

Chapter 7

Microbial Synthesis of Nanoparticles and Their Applications for Wastewater Treatment



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Abstract For the last few decades, with the emergence of nanoscience and nanotechnology, nanoparticles gained enormous attention due to their high surface/volume ratio and other novel, unique, and remarkable properties. Traditionally, nanoparticles are fabricated by either chemical or physical approaches which not only utilize toxic chemicals but also are energy-intensive and consequently costly. The microbe-based synthesis of nanoparticles is biocompatible, economical, eco-friendly, and energy-intensive. Metallic and nonmetallic, metal oxide, and sulfide nanoparticles are synthesized by bacteria, virus, fungi, and algae. These nanoparticles act as an adsorbent for the remediation of water and wastewater pollutants clearly due to their physicochemical properties, nano size, controlled growth, and surface modification. The carbohydrates, proteins, and enzymes present in such microbes act as surfactant and capping agents which reduce the use of harmful chemical surfactants. Nanoparticles find application in the remediation of dyes, heavy metals, microbial contaminants, and pesticides, in the area of wastewater treatment. The widely used adsorbents are iron oxide nanoparticle, zinc oxide,

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alumina, and titanium dioxide. The present chapter highlights the synthesis of nanoparticles from bacteria, algae, fungi, and viruses and their application for wastewater treatment.

Keywords Nanoparticles, bacteria · Metal oxide · Surface modification · Wastewater treatment

7.1 Introduction

Increasing population and advanced lifestyle have resulted in mounting pressure on environmental resources, deteriorating the process feasibilities of multiple natural environmental conservation pathways. The effects seem to be convincingly regulated, after the reports of environmental pollution assessment agencies, reflecting inescapable approaching threats. Though several efforts and resolving inroads are rapidly emerging to tackle this issue, nothing serious seems to shake the generating source, owing to which there is an urgent need to counteract the probable risk factors with powerful and stronger prediction technologies. For example, on a daily basis, more than half of the natural processes require conversion of harmful and complex materials into simpler and self-decomposable entities, through interventions that are increasingly energy sensitive, whereby stress levels and contaminants are bound to affect the environmental quality. So better and more powerful solutions, consuming little and effecting much higher problem domain, are urgently needed. In this context, the unique physical and chemical properties of nanoscale materials have been the focus of sheer interest ever since their inception. Nanotechnology and nanoparticles have drawn the attention of the scientific community towards them (Seqqat et al. 2019). Mostly nanoparticles due to their size tunable and fascinating properties find applications in the field of electronics, medicine, research, catalysis, and environmental cleanup (Khan et al. 2019), due to which their demand has increased drastically, and the load on the industries for their synthesis has increased manifold. The commercial synthesis of nanoparticles is generally carried out by various chemical and physical routes. Among the chemical approaches, the most common methods are sol-gel, coprecipitation, hydrothermal, solvothermal (Ganachari et al. 2017), etc. On the other hand, the physical synthesis method includes laser sputtering, chemical vapor deposition (CVD), and ball milling. In addition to their advantages, these chemical and physical methods have several disadvantages as well. Chemical methods utilize numerous hazardous chemicals as surfactant reducing agents, or reactants, which are toxic to the environment. While the physical methods require sophisticated machinery for their synthesis, they are highly energy-intensive, which makes them expensive. So there is a need for another alternative source which should overcome the drawbacks of the above-mentioned methods. A generalized method of nanoparticle synthesis is depicted in Fig. 7.1.

