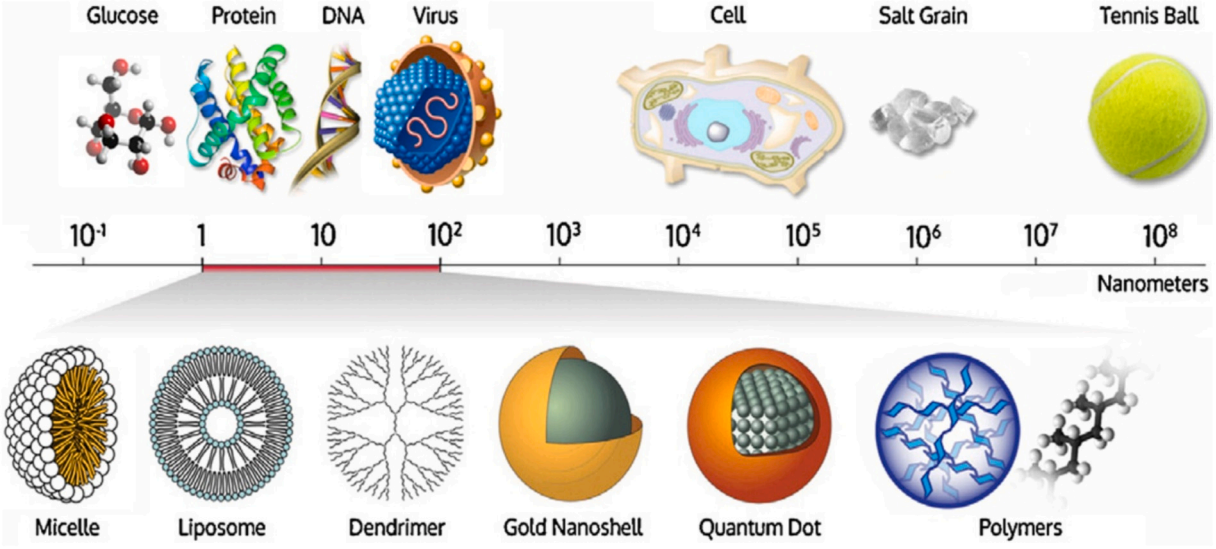

Fig. 1. Different materials at the nano-scale [Reprinted with permission from ref. [4] © 2014 Elsevier publisher]

means or ultrasonication and the subsequent removal of cell constituents by centrifugation, thus making the process cost-effective [16].

3. Phytoextracts in synthesis

Biosynthesis using phytoextracts is an extremely cost-effective method with potential for commercial scale-up to produce large quantities of nanomaterials. The biomolecules in plant extracts are environmentally benign, although chemically complex [3]. Plants abound in natural antioxidants that are mostly polyphenols such as anthocyanins, flavonoids, phenolic acids, lignans and stilbenes; carotenoids such as carotenes and xanthophylls; as well as vitamins-C and E [17]. The antioxidants along with other functional molecules like alkaloids, terpenoids, steroids, free amino acids and tannins have the ability to be natural bioreductants for the phytosynthesis of several nanomaterials for a plethora of applications [1]. Moreover, the phytoconstituents can also work as capping agents to passivate or protect the surface of particles, thereby making unnecessary the need for using synthetic stabilizing agents [18].

Various parts of plants including flowers, leaves, barks, stems, shoots, seeds, latex, roots, twigs and fruits may be used for extraction of the phytoconstituents [19]. A variety of modern extraction approaches have been developed based on the "green" concept in order to cut down the operational time and solvent consumption as well as to improve the extraction efficiency. They include extractions assisted by ultrasound, microwaves, enzymes, supercritical fluids, as well as those performed under pressurized conditions, pulsed electric field and high voltage electrical discharges. The selection of solvent for extraction depends on the polarity and chemical nature of the constituents to be extracted, along with other factors like the toxicity, availability and cost. Solvents such as water, methanol, ethanol, propanol and acetone are widely used for extraction of phytoconstituents [17].

The simplest method for the biosynthesis of nanomaterials with phytoextracts involves mixing of the precursor salt solution with the phytoextract in predetermined ratio and carrying out the reaction in ambient conditions or at elevated temperatures under mechanical agitation. On the other hand, modern approaches may employ microwaves, ultrasound and hydrothermal treatment to assist the bioreduction process. The reaction conditions during the reduction i.e. temperature, time and pH; ratio of the precursor salt solution to the phytoextract and the antioxidant capacity of the extract are the critical factors influencing the morphology of the synthesized nanomaterials [20].

3.1. Synthesis of metal nanomaterials

Noble metals like Ag, Au, Pt and Pd have gained enormous popularity on account of their widespread usage in biomedicine, catalysis, optics and biosensing [21,22]. Table 1 lists the various metal nanomaterials biosynthesized from phytoextracts. Several studies have reported significant cytotoxicity of Au nanoparticles (NPs) synthesized using cetyltrimethylammonium bromide, which is one of the most commonly used surfactants and highly toxic in nature. In contrast, Au NPs synthesized using phytoconstituents as natural reducing agents have demonstrated safety and biocompatibility essential for applications in nanomedicine [8,23].

Gold NPs were fabricated using an aqueous leaf extract of *Commiphora wightii* showed promising anticancer activity against MCF-7 breast cancer cells [24]. The biosynthesis was brought about with chloroauric acid as a precursor and the bioreduction was achieved in 5 min, confirmed by the color change of the solution from pale yellow to purple wine, thus indicating the formation of nanogold in solution. Similarly, an aqueous leaf extract of *Sansevieria roxburghiana*, a medicinal plant, was utilized to synthesize Au NPs with different morphologies by reacting the extract with varying concentrations of chloroauric acid in a water bath at 40 °C in the dark [25]. The polyphenols and proteins in the extract facilitated the bioreduction of Au^{3+} ions and the capping of Au NPs. In another work, *Plumeria alba* flower extract was employed for the size-controlled formation of Au NPs- 1% and 5% concentrations of the extract resulted in NPs with two different sizes, as indicated by the surface plasmon resonance (SPR) peaks at 552 and 536 nm, respectively [26]. The NPs exhibited a good catalytic degradation efficiency against hazardous dyes such as methyl red and 4-nitrophenol. Similarly, Au NPs of 15-40 nm size, fabricated using *Ganoderma lucidum* extract showed excellent catalytic activity of over 98% in 9 min and reusability of over 95% after five cycles in converting 4-nitrophenol to 4-aminophenol [27].

Despite of efforts to investigate the phytoconstituents responsible for

Table 1
Metal NPs biosynthesized using phytoextracts

NPs	Plant	Synthesis method	Morphology		Property or application	Reference
Au	*Capsicum annum* (leaves)	room condition synthesis	triangular pyramid, square & rectangular	13 nm	antimicrobial activity, toxic metal removal from water	[44]
Au	*Achillea bieber-steinii* (flower)	Heating at 25, 40 & 80 °C for 30, 60, 120 & 240 min	spherical	2-30 nm	cytotoxicity effects on embryonic carcinoma stem cells	[21]
Au	*Moringa oleifera* (flowers)	mixing at room temperature	spherical	3-5 nm	catalytic & anticancer activity	[45]
Au	*Capsicum annum* (green bell pepper)	heating at 95 °C for 40 min	spherical	3-12.5 nm	colorimetric sensing of Fe^{2+}	[46]
Au	*Mussaenda glabrata* (leaves)	mixing at room temperature for 5 min	spherical, triangular	10.59 nm	antioxidant, catalytic & antimicrobial activity	[47]
Au	*Aerva lanata* (leaves)	microwave-assisted synthesis	spherical, hexagonal, triangular	17.97 nm	catalytic reduction of 4-nitrophenol	[48]
Ag	Turmeric powder	overnight stirring of mixture	spherical	5-35 nm	antibacterial activity	[3]
Ag	*Anthemis atropatana*	heating & stirring the mixture	spherical	38.89 nm	cytotoxicity, induction of apoptosis	[49]
AgNi AgCo	*Withania coagulans (fruits)*	heating at 70 °C for 15-120 min	Cauliflower Lichen	20-140 nm 10-35 nm	photocatalysis, antibacterial activity, adsorption of lead	[50]
Ag	*Moringa oleifera* (flower)	stirring for 30 min	spherical	8 nm	antimicrobial activity and sensing of copper ions	[51]
Ag	Green tea	mixing and stirring in alkaline environment	spherical	34.68 ± 4.95 nm	antibacterial activity	[52]
Ag	*Araucaria angustifolia*	mechanical stirring at 45 °C for 35 min	spherical	91 nm	electrochemical sensing of paracetamol	[22]
Pt	Water hyacinth	heating at 90 °C for 1 h	spherical	3.74 nm	good colloidal stability of the NPs	[34]
Fe	*Moringa oleifera* (leaves/ seeds)	room temperature stirring for 30 min	spherical	2.6-7.4 nm	adsorptive removal of nitrate ion from water	[53]
Cu	*Eclipta prostrata* (leaves)	mixing & stirring for 24 h at room temperature	spherical, hexagonal, cubical	28-45 nm	antioxidant & cytotoxic activity	[54]

bioreduction using a number of analytical techniques including Fourier transform infrared spectroscopy (FTIR), gas chromatography and mass spectrometry as well as nuclear magnetic resonance; the exact mechanism of phytogenic synthesis still remains unclear [28]. Common plant polyphenols such as catechin, luteolin, ellagic acid, epigallocatechin-3-gallate and myricetin 3-O-galactoside have strong reducing potential with a high propensity to chemisorb on the particle surface, thus leading to reduction and stabilization of the NPs [29]. The pioneering work on the green synthesis of Ag NPs was done with *Pelargonium graveolens* (geranium) leaf extract, reducing Ag^{+} ions to produce spherical Ag NPs of 16-40 nm size [30]. Silver NPs of 3-15 nm were fabricated using *Cassia auriculata* bark extract at room temperature [31]. The biosynthesized AgNPs (at 25 to 125 μg/mL) showed good antioxidant activity in FRAP (ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assays. Interestingly, non-toxicity analysis revealed the least toxic effects of AgNPs, $AgNO_3$ and bark extract on microcrustacean *A. nauplii*, with mortality rates of 28.14, 32.26 and 38.42 %, respectively. The green synthesis of AgNPs using leaf extracts of neem, aloe vera, Indian mint and guava demonstrated biosensing and photocatalytic potential against mancozeb agro-fungicide [32]. Similarly, AgNPs of 14 nm size made from extract of flowers and leaves of *Onobrychis sativa* L. demonstrated good antioxidant activity and catalytic activity of methylene blue with 68 % degradation in 30 min [13].

Various phytoextracts have been explored for the synthesis of other metal nanoparticles such as Pt, Pd, Fe and Cu. For instance, the fabrication of Pt NPs has been performed using pomegranate peel [33], water hyacinth [34] and *Ocimum sanctum* [35]; while Pd NPs have been synthesized with *Origanum vulgare* [36] and *Filicium decipiens* [37]. Fe NPs synthesized using leaf extracts of *Mangifera indica, Murraya Koenigii, Azadiracta indica* and *Magnolia champaca* have demonstrated potential for domestic waste water treatment by simultaneous removal of chemical oxygen demand, nitrogen and phosphates [38]. In another work, successful removal of Cr^{6+} was achieved with Fe NPs fabricated from *Rosa damascene, Thymus vulgaris* and *Urtica dioica* [39]. The polyphenols, proteins and organic acids in the extracts led to both reduction and stabilization in the biosynthesis. Using *Plantago asiatica* leaf extract, Cu NPs were synthesized without the addition of any external reducing agent by mixing the aqueous plant extract and copper chloride solution with vigorous shaking at 80 °C [40]. ultraviolet-visible (UV-VIS) spectroscopy confirmed the formation of Cu NPs, indicated by a visual color change of the mixture within 5 min. The synthesized NPs were uniform and spherical in shape with a narrow size distribution in the range of 7-35 nm. It should be noted that the experimental conditions maintained during phytogenic synthesis play a critical role in the reaction kinetics as well as in tuning the particle morphology [41]. On similar lines, the phytogenic synthesis of Cu NPs has been accomplished with extracts from pomegranate seeds [42] and green/black tea leaves [43].

3.2. Synthesis of metal oxide nanomaterials

Semiconductors are multi-functional metal oxides or sulfides with a large band gap energy. The semiconducting materials have the ability to create electron-hole pairs when exposed to ultraviolet light, which makes them a promising candidate for use in opto-electronic devices such as solar cells, photocatalysis for degradation of toxic dyes, cosmetics, disinfection and sensing [55]. Table 2 shows the various semiconducting materials synthesized using phytoextracts. ZnO, with a band gap energy of 3.37 eV, is one of the most extensively explored metal oxides. The ZnO NPs have been synthesized with extracts of *Aloe vera, Solanum nigrum, Terminalia chebula, Agathosma betulina, Limonia acidissima* and *Phyllanthus niruri* [55]. The aqueous extract of *Cassia fistula* was employed for the synthesis of ZnO NPs by solution combustion method [56]. X-ray diffraction and transmission electron microscopy (TEM) analysis confirmed the formation of hexagonal wurtzite-structured NPs of 5-15 nm size. The NPs showed effective degradation of methylene blue under ultraviolet light as well as significant free radical scavenging activity. In a different work, ZnO NPs were fabricated with extracts of *Allium sativum* (garlic), *Allium cepa* (onion) and *Petroselinum crispum* (parsley) [57]. The FTIR spectrum indicated the existence of biomolecules such as phenolics, carbohydrates, amino-acids and organosulfur compounds, bound to the surface of the particles as responsible for reduction as well as stabilization of the NPs. The size of the NPs was found to be affected by the nature of plant extract used for synthesis. The phytoextract-mediated production of ZnO NPs also narrowed the optical band gap of the NPs and increased their photodegradation efficiency [57].

Similarly, the biosynthesis of titania (TiO_2) has been explored using phytoextracts from plant species like *Murraya koenigii, Aloe vera, Psidium*

Table 2
Semiconducting NPs biosynthesized using phytoextracts

NPs	Plant	Synthesis conditions	Morphology		Property or application	Reference
ZrO_2	Green tea (leaves)	Reaction at 90 °C for 3 h, centrifugation, calcination	spherical/ polygonal	7 nm	photocatalytic & antimicrobial activity	[74]
ZrO_2	*Wrightia tinctoria* (leaves)	75 °C for 3 h, centrifugation, calcination	tetragonal crystals	17 nm	photocatalytic dye degradation	[75]
ZnO	*Nelumbo nucifera (lotus leaf)*	solution combustion method	spherical	3-4 nm	sensing CO gas	[76]
ZnO	*Eucalyptus globulus* (leaves)	room temperature synthesis	spherical	11.6 nm	photocatalytic & antioxidant activity	[77]
TiO_2	*Trigonella foenum-graecum* (leaves)	stirring for 15 min, sintering 700 °C for 3 h	spherical	20-90 nm	antimicrobial activity	[78]
ZnS	*Stevia rebaudiana* (leaves)	reaction mixture incubated at 70 °C for 6 h	spherical	8.35 nm	cytotoxic effects on human cancer cell line (MCF-7)	[79]
Al_2O_3	*Prunus* × *yedoensis* (leaves)	room temperature synthesis	spherical, hexagonal	50-100 nm	nitrate ion removal	[80]
Fe_3O_4	*Anthemis pseudocotula* (aerial parts)	stirring for 1 h at room temperature	spherical	308-565 nm	petroleum oil spill collection	[81]
Fe_2O_3	*Platanus orientalis* (leaves)	reaction at 25 °C for 1 h	spherical	38 nm	antifungal activity	[82]
NiO	*Calotropis gigantea* (leaves)	reaction at 80 °C for 90 min	-	20-40 nm	catalytic & antimicrobial activity	[64]
PdO	*Aspalathus linearis* (leaves)	stirring at room temperature for 30 min	tetragonal	22.7 ± 4.3 nm	semiconducting properties	[65]
CeO_2	*Jatropha curcus* (leaves)	autoclave at 150 °C for 12 h, calcination	spherical	3-5 nm	photocatalytic degradation of acetaldehyde	[83]
Co_3O_4	*Manihot esculenta* (root)	reaction for 24 h at room temperature	octahedron	-	antiferromagnetic behaviour	[84]
Co_3O_4	*Trigonella foenumgraceum* (fenugreek leaves)	reaction at 80 °C, annealing at 500 °C	spherical	13.2 nm	high thermal stability, high purity of nanoparticles	[85]
CdS	*Camellia sinensis* (leaves)	incubation in dark for 7 days	spherical	2-5 nm	antimicrobial, bioimaging & therapeutics	[86]

guajava, Eclipta prostrata, Solanum trilobatum and *Catharanthus roseus* [19]. Room temperature fabrication of TiO_2 NPs of size 10-20 nm by mixing the titanium chloride precursor and the leaf extract of *Jatropha curcus* L. under agitation was recently reported [1]. A change in color from transparent to whitish-brown indicated the bioreduction of Ti^{4+} ions to TiO_2 NPs, which were precipitated out by dropwise addition of ammonia with subsequent filtration, washing and calcination. The FTIR analysis of the phytoextract revealed the presence of various bio-molecules such as phenols and tannins, which might be accountable for the green reduction of metal ions and their stabilization during the synthesis of TiO_2 NPs (Fig. 2). The green-synthesized NPs demonstrated a huge potential for removal of chemical oxygen demand (82.26%) and Cr^{6+} ions (76.48%) from tannery wastewater [1]. Several researchers have also developed titania composites with metals; for instance, Ag/TiO_2 nanocomposite was synthesized with leaf extract of *Euphorbia heterophylla* without the addition of external surfactants [58]. The bio-synthesized composite showed effective catalytic reduction of dyes such as methyl orange and Congo red with potential to be recovered and reused many times without significant loss of activity. In similar fashion, *Myrtus communis* leaves were used for the fabrication of Pd/TiO_2 NPs as catalysts for the Suzuki–Miyaura coupling reaction [59].

Iron oxide (Fe_2O_3) NPs have been synthesized in core-shell form from *Ficus carica* (common fig) dried fruit extract [60]. The metallic core was oxidized because of high phenolic content of the extract resulting in spherical NPs of 9 ± 4 nm size. Interestingly, the biosynthesized NPs were found to have moderate stability and lower side effects compared to the chemically synthesized ones [60]. Likewise, *Lagenaria siceraria* has also been successfully explored for the production of iron oxide NPs [61]. Cobalt-zinc ferrite NPs made using quince fruit extract showed potential for the adsorptive removal of dye pollutant and catalytic decomposition of H_2O_2 [62]. NiO NPs have been fabricated from the leaf extract of a medicinal plant, *Aegle marmelos* [63]. The synthesis was carried out on a hot plate, where a homogeneous solution of the reactants was uniformly heated to 250 °C for 15 min leading to combustion of the mixture to form spherical or cubical NPs of 8-10 nm size. Cytotoxicity and apoptosis were induced due to the generation of reactive oxygen species and oxidative stress effects were augmented by reduced particle size, specific surface area and release of more Ni^{2+} ions (Fig. 3a). Interestingly, the NiO NPs were more effective over Gram positive bacteria than the Gram-negative bacterial strains. The enhanced antibacterial activity was attributed to the high stability, smaller size and spherical morphology of the NiO NPs synthesized via the green route. Further, the NPs demonstrated photocatalytic degradation of 4-chlorophenol initiated by branch dissociation, where the hydroxyl free radicals reacted with the organic molecules on the catalytic surface (Fig. 3b) [63]. Other works have demonstrated the synthesis of NiO NPs using *Calotropis gigantea* leaves [64] as well as *Aspalathus linearis* leaves [65].