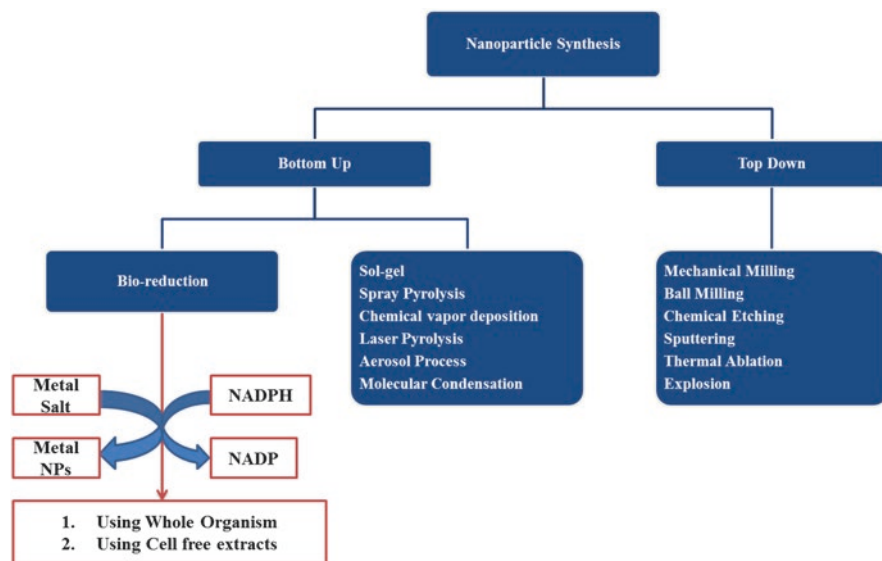


Fig. 7.1 Generalized synthesis of nanoparticles

The biological synthesis of nanoparticles is the best alternative method which will overcome the drawback of the abovementioned problems. Among the biological methods, microbial-based synthesis of nanoparticles is of utmost importance due to its cost-effectiveness, ease of synthesis, and eco-friendly nature (Singh et al. 2016a; Prasad et al. 2016). Since the source of preparation is economically viable and renewable, interest in their scientific evaluation has attracted unconditional support from different parts of the globe. Besides this, the biosynthesized nanoparticles are also biocompatible which can be used directly in the field of medicine and drug delivery. Microorganisms are the organisms whose sizes fall in the range of microns. Generally, they can be classified into two classes: prokaryotic and eukaryotic. Prokaryotic involves bacteria and *Actinomycetes*, whereas eukaryotes involve algae, fungi, and yeast. There were several reports of using viruses as the templates for the synthesis of several nanoparticles. Microorganisms have numerous biomolecules such as enzymes, organic acids, polysaccharides, etc. known to play a key role in the synthesis process. Moreover, such biomolecules encapsulate the nanoparticle which further prevents the aggregation by acting as a capping agent. Microorganisms can synthesize all types of nanoparticles by reduction, for instance, single metallic nanoparticles (like gold, silver, copper, Se, Fe, or NZVI) (Das et al. 2017) or metal oxides (titanium oxide, zinc oxide, iron oxide, silica, and aluminum oxide) or metal sulfides (like PbS, CdS, PbSe, CdSe, ZnS). Metallic nanoparticles have almost replaced conventional catalysts in different biochemical and even industrial activities. The generalized flowchart for extracellular and intracellular biosynthesis is shown in Fig. 7.2.