Bismuth oxide (BiO) nanoparticles, synthesized using almond gum as the reducing and stabilizing agent, showed over 90% dye degradation under visible light [7]. Also, BiO NPs showed catalytic reduction of 4-nitrophenol to 4-aminophenol, attaining 92% reduction efficiency in 32 min. The reduction comprised of adsorption and diffusion of 4-nitrophenol molecules onto active hydrogen surface of BiO NPs, followed by an electron transfer from BH_4^- ions to 4-nitrophenol ions, as depicted in Fig. 3c [7]. The phytogenic synthesis of ZrO_2 with *Rosmarinus officinalis* [66] and *Lagerstroemia speciosa* [67] has been reported. Manganese oxide NPs with different Mn/O ratios (Mn_3O_4, Mn_2O_3 and MnO_2) synthesized using *Sapindus mukorossi* exhibited different morphologies such as needle-shape, spherical as well as cubical and showed potential as oxidation catalysts [68]. CuO NPs fabricated using leaf extract of *Catha edulis* have shown promising antibacterial activity against the pathogens- *S. typhimurium* and *E. coli,* with zones of inhibition of 12.5 mm and 8 mm, respectively [69]. CuO NPs of 2-6 nm size, synthesized from *Psidium guajava* leaf extract as reducing agent have demonstrated excellent degradation efficiency for the industrial dyes [70]. Similarly, plant extracts have also aided the synthesis of other semiconductors such as CdS with *Calotropis gigantea* leaves [71], CdSe with *Lawsonia inermis* leaves [72] and CdTe with *Ficus Johannis* fruits [73].

4. Microbes and other organisms in synthesis

Various classes of microbes including bacteria, fungi, algae and yeast have been explored for the biogenesis of nanomaterials for applications in agriculture, biomedicine, cosmetics, drug delivery and sensors. The microbial production of nanomaterials is a slow affair, taking several hours to days, as compared to the physical, chemical and phytoextracts-mediated approaches. Fig. 4 shows the different pathways for microbial production of nanomaterials. In the intracellular synthesis, the nanomaterials are formed within the cell and subsequently diffused into the cell wall. Herein, electrostatic interaction facilitates the positively charged metal ions to be trapped on the cell wall surface or inside the cytoplasm with negatively charged proteins or enzymes. Thereafter, the enzymes present in the cell wall cause the reduction of these ions into

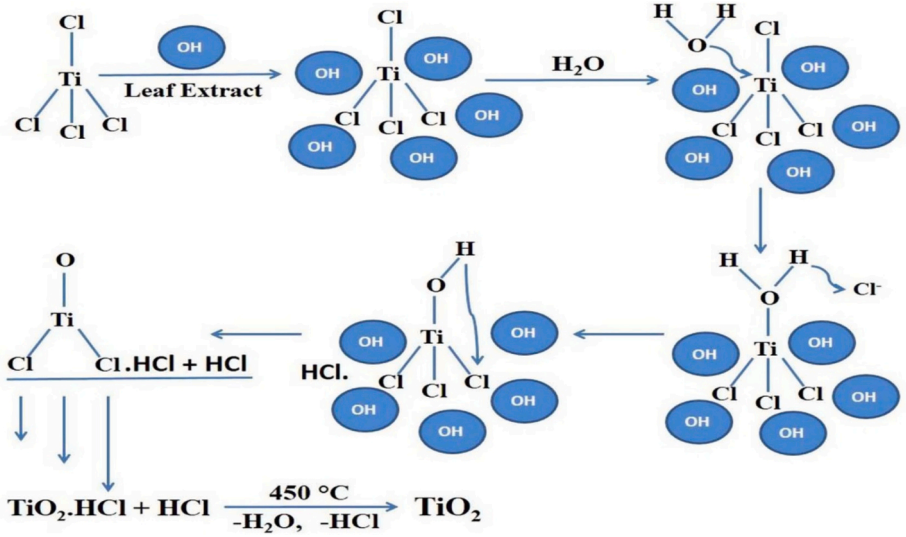

Fig. 2. Proposed mechanism for the biosynthesis of TiO_2 NPs using leaf extract of *Jatropha curcas* L.; the hydroxyl groups from the polyphenolic tannins in the phytoextract served as capping agents for TiO_2 NPs[Reprinted with permission from ref. [1] © 2018 Elsevier publisher]

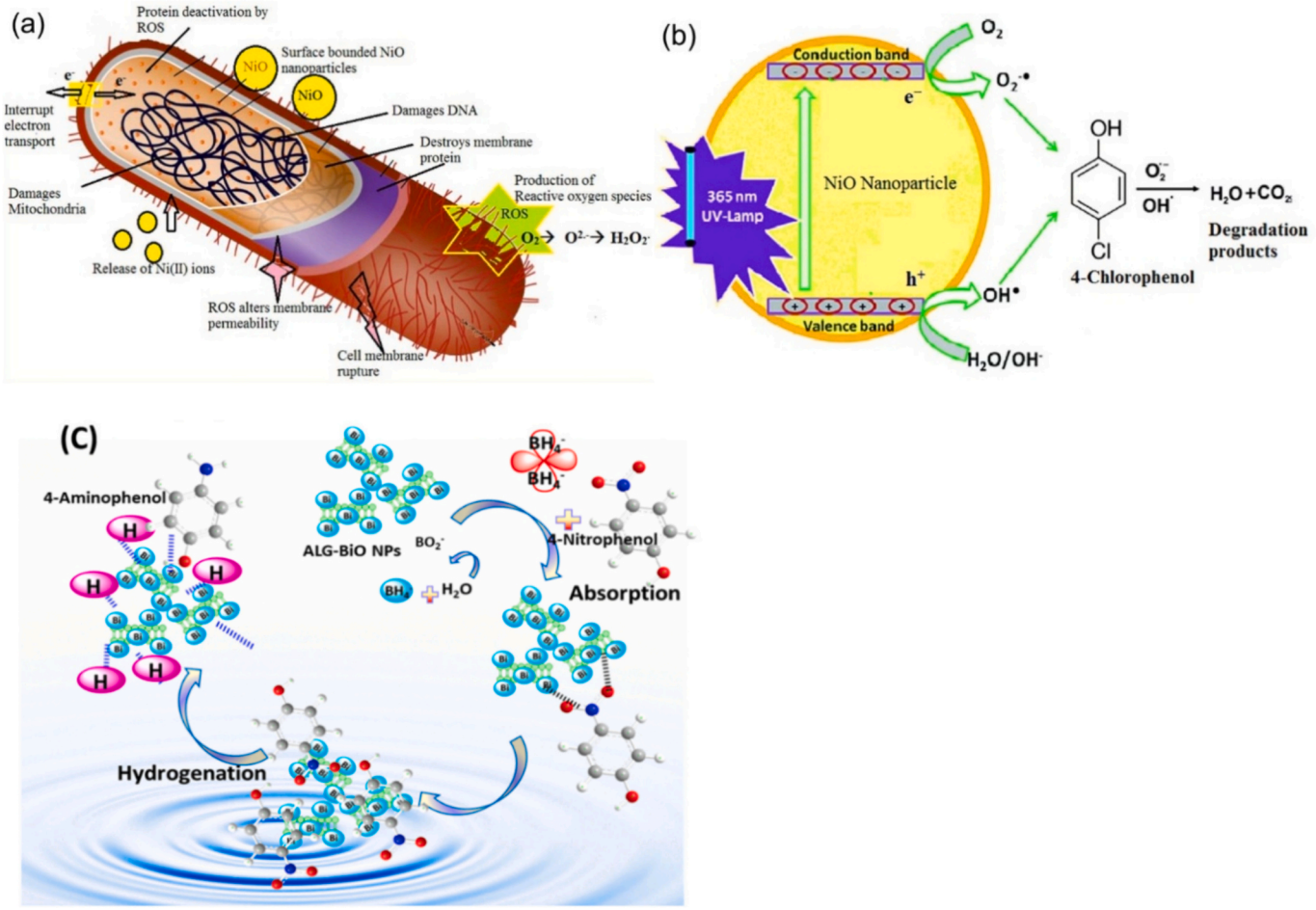

Fig. 3. (a) Toxicity effect on bacteria by NiO NPs- production of reactive oxygen species (ROS) damages mitochondria, nucleic acid, proteins and cell membrane eventually leading to cell lysis; (b) Degradation mechanism of 4-chlorophenol by NiO NPs under ultraviolet light- excitation of electrons occurs from the valence band to the conduction band, causing a hole in the former that reacts with water to form hydroxyl free radicals resulting in degradation[Reprinted with permission from ref. [63] © 2018 Elsevier publisher]; (c) Mechanism for the catalytic reduction of 4-nitrophenol on BiO nanoparticles [Reprinted with permission from ref. [7] © 2025 Elsevier publisher]

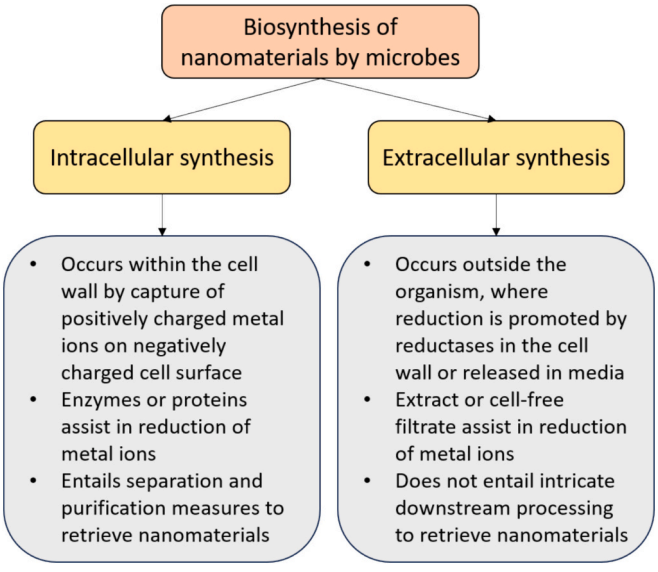

Fig. 4. Mechanisms of microbe-mediated biosynthesis of NPs by intracellular and extracellular pathways

small nuclei that eventually form NPs and are diffused via the cell wall. In contrast, in the extracellular synthesis, the microbial extracellular constituents such as proteins and enzymes play a critical role. The vital role of nicotinamide adenine dinucleotide hydrate (NADH)-dependent nitrate reductase enzyme to reduce the metal ions to NPs has been reported by several researchers. The transfer of electrons from NADH by NADH-dependent reductase, which is secreted by the microbe leads to the reduction of metal ions. Diverse biomolecules with carboxyl groups, amides, primary and secondary amines act as bioreductants and capping agents in order to stabilize the particles by preventing their agglomeration. The morphology of the NPs varies with the organism employed as well as the conditions employed during synthesis including temperature, pH and substrate concentration [4,87,88].

The approach of biogenic synthesis is inspired from its safety, reliability, eco-friendliness and cost-effectiveness. Furthermore, it minimizes the need for surfactants, polymers and ionic liquids that are associated with a number of biological and environmental risks [89,90]. Nevertheless, the use of microbes for the biosynthesis of nanomaterials comes with certain disadvantages and limitations. The screening of microbes could be an arduous task and the construction of vector may be time consuming. Dealing with microbes always requires highly aseptic conditions and proper maintenance of the system. Despite the recent advances in the microbial biosynthesis materials, finding a microbe capable of synthesizing the desired morphology still poses a challenge for the scientific community [2].

4.1. Bacteria-mediated synthesis

Table 3 lists the various nanomaterials fabricated from different strains of bacteria. The bacterial synthesis of Au NPs has been investigated from bacterial species such as *Pseudomonas aeruginosa* [91], *Shewanella oneidensis* [92], *Paracoccus haeundaensis* [16] and cyanobacterium such as spirulina [93]. *Rhodopseudomonas capsulata*, commonly existing bacteria in the natural environment, has shown potential for the reduction of Au^{3+} ions in a single step in ambient conditions by extracellular pathway [94]. The reduction was confirmed by a color change from yellow to purple indicating the synthesis of Au NPs. The NPs exhibited an absorption maximum at 540 nm assigned to the SPR band of Au NPs. The pH value was a critical parameter controlling the size and shape of Au NPs. In a different work, *Shewanella algae* was employed not only as a reducing agent but also as a shape-controlling agent for the fabrication of Au NPs and Au nanoplates at ambient temperature [95]. The bacterial cell extract could reduce aqueous $AuCl_4^-$ ions into elemental gold within 10 min in the presence of hydrogen gas as an electron donor. The morphology of the NPs was found to be a function of time- spherical Au NPs of 9.6 nm mean size were formed in the beginning of the reaction whereas; Au nanoplates of 100 nm edge length resulted as the reduction proceeded. The FTIR spectrum of gold particle suspension suggested the contribution of carbonyl groups in the extract in the synthesis of Au nanoplates [95]. Spherical Au NPs of 20.9 nm were synthesized using cell-free supernatant of a marine bacterium, *Paracoccus haeundaensis* [16]. The formation of NPs was indicated by color change of the reaction mixture to ruby red due to SPR after 15 min of reaction at 70 °C. The NPs showed concentration-dependent antioxidant activity against the DPPH radical as well as concentration-dependent growth-inhibition in A549 and AGS cancer cells. Importantly, no toxic effects were seen on HaCaT and HEK293 normal human cells, thus they could be used for biomedical application.

The biogenic synthesis of ultrafine Ag NPs of size 6-13 nm has been accomplished using cell-free culture supernatants of five strains of psychrophilic bacteria viz. *Arthrobacter kerguelensis, Pseudomonas antarctica, Pseudomonas proteolytica, Pseudomonas meridiana* and *Arthrobacter gangotriensis* [96]. The synthesis and stability of the extracellularly synthesized Ag NPs was found to depend on the temperature, pH as well as the bacterial strain. Further, it was observed that constituents of the nutrient broth also assisted the formation of Ag NPs, which is contrary to the commonly accepted mechanisms of bioreduction by nitrate reductase and biomolecules or proteins. Therefore, it was proposed to test the medium and its individual components as a control to ascertain that the NPs were synthesized by the culture supernatant and not by the medium or its components [96]. A silver-resistant psychrophilic bacterium, *Morganella psychrotolerans* has shown potential for shape-directed synthesis of Ag NPs to give nanospheres and nanoplates [97].The shape anisotropy of Ag NPs was achieved by controlling the kinetics of bacterial growth at different temperatures during synthesis. At optimum bacterial growth temperature of 20 °C, Ag NPs with spherical morphology and 2-5 nm size were observed during TEM imaging. However, at temperatures other than the optimum, the synthesis of Ag nanoplates was observed. The electrochemical measurements revealed the possibility of a silver reductase enzyme in silver-resistant microorganisms to contribute to the reduction of Ag^+ ions, as illustrated in Fig. 5 [97]. Biosynthesis of Ag NPs has also been achieved with cyanobacteria such as *Nostoc carneum* [98] and *Leptolyngbya* sp. [99]. Cyanobacteria produce biomolecules including polysaccharides, proteins, carotenoids, flavonoids and pigments that act as reducing and capping agents during biosynthesis. They have the ability to sequester metal ions from aquatic environments through biosorption or bio-accumulation, and thus provide an ideal platform for the synthesis of metal nanoparticles [98].

Similarly, studies on bacterial biogenesis have also reported the production of other metal NPs such as Cu [100], Pt [101], Se [102] and Hg [103]. Aqueous phase biosynthesis of quasi-spherical Cu NPs of 15-20 nm size has been described using a silver resistant bacterium, *Morganella morganii* [100]. The formation of Cu NPs was indicated by the appearance of an SPR band at ca. 550-600 nm in the UV-VIS spectrum and further confirmed by X-ray photoelectron spectroscopy- a highly surface-sensitive technique, in order to negate the possibility of CuO formation. The intracellular reduction of Cu^{2+} ions indicated a profound correlation between the silver and copper resistance machinery in the bacterial cells in the context of metal ion reduction [100]. In a different work, bioaccumulation of mercury with simultaneous synthesis of spherical Hg NPs of 2-5 nm size was achieved using *Enterobacter* sp. [103]. The heavy metal bioaccumulation in the cytoplasm was confirmed from TEM micrographs of the bacterial cells as well as from energy dispersive X-ray analysis. The pH of the medium was found to affect the size and distribution of the NPs. At pH 7.0, the NPs were uniformly dispersed on the cell wall as well as inside the cytoplasm whereas; at pH 9.0, the NPs were smaller and less dense. The Hg NPs trapped in the cell were unable to vaporize back into the environment, thus assisting the remediation process [103].

The biosynthesis of semiconductor oxides such as ZnO and TiO_2, as well as semiconductor sulfides has been explored with diverse range of bacterial species. The production of nearly spherical ZnO NPs of 7-19 nm size has been accomplished using *Lactobacillus plantarum*, which is the most abundant lactic acid bacteria in majority of the food products worldwide [104]. The negative electrokinetic potential of the bacteria easily attracted the cations, which acted as a trigger for the biosynthesis of the NPs. A zeta potential value of -15.3 mV for the synthesized

Table 3
Nanomaterials synthesized from different bacterial strains

NP	Bacteria	Type of synthesis or localization	Morphology	Size	Reference
Au	*Pseudomonas aeruginosa*	extracellular	spherical	15-30 nm	[91]
Au	*Shewanella oneidensis*	extracellular	spherical	12 ± 5 nm	[92]
Au	*Escherichia coli*	on the bacterial surface	spherical	25 ± 8 nm	[117]
Ag	*Staphylococcus aureus*	extracellular	spherical	160-180 nm	[118]
Ag	*Lactobacillus rhamnosus*	extracted exopolysaccharide-led synthesis	spherical, triangular, hexagonal	2-15 nm	[119]
Ag	*Bacillus flexus*	extracellular	spherical, triangular	12-65 nm	[120]
Pt	*Shewanella algae*	periplasmic space of bacterial cell	spherical	5 nm	[101]
Se	*Shewanella* sp.	extracellular	spherical	181 ± 40 nm	[102]
TiO_2	*Aeromonas hydrophila*	-	spherical	40 nm	[121]
ZnO	*Bacillus subtillis*	-	hairy	10-15 nm	[122]
ZnO	*Aeromonas hydrophila*	-	spherical	57 nm	[123]
Fe_3O_4	*Bacillus subtilis*	extracellular	spherical	60-80 nm	[124]
Fe_3O_4	*Desulfovibrio magneticus*	intracellular	bullet-shaped	53.8 ± 14.2 nm	[125]
γ-Fe_2O_3	*Actinobacter* sp.	extracellular	spherical	5-7 nm	[126]
Fe_3S_4	*Actinobacter* sp.	extracellular	spherical	19 nm	[126]
PbS	*Rhodobacter sphaeroides*	extracellular	spherical	10.5 ± 0.15 nm	[110]
ZnS	*Rhodobacter sphaeroides*	-	spherical	12 nm	[111]
CdS	*Rhodopseudomonas palustris*	extracellular	spherical	8.01 ± 0.25 nm	[112]

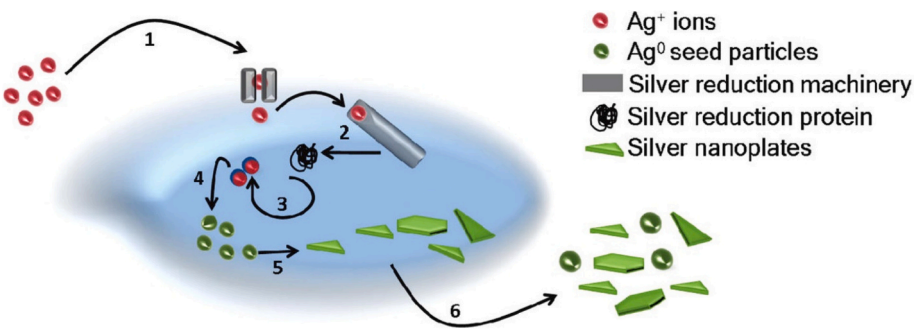

Fig. 5. Mechanism for extracellular biosynthesis of Ag NPs by silver-resistant bacterium, *Morganella psychrotolerans*: 1- uptake of Ag^+ ions by bacterium, 2- exposure of Ag^+ ions to bacterial silver reduction machinery, 3- binding of the biomolecules synthesized during silver reduction to Ag^+ ions, 4- reduction of Ag^+ ions to Ag^0 nuclei, 5- growth and assembly of Ag seed particles within the bacterial cell leading to spherical or plate-like Ag NPs, 6- release of the NPs from the cell by a cellular efflux system [Reprinted with permission from ref. [97] © 2010 ACS publisher]

colloidal system indicated that the NPs were moderately stable. Another study reported the fabrication of ZnO NPs on cotton by activated ammonia formed in the presence of the ureolytic bacteria, *Serratia urelilytica* [105]. The synthesis was achieved by immersing the cotton fabric in zinc ammonium complex and subjecting it to heat treatment at 50 °C for time periods of 30, 60 and 90 min. Interestingly, the structural morphology of ZnO NPs changed from spherical to flower-like with increasing treatment times. The ZnO NP-loaded cotton fabric revealed substantial antibacterial efficiency against *E. coli* and *S. aureus* due to consistent interfacial contact between the bacteria and the NPs [105]. The biosynthesis of ZnO NPs (40 nm) has also been accomplished with cyanobacterium species such as *Oscillatoria* [106]. The extracellular synthesis was indicated by a visual color change from blue to pale white and confirmed by an absorption peak at 372 nm by UV-VIS spectroscopy. The biogenic NPs demonstrated enhanced antioxidant and antibacterial activity against commercial chemically-synthesized NPs due to synergistic effect of biomolecules in the cell extract.