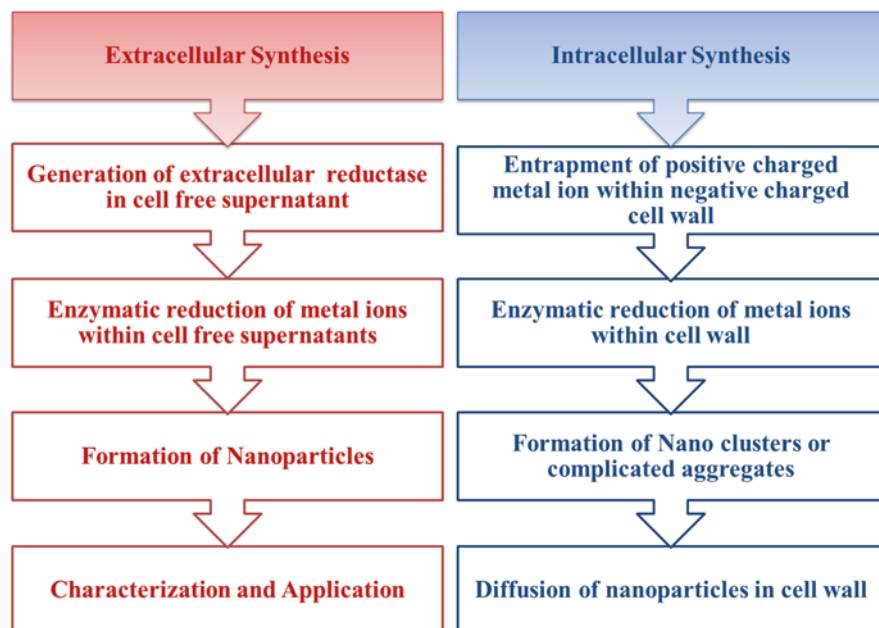


Fig. 7.2 Schematic flowchart of extracellular and intracellular biosynthesis

The biologically synthesized biocompatible and eco-friendly nanoparticles have potential for wastewater treatment like remediation of water pollutants such as dyes, heavy metals, pesticides, and other organic/inorganic pollutants (Das et al. 2017). The nanoparticles being small in size have a high surface/volume ratio (SVR) and comparatively high surface energies have a special role in wastewater treatment (Shah et al. 2015a). Moreover, their microbial origin provides them special advantage like capping by biomolecules, which not only prevents their aggregation but also provides different functional active sites. These active sites help in the adsorption of pollutants from the wastewater.

The contamination of wastewater streams and its associated risks of environmental degradation is the source of polluting water bodies owing to which aquatic organisms are at risk (Bhateria and Jain 2016). The risk factors are enhanced significantly due to the heterogeneous nature of contaminants, requiring differential treatment measure for each specific kind of pollutant. The NPs with their high surface areas (SA) are able to initiate multiple interactions with chemically distinct pollutants at the same instant of time, owing to which in the same time interval, pollutant load is reduced to a greater extent as compared to conventional technologies (Lin et al. 2014). Keeping such characteristics of metal/metal oxide NPs in observance, the present chapter focuses on the advances in the field of microbial synthesis of single metals and metal oxide nanoparticles and their potential applications in the field of remediation of various organic and inorganic pollutants from the water and wastewater.

7.2 Advantages of Bacteria-Mediated Synthesis of Nanoparticles

Biological systems especially bacteria, due to their high growth rate, act as a unique candidate to achieve nonhazardous nanoparticle synthesis. In chemical methods, the growth of NPs is usually expensive and ends with toxic end products released into the environment. In biological systems, an individual bacterium can be a center and a matrix for the controlled growth on NPs.

Studies on bacterially synthesized NPs reveal interesting variations, with extracellular as well as intracellular formation sites, variability in the shapes and sizes, and numerous others. A key concern in all the cutting-edge applications of NPs (ranging from industrial, chemical, biological, and many others) is the extent for which these entities retain their low sizes (Nath and Banerjee 2013). The diverse chemical environments of application sites often lead to the aggregation of these moieties, so it is very important that these nanomaterials retain their native sizes for sufficiently longer durations. That is why physically prepared NPs are often ineffective in most applications and the problem of aggregation is frequently encountered. With biological sources, there is an advantage that an aggregation preventing agent does not need to be added separately, and the polysaccharides, protein fractions, and fatty acid conjugates of microbes serve as aggregation stabilizing agents (Siddiqi et al. 2018). Apart from this, culturing bacteria is relatively easy (since it is the largest active microbe among all varieties) and relatively less costly as compared to reducing agents of the chemical method. Furthermore, an incentive with the bacterial synthesis is that even if we have one bacterial strain, culturing it in different chemical environments (characterized by pH and temperature variation) could form different NPs at the same instant of time. For example, if the salt precursor for Fe is added in the culture medium of magnetotactic bacterium, we will get Fe-O NPs, while if a cobalt salt is added, the likely product would be Co (as such) or in some combined form as NPs. The reason is that both Co and Fe are magnetically sensitive and have unpaired electrons in their molecular orbitals. Similarly, the NPs of two metals belonging to the same group of the periodic table could be formed in a simplistic manner as they would have similar chemical characteristics. The only distinction is the presence of salt (for each metal type) in the culture medium of the chosen bacterial strain. So, low cost lesser synthesis time, variability, and better stability of the product (here NPs) are some of the reasons to choose a bacterial route for NP synthesis (Shah et al. 2015a).

The exact mechanism of microbial-based nanoparticle formation is not known and most of the studies project consensus for the metabolism of toxic compounds by bacteria after which a whole range of defensive genes is activated to combat the resultant toxicities. The activation of these genes is accompanied by the sequential modulations in oxidation and reduction chemical balances which ultimately result in the prevalence of a peculiar form that neutralizes the toxicity to a maximum extent. In this reference, some authentic claims are put forward by Ghashghaei et al. in their 2015 compilation, describing distinctive three-step nanoparticle formation

by bacteria (Gahlawat and Choudhury 2019). The first discusses the bacterial self-assembled nanostructures such as pilus, flagellum, S-layer bipolyester, cyanophycin inclusions, phage, rhodopsin, and alginates. The second possibility projects the metal and metal oxide nanoparticle formation as bacterial metabolism by-products. In this mechanism, there seems to be an implicit role of bacterial enzymes. The third step of nanoparticle formation by bacteria relies on bacterial polymers (using polysaccharides and polysaccharide derivatives) which can be thereafter processed to NPs. Derivatives of plant products are highly reliable sources of preventing aggregation owing to their natural mode of existence, least energy requirements for operation, and much lower toxicity than chemical agents accomplishing the same. Discussing the diversity of mechanisms explaining nanoparticle formation, they concluded one common aspect in all approaches, involving the trapping of metal ions either on the surface of or inside microbial cells. Subsequently, these are reduced to NPs through enzymatic or nonenzymatic methods. Holding detoxification pathways as prime sources responsible for bacterial (and even other) nanoparticle formation, they illustrate a nonspecific uptake of toxic metal ions via the cationic membrane transport systems, which regulate the transport of metabolically important cations. Now since these uptaken metal ions are toxic, they are not tolerated by bacteria beyond a threshold concentration which is accomplished either via mutagenesis of metal-resistant bacterial strains or by complementation to identify the resistance restoring genes. The identification of genes responsible for causing toxic responses paves the way for their enzymatic inactivation of toxic ionic forms of the metal into nontoxic metal salts, modulation in metal ion efflux system activities, and diminishing membrane permeability. Figure 7.3 discusses the concentration-dependent enzymatic induction controls in a bacterium to counter the toxicity threats to the bacterium itself.

The higher the toxicity (caused by an increasing population of metal ions), the more pronounced is the activation of enzymes restoring the normal pH and chemical balance. Diversity of the product manifested by changes in the process parameters, such as pH, temperature, duration of culture, type of strain, culture conditions, and their mutual optimization, is a key factor affecting the nature of NPs formed. Interesting aspects within these predictors are the differences in the product's nature attained through differential regulation of comprising factors. For example, maintaining the temperature at 45 °C for half an hour is likely to confer different features compared to assuring the same for an hour or more. Similarly, the concentration of a characteristic protein source in the culture medium could be responsible for a peculiar nature of the product which may vary with the change in the protein source. The obvious reason is that proteins are composed of amino acids that play decisive roles in regulating 1:1 dispersion. Constituent amino acids may exhibit an altogether distinct response if they are dextro-rotatory instead if leave or vice versa. Likewise, composition of dispersion medium in the culture plays a critical role where if the water is the major source, polar activities are likely to be aggressive contrary to aqueous ethanol or some other additive (organic) source. On a similar note, if we change the pH, the dispersion trend is likely to be varied that is reflected in the ζ -potential trend. It may be possible that the shape and size of NPs being