The magnetotactic bacteria are a nature-perfect example of biogenesis of magnetite. These bacteria take up the iron from the environment, in either ferric or ferrous form and transport it inside the cell. The bacterial magnetosomes that are intracellular vesicles resulting from invaginations of the cytoplasmic membrane, have an invaluable role in the biomineralization of magnetite (Fig. 6) [107]. The biosynthesized magnetite has technological applications in water purification, catalysis, data storage; as well as in the biomedical sector for cancer therapy, magnetic resonance imaging, drug-delivery and biosensing [108]. Nanocrystalline magnetite of 10-40 nm size has been synthesized by reacting *Actinobacter* spp. with an aqueous mixture of potassium ferricyanide/ferrocyanide under aerobic and ambient conditions [109]. The formation of quasi-spherical magnetite NPs with excellent magnetic properties was observed within 24 h of reaction by extracellular route. The biotransformation was induced by proteins secreted by bacteria on exposure to the iron complexes, as revealed by the protein profile of the bacterial culture supernatant. The gel electrophoresis method showed no proteins in the culture grown in absence of the iron complexes. This mechanism of magnetite biosynthesis was found to be different from that observed in case of magnetotactic and iron-reducing bacteria [109]. Other works have demonstrated the synthesis of semiconductor sulfides-PbS [110], ZnS [111], CdS [112], iron sulfide [113,114] and CdTe quantum dots [115].

4.2. Fungi-mediated synthesis

Mycosynthesis of Au, Ag, Pt, Pd, Se, Te, titania, silica, magnetite, zirconia and quantum dots has been studied by many scientists. Lately, several species of fungi have been probed for their capability to produce Ag NPs, which include *Pleurotus ostreatus* [127] and *Cladosporium cladosporioides* [128]. The synthesis of Ag NPs can be confirmed by a typical SPR band occurring at around 440 nm in the UV-VIS absorption spectrum [127]. The extracellular synthesis of Ag NPs has been extensively studied using *Metarhizium anisopliae* [129] and white rot fungi [130] for a range of biological activities such as antimalarial, antibacterial and anticancer. Silver NPs of 33 nm fabricated using an endophytic fungus, *Letendraea sp.* demonstrated strong catalytic activity with 97.29%, 98.96%, 95.83% degradation of methyl orange, rhodamine B, and methylene blue in 3-, 9-, and 16-min, respectively [15]. Nanosilver biosynthesized with *Aspergillus sydowii* showed effective antifungal activity against pathogenic fungi (*Candida* spp. or *Aspergillus* spp.) and antiproliferative activity to HeLa cells and MCF-7 cells in vitro [131].

A number of fungi such as *Rhizopus oryzae*, *Aspergillus oryzae*, *Colletotrichum* sp., *Aspergillus niger, Penicillium brevicompactum* and *Phanerochaete chrysosporium* have been investigated for the fabrication of Au NPs, either extracellularly or intracellularly [132]. However, owing to easy downstream processing and cost-effectiveness, the extracellular synthesis finds more widespread application commercially than the intracellular one. For instance, Au NPs with different sizes in the range

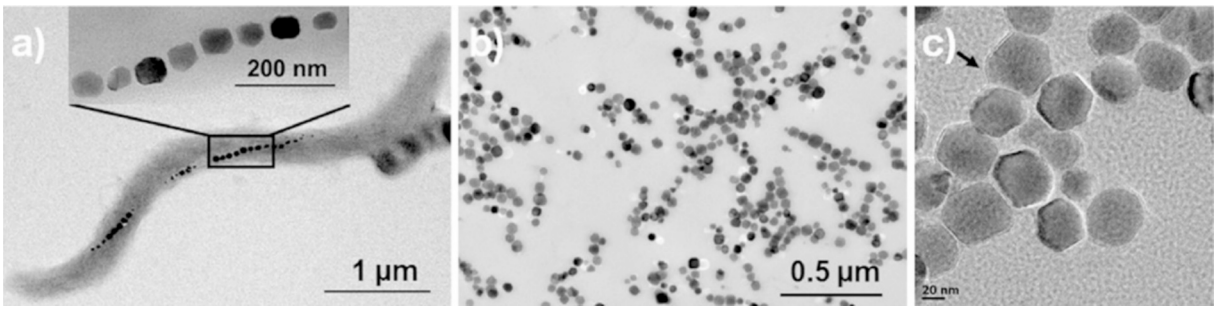

Fig. 6. (a) TEM image of *Magnetospirillum magneticum*, a magnetotactic bacterium, showing magnetite nanocrystals aligned along the cell axis (b) TEM and (c) high resolution TEM images of magnetite NPs [Reprinted with permission from ref. [116] © 2016 Elsevier publisher]

of 6-40 nm and size distribution between 30-70% standard deviations, have been synthesized using multiple strains of thermophilic filamentous fungi at varying experimental conditions [133]. The synthesis of multi-shaped Au NPs at ambient temperature was achieved by reaction between chloroauric acid and the mycelial extract of *Rhizopus oryzae*, which functioned as a reducing, shape-directing and capping agent [134]. The different geometries were created by adjusting the key growth parameters including Au^{+} ion concentration, pH and time (Fig. 7). The intrinsic property of the proteins or enzyme activity depends on these reaction parameters, which is the main factor governing the formation of anisotropic Au nanoplates. At low precursor to extract ratios, small NPs were formed due to the high rate of nucleation because of high protein concentration. On the other hand, at high Au^{+} concentration, the proteins of the extract were adsorbed on energetically favorable facet of the nuclei, thus resulting in nanoplates and nanorods [134]. The same group later proposed a detailed mechanism for the biotransformation of gold in *R. oryzae* by two reduction routes: (a) binding of Au^{3+} on the cell wall and subsequent reduction to Au NPs caused by enzymes, and (b) diffusion of Au^{3+} in cytoplasmic space and formation of Au NPs by enzymatic reduction (Fig. 8). The negatively charged gold ions bind to the positively charged *R. oryzae* mycelia via electrostatic interaction and are further reduced to Au^{+} due to the high redox potential of Au^{3+} [135].

Similarly, studies have also reported the mycofabrication of iron oxide NPs of 13-30 nm size with filamentous fungi (*Trichoderma asperellum*, *Phialemoniopsis ocularis* and *Fusarium incarnatum*) [136] and cobalt oxide NPs of 20 nm size by *Aspergillus nidulans* [137]. The fungus, *Coriolus versicolor*, was investigated for the bioremediation of cadmium with simultaneous synthesis of CdS NPs [138]. It could reduce Cd to stable CdS NPs extracellularly in continuous column by a purely enzymatic process. Interestingly, no external sulfur supply was needed for the biotransformation of Cd to CdS. The thiol groups in the fungal protein were responsible for the synthesis of non-toxic CdS NPs with uniform nanospheres in the range of 5-9 nm, as revealed by TEM imaging. The thermogravimetric analysis revealed that CdS NPs were very stable and could be stored under ambient conditions without deterioration. The protein molecules bind to the NPs through free amine groups or cysteine residues to form a coating, which in turn stabilizes them [138]. Another work performed heavy metal remediation of cadmium and lead using *Fusarium oxysporum* by converting the heavy metals into corresponding metal carbonates [139].

4.3. Algae-mediated synthesis

Algae are eukaryotic aquatic oxygenic photoautotrophs, which include genera of organisms from the unicellular *Chlorella* to the multicellular giant kelps [140]. Seaweeds are the largest and the most complex marine algae with distinct advantages of having a high metal uptake capacity, macroscopic structure and low cost, which provide them an edge over other bioreductants. Algae have been explored as the "biofactory" for the facile and ecofriendly synthesis of nanomaterials from Au [140,141], Ag [141], Cu [142], CuS [143] and Pd [144,145]. For instance, *Chlorella vulgaris*- common green microalgae, manifested the ability to reduce Pd^{2+} to Pd^{0} in an aqueous solution of $Na_2(PdCl_4)$ to generate spherical Pd NPs of size 2-15 nm (Fig. 9a,b) [145]. The chlorophyll-a and chlorophyll-b pigments in the green algae facilitate oxygen-evolving photoautotrophic reactions using water molecules as electron donors, as illustrated in Fig. 9c. The NPs were adsorbed onto a chitosan mat as catalyst support to show high catalytic activity (68%) for the standard Mizoroki–Heck reaction [145].

Phycosynthesis of Au NPs has been reported from algal species like *Sargassum wightii, Laminaria japonica, Stoechospermum marginatum* and *Turbinaria conoides* [140]. Rapid fabrication of Au NPs through extracellular biosynthesis was accomplished from a brown seaweed, *Stoechospermum marginatum* [146]. The photoluminescent Au NPs were crystalline in nature with mostly spherical morphology and 18-94 nm size. The hydroxyl groups present in the diterpenoids of the seaweed were responsible for reducing and stabilizing the NPs, indicated by FTIR analysis of the extract. Au NPs of size 5-35 nm, with spherical and triangular morphologies were produced intracellularly when $AuCl_4^-$ ions were reduced by *Tetraselmis kochinensis* [147]. In the intracellular synthesis, the NPs are more concentrated on the cell wall compared to the cytoplasmic membrane, thereby making them easily accessible for catalysis, coating and drug delivery applications.