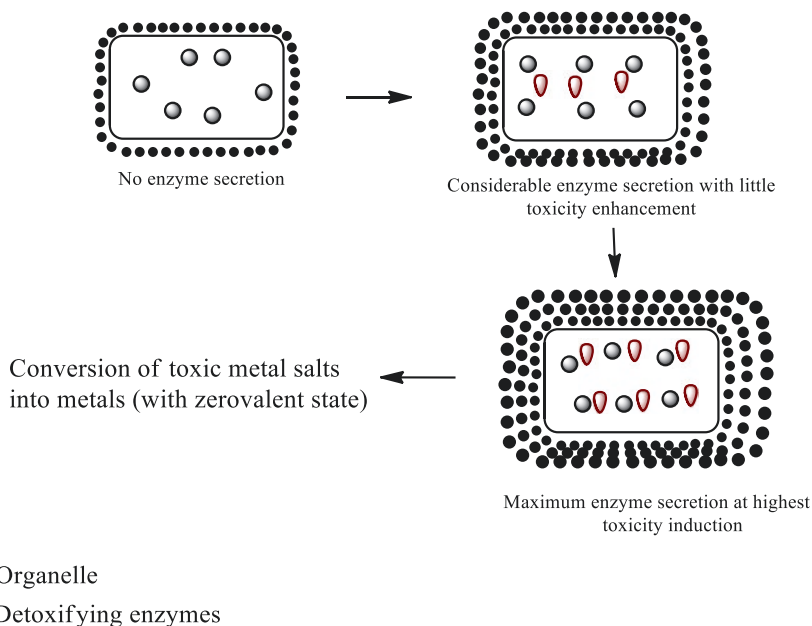


Fig. 7.3 Correlative regulation of toxic metal accumulation and the intracellular enzyme activation in microbial cells

prepared are more regulatory and optimized at acidic pH which the alkaline pH is unlikely to support. The discussed factors present preliminary aspects, which, if replicated on a molecular scale, can have quite significant consequences. For instance, acidic pH may be supportive inactivating a particular bacterial gene/enzyme in a specific manner that the basic pH is unable to do. At the same point, it is imperious to elucidate the consequence of a peculiar pH on the expression of neighborhood genes and enzyme secretion, so that growth remains unaffected. An accurate generalization of the bacterial activities in different growth phases is the key to the choice of a bacterial species for a certain kind of nanoparticle synthesis. Generally, in the log phase, the most active nutrients are synthesized and the stationary phase is the domain where the functional activities could be stimulated.

Thus, exploiting the bacterial genetic makeup is the groundwork required for thoughtful and optimized regulation of nanoparticle formation. The mechanistic insights probably offer incentives that, with the variations in culture ingredients, even hybrid or core-shell NPs could also be prepared. Similar robustness is not attainable with fungal cultures since fungi require adequate growth of mycelia to exhibit the desired metabolic performance. Apart from this, the protein content of fungi is very much different from that of a bacterium, owing to which growth conditions remain less controlled with the former. Nevertheless, the logical estimation of obtaining still better results with consortium cultures suits well for the kind of NPs being formed.

7.3 Bacteria-Mediated Synthesis of Metallic Nanoparticles

Microbes used for preparing metal and metal oxide NPs comprise bacteria, fungi, virus, and algae, thanks to the robust chemical actions of microbial enzymes which remain operational even at extreme temperature and pH conditions. An interesting aspect of microbial biochemical activities is that the expression of enzymes could be modulated by changing the chemical compositions of their culture media. Optimization of culture parameters such as pH, light exposure, temperature, medium salt content, and mixing speed is a pivotal factor which can significantly modulate the enzyme activity (Sarker et al. 2013; Kirthi et al. 2011a).

Bacteria can significantly reduce heavy metal ions to produce nanoparticles; many researchers proved that bacteria facilitated interactive pathways accountable for the reduction of metal ions and their ability to precipitate metals to the nanometer scale (Kirthi et al. 2011a). One of the major benefits of bacteria-mediated nanoparticle synthesis is their large-scale production with negligible use of hazardous chemicals; however, there are firm boundaries like laborious bacterial culturing procedures and less control over their shape, size, and distribution.

So keeping track of media composition-dependent enzyme expressions easily serves as the lead for facilitating the requisite extent of chemical reduction. For example, the most common bacteria, *E. coli*, acts as a probiotic within the physiological environment, hinting that changing the pH and the chemical composition of the surrounding environment could activate another set of *E. coli* enzymes whereby functions other than the probiotic activities could be exercised (Fijan 2014). Another important feature responsible for regulating nanoparticle formation by bacteria is their ability to interact with their nearby environment arising due to the polarity of their lipidic membranes that catalyzes diverse oxidation-reduction processes (most of the nanoparticle preparation is enacted through a chemical reduction of metallic salts) (Singh et al. 2018). The distinction in cell wall polarities of gram-positive (GP) and gram-negative (GN) stains (dissimilar peptidoglycan extents) is, in itself, quite a significant prospect of bacterial structural diversity, which is missing in all other microbes. Another critical factor affecting NP formation by bacteria is the choice of a particular species (Singh et al. 2018; Wang et al. 2017). This is because all bacteria do not have a similar genetic makeup and that is why culturing two different species in the same media is likely to result in differential functional expressions of similar redox enzymes. To meet such challenges, a consortium of the bacterial population, comprising two or more species, generally performs better than a single bacterium. Furthermore, studies on the microbial synthesis of NPs have notified that only those biological moieties that have potential to accumulate the metallic precursors in the form of oxides, hydroxides, sulphates, etc. have the best chance of forming metal NPs (Zargar et al. 2011).