Silver NPs have been biosynthesized with ulvan, a sulfated polysaccharide, extracted from green algae of *Ulva armoricana* sp. as a reducing and stabilizing agent [148]. The enhanced stability of the NPs was achieved by the formation of a thick polysaccharide shell around the inorganic nanoparticle core. Marine red algae, *Gelidium amansii* facilitated the production of spherical Ag NPs of size 27-54 nm [149]. The strong affinity of the carbonyl groups to silver resulted in the capping of the NPs, thus stabilizing them and preventing their agglomeration. Likewise, an aqueous extract of red algae, *Portieria hornemannii*,

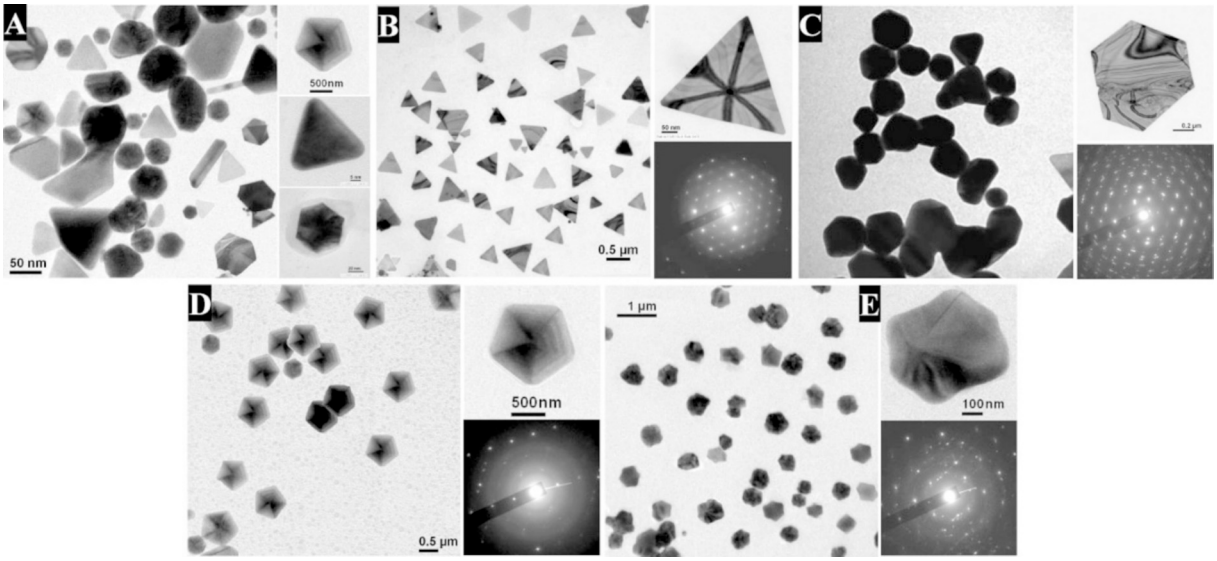

Fig. 7. TEM images of various geometries of Au nanoplates: A) Mixed nanoplates, B) triangle-, C) hexagon-, D) pentagon-, and E) star-shaped Au nanoplates. (the upper and lower parts on the right of each image indicate the high-resolution single-crystalline nanoplates with their selected area electron diffraction patterns, respectively) [Reprinted with permission from ref. [134] © 2010 John Wiley and Sons publisher]

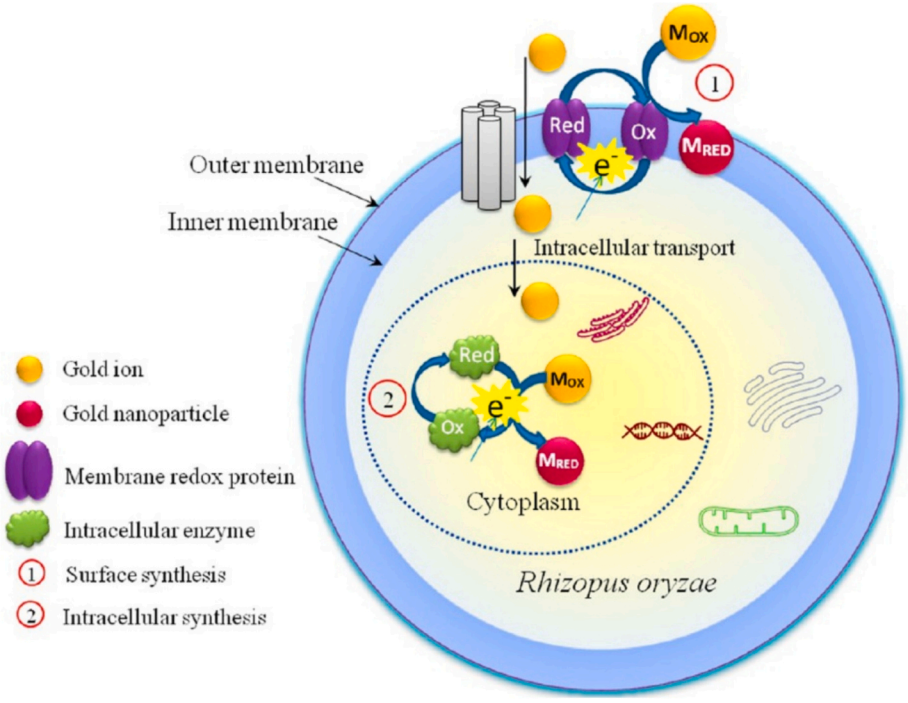

Fig. 8. Proposed mechanism of Au biomineralization in *R. oryzae* [Reprinted with permission from ref. [135] © 2012 ACS publisher]

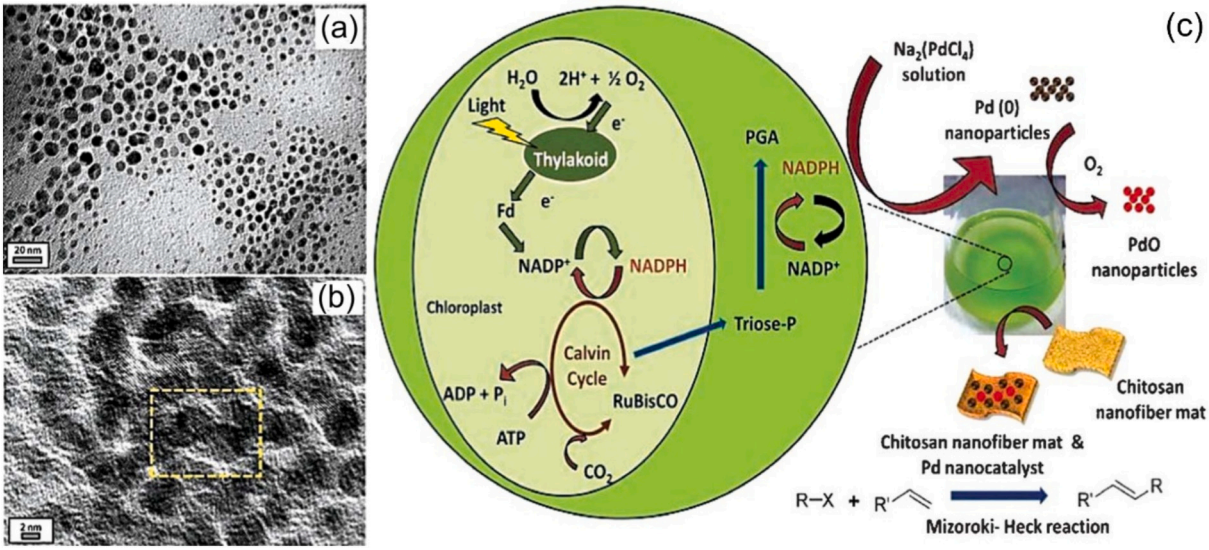

Fig. 9. (a) TEM image of Pd NPs precipitated in a four-week old microalgae solution (scale bar: 20 nm), (b) High resolution TEM image of Pd NPs, (c) Mechanism of phycosynthesis of Pd NPs leading to the generation of reducing agents via photosynthetic reactions within the algae and their immobilization on chitosan for catalytic application (ADP: adenosine diphosphate, Fd: ferredoxin, ATP: adenosine triphosphate, $NADP^+$/NADPH: oxidized/reduced forms of nicotinamide adenine dinucleotide phosphate, RuBisCO: rubilose biphosphate carboxylase, PGA: phosphoglycolic acid) [Reprinted with permission from ref. [145] © 2013 RSC publisher]

harvested from the Gulf of Mannar was used to synthesize Ag NPs at room temperature, confirmed by a change in color of solution from pale pink to dark brown after 1-2 days of incubation [150]. The spherical NPs of 35-50 nm size showed inhibition against fish pathogens, *Vibrio harveyii* and *Vibrio parahaemolyticus*. In another study, fresh water green algae, *Pithophora oedogonia* reduced Ag^+ ions to generate cubical and hexagonal Ag NPs of size 34 nm [151]. The terpenoids, fatty acids and secondary amide derivatives resulted in the efficient stabilization of the NPs. Similarly, phycosynthesis of Ag NPs for antibacterial applications has been done using polysaccharides extracted from *Jania rubins, Colpmenia sinusa, Ulva faciata* and *Pterocladia capillacae* that played a vital role in the reduction of the metal ions and stabilization of the Ag NPs [152].

Algae have also been explored for the fabrication of metal oxide NPs. *Bifurcaria bifurcate* was employed for the synthesis of copper oxide NPs [153]. The synthesis was achieved by mixing the algal extract with the precursor solution at 100-120 °C. The formation of the NPs was indicated by the disappearance of deep blue color, which gradually turned to brick red. The spherical NPs of size 5-45 nm, exhibited high antibacterial activity against *E. aerogenes* and *S. aureus* bacterial strains [153]. Iron oxide (Fe_3O_4) NPs were synthesized using aqueous extracts of brown (*Colpomenia sinuosa*) and red (*Pterocladia capillacea*) algae harvested

from the Mediterranean Sea with particle size in the range of 11.24-33.71 nm and 16.85-22.47 nm, respectively [154]. The NPs showed promising antifungal potency against *Aspergillus flavus* and *Fusarium oxysporum*. In another work, magnetite NPs were synthesized using seaweed extract of *Kappaphycus alvarezii* in ambient conditions at an alkaline pH, resulting into the precipitation of black-colored spherical NPs of 14.7 nm average size [155]. The positively charged magnetite NPs and the negatively charged functionalities in the seaweed were held by van der Waals forces. Zinc oxide NPs were synthesized from the extract of *Ulva lactuca,* an edible green seaweed, which served as a reducing and capping agent [156]. TEM analysis revealed different morphologies of the NPs including rods, triangles, rectangles as well as hexagons having an average crystallite size of 15 nm. The green-synthesized NPs displayed a photocatalytic degradation efficiency of over 90% for methylene blue, irradiated under sunlight for 2 h. Further, they also demonstrated a high insecticidal activity leading to complete mortality of *Aedes aegypti* fourth instar larvae at an initial concentration of 50 μg/ml within 24 h [156]. Similarly, anticancer activity of zinc oxide NPs fabricated from a brown seaweed, *Sargassum muticum* [157] and a red seaweed, *Gracilaria edulis* [158] has been investigated with beneficial cytotoxic effects on human cancer cells, inhibiting angiogenesis and inducing apoptosis.