Synthesis of NPs using biological moieties (enzymes, sugar, and proteins secreted by a bacterial strain) is a bottom-up approach, producing less waste material and enabling the formation process to be stopped as per the requirement. This is particularly exciting, as applications of NPs are substantially expressed through

their shape and size variations, which acutely affect their interaction abilities. Another advantage with bacterially synthesized NPs is that the enzymes responsible for initiating biochemical reduction of corresponding metallic precursors could be amplified in their expression through knowledge of their regulatory genes and pathways. Similarly, the introduction of these enzymes from the outside could facilitate an extracellular synthesis of both, i.e., pure metallic and their oxides NPs, the absence of which drives the synthesis at intracellular locations (Patra and Baek 2014; Malik et al. 2014).

7.4 Inception and Progression of Bacterially Formed NPs

7.4.1 Bacterial Synthesis of Metallic Nanoparticles

Bacteria bear exceptional abilities for reducing metallic ions into their zerovalent forms (the nanoparticle state) and are perhaps the most befitting candidates for nanoparticle synthesis attributed to their ease of handling and robust culture medium requirement (Ruttikay-Nedecky et al. 2017a). In comparison to other microbes, it is very easy to mold and manipulate genes in bacteria for retrieval of metal ions. Generally, bacteria are exposed to harsh and increased heavy metal ion concentration in their surroundings. To combat these stressful conditions, bacteria have developed numerous defense processes, for instance, intracellular sequestration, efflux pumps, fluctuation in concentration ions of metals, and extracellular precipitation. These defense mechanisms form the basis of shape- and size-specific nanoparticle synthesis by bacteria. Table 7.1 comprises the various bacterial strains used for the synthesis of NPs along with their specific applications. The first incidence of bacterial synthesis of Au NPs was reported by *Beveridge and Murray*, who noted the extracellular deposition of Au NPs on *Bacillus subtilis* cell wall when AuCl₄ solution was exposed to its colonies (Beveridge and Murray 1980). Almost at the same time, *Klaus-Joerger* intracellularly synthesized AgNPs < 200 nm using an NADH-dependent reductase as an electron source and, in course, itself getting oxidized to NAD⁺. It was noted that e⁻ transfer from NADH results in a biological reduction of Ag⁺ ions to their zerovalent form (the nanoparticle form) (You et al. 2013). The emergence of bacteria-mediated nanoparticle synthesis acquired attention in 2012 when Srivastava et al. intracellularly synthesized Pd, Ag, Rh, Ni, Fe, Co, Pt, and Li NPs (Srivastava and Constanti 2012). Interestingly, these synthesis procedures do not require an external stabilizing agent and electron donors and also did not need any pH modification in the course of biomineralization of different metal ions. Various metallic nanoparticles synthesized using various microbes are summarized in Table 7.1.

Some of the bacteria have shown exceptional phenomenon of nanoparticle synthesis, like synthesis at extremely high concentration of metal ions, acidic pH, and higher temperature. *Ps. stutzeri* and *Ps. aeruginosa* are bacteria that can grow at higher metallic concentration (Lu et al. 2016).