4.4. Yeast-mediated synthesis

The yeast genera have shown the potential to amass considerable quantities of toxic heavy metals by enzymatic oxidation or reduction, cell wall sorption, controlled transport of heavy metals through cell membrane and their active efflux from the cell. Detoxification in yeast cells is brought about by glutathione and the metal-binding ligands-metallothioneins and phytochelatins, which determine the mechanism for the formation and stabilization of the NPs [4]. Yeasts are advantageous in the biosynthesis of nanomaterials on account of their abundance and high yield. Furthermore, they are easy to handle in laboratory conditions, synthesize large amounts of enzymes and grow rapidly with simple nutrients [159].

The yeast cells have been employed as a biotemplate due to their nontoxicity, easy degradability and environmentally friendly chemistry [160,161]. The fabrication of mesoporous zirconium phosphate, having 217.64 m^2 g^{-1} specific surface area, has been achieved at ambient conditions with remarkable electrocatalytic activity for oxygen reduction reaction using instant dry yeast as a template. The FTIR analysis revealed the critical role of amide carboxyl groups in yeast in the chemical interaction between proteins and zirconium phosphate NPs (Fig. 10a). The yeast cells provided nucleation sites for the NPs to bind Zr^{4+} ions through electrostatic and chemical interactions, as depicted in Fig. 10b [160].

Saccharomyces boulardii yeast cells have been employed for the intracellular fabrication of Se NPs with a mean particle size of 235 nm [162]. The natural organic molecules present in the yeast culture stabilize the NPs, thus eliminating the need for any external stabilizing agents. In another study, *Saccharomyces cerevisiae* or the Baker's yeast was used for the synthesis of MnO_2 NPs by a simple, low-temperature reduction of potassium permanganate, indicated by a change in color of the permanganate solution from purple to yellow [163]. The biosynthesis of hexagonal and spherical NPs of average size 34.4 nm was facilitated by proteins and alcoholic compounds as well as the cell walls of the yeast cells, as revealed by FTIR analysis. The baker's yeast has also been used for the synthesis of TiO_2 NPs [164] and Sb_2O_3 NPs [165]. ZnO NPs of 10-61 nm size, produced by *Pichia kudriavzevii* yeast strain, have demonstrated strong free radical scavenging activity for 2,2-diphenyl-1-picrylhydrazyl (DPPH) as well as a dose-dependent cytotoxicity in Vero cells [166].

Au NPs have been fabricated with yeast cells from *Candida albicans* [167], *Hansenula anomala* [168] and *Yarrowia lipolytica* [169] by extracellular or intracellular routes. Using an aqueous extract of Baker's yeast, Au NPs of 13.0 ± 0.9 nm size were synthesized under visible light [170]. The gas chromatography and mass spectrometry analysis of the extract showed the presence of trimethylsilyl derivatives of metabolites that acted as a capping and reducing agents for the NPs. The comparative cytotoxicity evaluation of the yeast extract, chemically synthesized Au NPs and the photo-biosynthesized Au NPs towards Ehrlich ascites carcinoma cancer cells demonstrated the highest toxicity of 86.5% for the lattermost under visible light incubation for 1 h. The toxicity enhancement in visible light was attributed to the photothermal properties of the plasmonic Au NPs that conjugated with the anti-epidermal growth factor receptor antibodies beside the phagocytosis of the excess yeast extract present in the Au NPs [170]. Instant dry yeast has been used to produce different morphologies of gold such as nanoplates and nanoflowers by altering the pH of the medium [159]. Biosynthesis of Au NPs has also been reported using yeast cells of *Magnusiomyces ingens* LH-F1, where the biomolecules absorbed on the surface of the NPs played a key role in synthesis as well as enhancement of the catalytic activity of the NPs [171].

The nitrate reductase activity of two epiphytic yeasts- *Cryptococcus laurentii* and *Rhodotorula glutinis,* isolated from apple peel, was exploited for the generation of Ag NPs having size-dependent antifungal activity against a phytopathogenic fungus [172]. Correlation was observed between the enzyme activity and the synthesis of NPs as well as the physiological state of the culture, particularly the redox state. Ultrafine spherical Ag NPs have been synthesized from freshly cultured Baker's yeast [173,174]. TEM showed the presence of the NPs in the cells, within

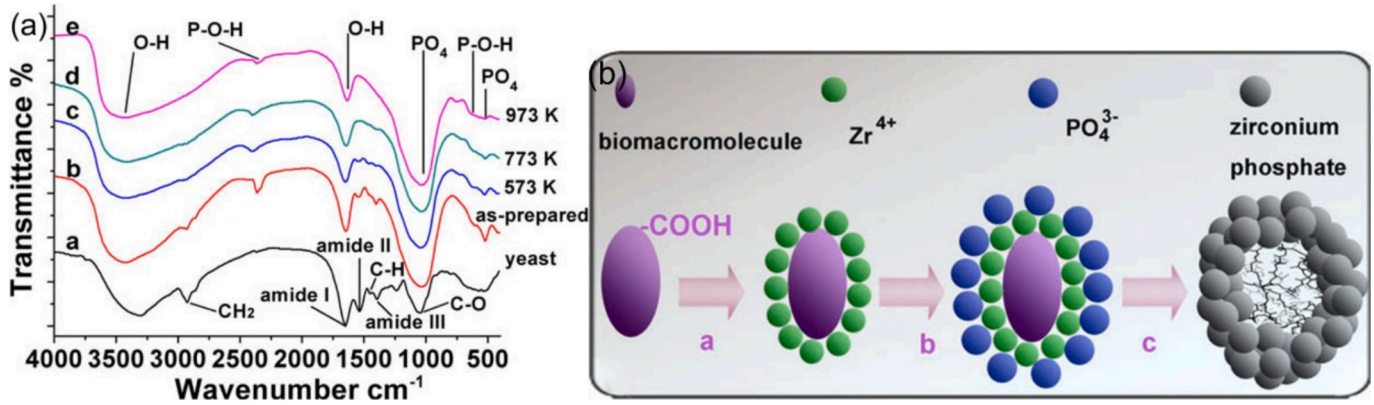

Fig. 10. (a) FTIR spectra of pure yeast, zirconium phosphate (with 0.4 g yeast) at varying temperatures; indicating the functional groups involved in the biosynthesis (b) Schematic for the synthesis of mesoporous zirconium phosphate with yeast as a biotemplate [Reprinted with permission from ref. [160] © 2010 Elsevier publisher]

the cell membrane as well as outside the cells [174]. The NPs displayed a good photocatalytic activity by completely degrading a solution of methylene blue in 6 h of exposure to sunlight, monitored by the decrease in UV-VIS peak intensity at 660 nm [173]. Similarly, studies have reported promising antibacterial or antiproliferative activity of Ag/AgCl NPs synthesized using yeast cultures [175,176]. For instance, AgCl NPs synthesized using commercial yeast extract have shown antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv [175].

Yeasts have also shown potential for the fabrication of semiconductors, which are extensively used in field-effect transistors, solar cells, light emitting diodes, photovoltaics, photocatalysis, photoluminescence and sensors [177,178]. The yeasts, *Schizosaccharomyces pombe* and *Candida glabrata* cultured in the presence of cadmium salts produced CdS quantum dots with higher monodispersity than the chemically-synthesized particles [177]. The short chelating peptides were found to control the nucleation and growth of CdS crystallites to protein-capped particles.

4.5. Other organisms in synthesis

Actinomycetes, although prokaryotes, possess characteristics of both bacteria and fungi. *Thermomonospora* sp. and *Rhodococcus* sp. were used for the extracellular and intracellular biosynthesis of Au NPs, respectively [179,180]. Other species like *Streptomyces hygroscopicus* and *Streptomycetes viridogens* have also been investigated for the fabrication of Au NPs [181]. The synthesis of Ag NPs has been achieved using *Streptomyces aureofaciens, Streptomyces rochei, Rhodococcus* sp. and *Nocardiopsis* sp. [181].

Marine organisms such as sponges [182] and fishes [183,184] have also been explored for the ecofriendly synthesis of nanomaterials. The green synthesis of Ag NPs has been accomplished with extract of a marine sponge, *Haliclona exigua* as a reducing agent [185]. The single-step reaction was performed in an ultrasonic bath leading to the formation and growth of flower-like Ag nanocolloids of size 100-120 nm. Another sponge, *Acanthella elongate* has been described for the production of spherical Au NPs of size 7-20 nm [182]. The secondary metabolites from the extract such as primary amines formed a coating on the metal particles, thus preventing their agglomeration and stabilizing them. The biosynthesis of Ag NPs using cod liver oil as a reducing agent as well as a surfactant has been reported. [183]. The carboxylate and amine functionalities present in the fish oil triggered the in-situ formation of Ag NPs. Similarly, the fish scales of *Labeo rohita* were utilized for the synthesis of spherical Ag NPs with catalytic properties [184]. The gelatin in the extract aided in reducing and stabilizing the self-assembled Ag NPs.

5. Emerging technologies in biosynthesis

Although viral technology has been used for a long time for gene delivery and therapy, it is only lately that other pathogens like bacteriophages and plant viruses have found increasing applications in nanobiotechnology. Viruses consist of nucleic acids, surrounded by a protein shell and are known to hijack the replication machinery of the host cell. Viruses hold a good potential for nanofabrication and can be exploited for controlling inorganic material nucleation, assembly, phase stabilization and pattern formation at molecular scale. This is on account of their ideal nanotemplate attributes such as size, monodispersity, composition, surface chemistry amenable to genetic manipulation and chemical tunability [186,187].