Table 7.1 Various metallic nanoparticles synthesized using bacteria

Sr. no.	Name of bacteria	Specific nanoparticle formed	Size, shape, and location	Reference
1.	<i>Pseudomonas aeruginosa</i>	Au	15–30 nm, spherical	Sarker et al. (2013)
2.	<i>Pseudomonas stutzeri</i> , <i>Bacillus cereus</i>	Ag	Up to 200 nm, various shapes	Kirthi et al. (2011a)
3.	<i>Alcaligenes faecalis</i>	Ag	30–50 nm, spherical	Fijan (2014)
4.	<i>Bacillus</i> sp. CS11	Ag	42–92 nm, spherical	Singh et al. (2018)
5.	<i>Kocuria flava</i>	Cu	5–30 nm, spherical	Wang et al. (2017)
6.	<i>Ochrobactrum rhizosphaerae</i>	Ag	10 nm, spherical	Zargar et al. (2011)
7.	<i>Alteromonas macleodii</i>	Ag	70 nm, spherical	Patra and Baek (2014)
8.	<i>Deinococcus radiodurans</i>	Ag	4–50 nm, spherical	Malik et al. (2014)
9.	<i>Bacillus subtilis</i>	Au	20–25 nm, spherical	Rutt kay-Nedecky et al. (2017a)
10.	<i>Bacillus brevis</i> (NCIM 2533)	Ag	41–68 nm, spherical	Beveridge and Murray (1980)

Besides this, there are a few iron-reducing bacteria that can reduce ferric to ferrous form when grown on elemental sulfur, i.e., *Thiobacillus ferrooxidans*, *T. thiooxidans*, and *Sulfolobus acidocaldarius*. This was reported by Brock and Gustafson, and the detailed process is given in IONP synthesis by non-magnetotactic bacteria (Brock and Gustafson 1976). Other biomineralization phenomena, such as the formation of tellurium (Te) in *Escherichia coli* K12 (Irvani 2014), the direct enzymatic reduction of Tc (VII) by resting cells of *Shewanella putrefaciens* and *Geobacter metallireducens*, and the reduction of selenite to selenium by *Enterobacter cloacae*, *Desulfovibrio desulfuricans*, and *Rhodospirillum rubrum* (Kessi et al. 1999), have been reported as well.

Mullen et al. reported the leaching capability of Ag⁺, Cd²⁺, and Cu²⁺ from solution by *Bacillus cereus*, *P. aeruginosa*, *B. subtilis*, and *E. coli*. Some of them can bind to large metallic ions, while some can form magnetic nanoparticles (Kessi et al. 1999) which are shown below in Table 7.1.

Multiple studies of the past 5 years have reported the quick and fast synthesis of NPs possessing varying size and shape from several bacterial strains, such as *E. coli*, *B. subtilis*, *B. cereus*, *B. megaterium*, *Ps. aeruginosa*, *Klebsiella pneumoniae*, *Alteromonas*, and *Ochrobactrum*. The idea of bacterial enzymatic functions can be well judged from the simplistic procedures, involving minimum time periods without any sophisticated pH and temperature optimization. For example, Das et al. reported that *B. cereus* could synthesize AgNPs extracellularly at ambient temperature within 24 h (Lakshmi Das et al. 2014). Kumari et al. reported enhanced

antifungal activity of AgNPs prepared using biological route (fungus, *Trichoderma viride*) contrary to that of chemical method, although, in both modes, the sizes and shapes of produced NPs were similar. It was observed that biologically prepared particles effected (20 and 48.8)% higher decrements (compared to those prepared chemically) in the dry weight of fungal pathogens, namely, *Fusarium oxysporum* and *Alternaria brassicicola*. Staining assays using nitro blue tetrazolium and propidium iodide dyes revealed an enhanced generation of superoxide radicals, as the probable cause of death for the biologically prepared NPs. Scanning electron microscopy (SEM) analysis revealed altered osmotic balance and membrane disintegrity as the primary source of cell death (Kumari et al. 2019). This study, therefore, concluded that compared to the chemically produced NPs, the bacteria synthesized NPs effected comparatively higher ROS generation, antioxidant pathway downregulation, and disruption of osmotic balance and cellular integrity. From this research, the observations infer a higher toxic expression of biologically prepared NPs, which confers them a preference compared to the chemically prepared NPs in the treatment of wastewater streams. The hidden prospect of this study is the size of the synthesized NPs, which have an intricate effect on their functional properties. It is well known that smaller size at the nanoscale argues for stronger quantum confinement which has a direct correlation with the particle energies. Since this study observed higher toxicity for biologically synthesized NPs, it seems certain that biological sources could have produced a lower size as lower size could have increased the kinetic stability owing to which interactive potential of the formed NPs increases. Such size controls are attainable with chemical methods where the stabilizing or aggregation preventing agent needs to be added separately to prevent the size growth beyond a stage.