Genetically engineered bacteriophages exposing recombinant proteins, have demonstrated the potential to not only bind but also synthesize various nanostructures. Viruses and bacteriophages have been used for the biosynthesis of Au NPs [188,189], Fe NPs [190], Fe-Pt NPs [191], Co-Pt NPs [192], Co_3O_4 and Au-Co_3O_4 nanowires [193], ZnS and CdS [194]. Recently, modified phages specifically binding a lanthanide were demonstrated as factories for the synthesis of Eu_2O_3 NPs [195]. The peptides exposed on virions revealed very strong binding to Eu_2O_3 NPs and the ability to catalyze the formation of Eu_2O_3 NPs. The isolated peptide from M13 bacteriophage could mineralize europium oxide in the form of NPs from the precursor salt solution $Eu(NO_3)_3$ as well as $Eu(OH)_3$ solution. The synthesis was solely dependent on the presence of the peptide-presenting phage and Eu^{3+} precursors (Fig. 11). Scanning electron microscopy analysis revealed spectacular cauliflower-like nanostructures composed of very small (5 nm) Eu_2O_3 NPs. On similar lines, M13 bacteriophage has been employed to synthesize a ZnO-binding peptide (TMGA- NLGLKWPV) for use as a biotemplate for the production of ZnO NPs, as shown in Fig. 12 [196]. The synthesis occurred on phages which exposed the ZnO-binding peptides on the phage proteins- pIII or pVIII. Depending on the recombinant phage type used (M13-pIII or M13-pVIII), ZnO NPs of 20-40 nm size were generated in ambient conditions. The ZnO NPs emitted light close to the short wavelength end of the visible spectrum at 400 nm.

6. Challenges in biosynthesis

The morphology of the biosynthesized nanoparticles depends highly on the chemical constitution of the extract and its potential to reduce and stabilize the nanoparticles [32]. This makes it challenging to control the size and shape of the nanomaterials for targeted applications. Secondly, it is possible that the biomolecules present in the extract may mask the intrinsic properties of the nanomaterials by forming a coating layer on them. Isolation and purification may be required in such cases thus adding to additional steps in synthesis. Thirdly, the biomass required for synthesis of nanomaterials depends on the type of nanoparticle to be synthesized and the biomolecular profile of the extract. This adds to the complexity of biosynthesis over chemical synthesis, as there are no stoichiometric equations to estimate the conversion. Furthermore, biosynthesis may result in inconsistent or poor yield of the nanomaterials compared to conventional methods, depending on the phytochemicals in the extract. Although, recent studies have demonstrated the non-toxic and environmentally-benign nature of green-synthesized nanoparticles such as iron oxide [197] and bismuth oxide [7], critical aspects including bioavailability, cellular interactions, adverse responses, and biodegradation need to be taken into account for translating nanomedicines into clinical practice.

As for microbial synthesis, the likelihood of the microbes employed in biosynthesis getting retained on the surface of nanoparticles may cause serious adverse effects to human cells in biomedical applications [14]. The production of nanomaterial by microbes faces several operational impediments that entail selection of species, optimal process conditions for culturing and cell growth, longer time for reduction to occur, complex steps for retrieving nanoparticles from biomass involving downstream processing such as extraction and purification.

The effectiveness of biosynthesis by plants or microbes is by far limited to laboratory-scale. Upscaling of process to produce homogeneous and monodisperse nanomaterials is a major challenge due to batch-to-batch variations that may hinder their suitability for many applications such as in drug delivery. The lack of synthesis standards, insufficient understanding of adverse effects of nanomaterials, stringent regulatory hurdles and dynamic market demands pose additional challenges that need to be addressed for commercialization of biosynthesized nanomaterials. For healthcare applications, the knowledge on biocompatibility and toxicity of biosynthesized nanomaterials is still in its infancy, and further studies are required to elucidate the underlying mechanisms and establish their use as potential therapeutic agents in future nanomedicine.

7. Summary & future perspectives

The growing consciousness towards sustainability and ecofriendly materials as alternatives to the conventional chemical-based materials for the synthesis of nanomaterials has paved the way for green

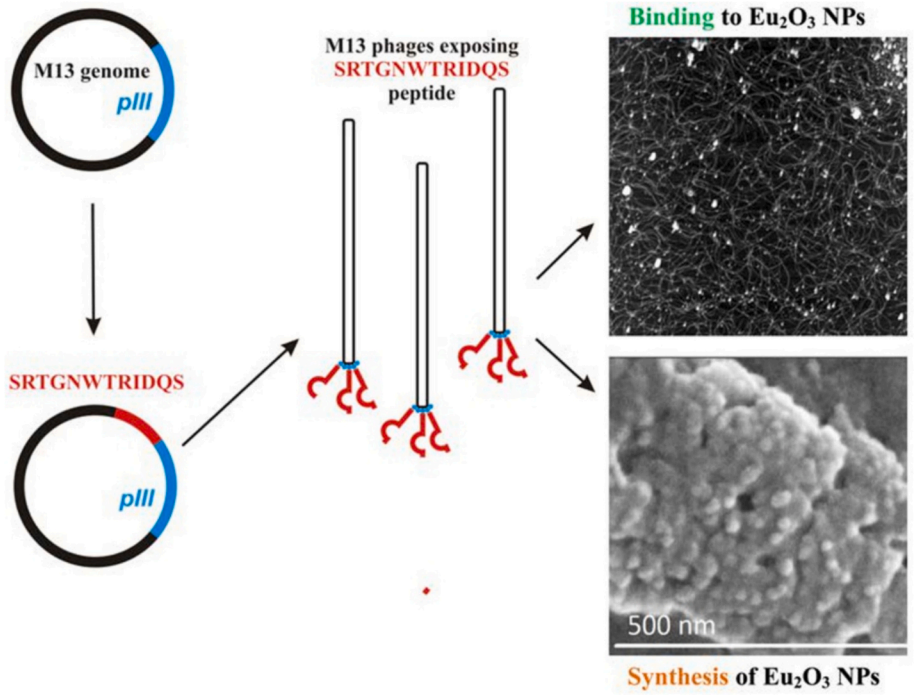

Fig. 11. Bacteriophage-assisted synthesis of Eu_2O_3 NPs- (top right) atomic force microscopy image with the white spots representing Eu_2O_3 NPs, and the filamentous structures are M13 bacteriophages, (bottom right) Scanning electron microscope image of Eu_2O_3 NPs [Reprinted with permission from ref. [195] © 2017 ACS publisher]

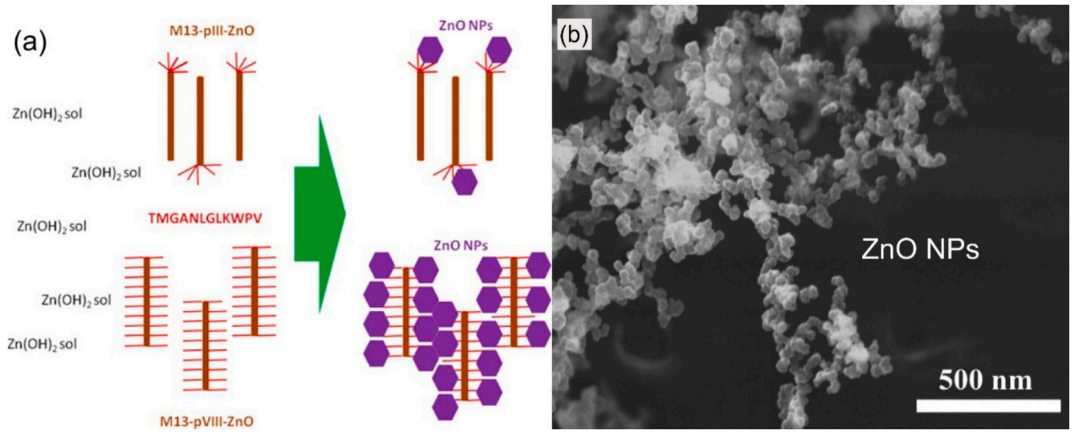

Fig. 12. (a) Bacteriophage-directed synthesis of photoluminescent ZnO NPs, (b) Scanning electron microscope image of ZnO NPs by M13 bacteriophage [Reprinted with permission from ref. [196] © 2016 ACS publisher]

nanotechnology. The biogenic synthesis of nanomaterials, still in the incipient phases of development, uses non-toxic and renewable materials such as plant extracts and microbes, as sources ofsecondary metabolites such as terpenoids, alkaloids, polyphenols, flavonoids; and biomolecules including enzymes, proteins, amines for the reduction as well as stabilization of the nanomaterials. Although, plant extracts have inherent advantages such as easy handling, quick and non-laborious processing, the microbes have been explored as potential biofactories for the biosynthesis of nanoparticles due to their diversity and unique biochemical profile. Another advantage of using the biomaterials in synthesis is their wide availability and cost-effectiveness. Also, the fact that they serve as stabilizing or capping agents for the nanomaterials to prevent their agglomeration, eliminates the need for synthetic chemical stabilizers. The reaction parameters such as substrate concentration, temperature and pH are critical factors influencing the particle morphology and characteristics. However, in order to increase the monodispersity of nanoparticles and rate of synthesis, the microbial cultivation methods and downstream processing techniques need to be improved. The green-synthesized noble metal nanoparticles and metal oxide semiconductors have found diverse applications in agriculture, electronics, catalysis, biomedicine, cosmetics and environmental remediation. Further, knowledge on the cellular and molecular mechanisms underlying the biogenesis of nanoparticles plays an instrumental role in controlling the morphology and crystallinity of nanoparticles and thus, needs to be well-established with further research. Studies on biocompatibility and *in vivo* cytotoxicity profiles of biogenically engineered nanomaterials to assess their safety and efficacy is still limited, thus restricting their translation into clinical practice. The scale-up and commercial implementation of the biogenic approaches for synthesis is still a challenge, with plenty of room for future efforts to improve the material yield and reaction efficiency. Thorough scrutiny of the whole life cycle of nanomaterials is required to mitigate risks during

production, handling, storage and disposal.

CRediT authorship contribution statement

Charu Agarwal: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Levente Csóka:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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Data availability

No data was used for the research described in the article.

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