Further, AgNPs was also synthesized extracellularly by Kulkarni et al. using *Deinococcus radiodurans*. *D. radiodurans* are an extremely robust bacterium species which can survive cold, dehydration, vacuum, radiation, and acid exposures (Kulkarni et al. 2015). The NPs were synthesized by the reduction of AgCl solution, following which these were screened for their antibacterial and antibiotic responses towards GN and GP bacteria. The radiation resistance properties of *Deinococcus radiodurans* enabled its activity even in extreme environments, owing to which good results were obtained with regard to anticancer studies. Results of this study have encouraged the scientific community to optimize *Deinococcus radiodurans* growth kinetics (via changes in the culture medium composition) so that palladium, platinum, and several other NPs could also be produced with similar control and ease. An incentive with such microbial species is that they can be as such added to wastewater streams where they can feed and regulate their metabolism through processed consumption of organic contents, such as discarded proteins, carbohydrates, fats, nitrogen sources, and several others. The temperature-resistant functioning of *Deinococcus radiodurans* offers special benefits as it is likely to cause minimal structural changes of synthesized NPs owing to which the NPs retain an unaltered application potential to a longer extent. Digging for the mechanism of bacterial NP formation, Sneha et al. replicated the attempt of Liu et al. (synthesis of AuNP *Bacillus megaterium* dried cells) using *Corynebacterium* species to obtain NPs and

proposed a nonenzymatic reduction mechanism to be the source of nanoparticle formation (Liu et al. 2000). They noted that the formation of NPs by bacteria depended on two major requirements, where the first was the presence of organic functional groups in the bacterial cell wall, while the second factor was the maintenance of optimum temperature and pH in the surrounding environment. Studies by He et al. provide the most significant justification of this fact where working on *Rhodopseudomonas capsulata* bacterium growth optimization, they noted that the peculiar morphology of the formed particle can be governed by concentration of metallic salts and pH of the medium. There was formation of spherical AuNPs within 10–20 nm at pH 6 using diluted AuCl₄ solutions. However, when the salt concentration was increased, the reaction formed Au nanowires (He et al. 2008). Interestingly, reduction of pH to 4 (enhancing the acidity), diluting salt concentrations, formed both spheres and triangular nanometer-scale plates. This study clearly established the role of pH and precursor concentration in the formation efficacy, size, and shape regime of NPs. So the same bacterium being fed on a similar metallic precursor could produce differently shaped NPs with the variation in medium pH. Similar attempts with *Lactobacillus* sp. A09 and *B. megaterium* D01 produced AgNPs through distinct reduction of silver ions (Gowramma et al. 2015). For details on the bacterial potential to synthesize NPs that are not discussed above (palladium, platinum, and some other metals), readers can refer to more specific literature sources (Shah et al. 2015b).

7.5 Bacterial Synthesis of Gold (Au) and Silver (Ag) Nanoparticles

Both Ag and AuNPs have drawn huge attention towards them due to their applications in medicine, electronics, cosmetics, coatings, packaging, and biotechnology. The production of nanoparticles emerges as an environment-friendly and exciting approach due to the use of microorganisms. Kushwaha et al. (2015) synthesized silver nanoparticles using *E. coli* and assessed their antibacterial potential against bacteria *Bacillus subtilis* and *Klebsiella pneumoniae* after confirming by UV-Vis and TEM (Sabri et al. 2016).

7.5.1 Gold (Au) Nanoparticles

In the biotechnology and biomedical field, the gold and silver NPs are considered very essential and interesting for several applications. Gold and silver NPs mainly find applications in the biomedical field such as diagnosis and therapy. Gold nanoparticle synthesis gained a lot of interest from many researchers. *Rhodopseudomonas capsulata*, *Shewanella oneidensis*, *Escherichia coli*, *Yarrowia*

lipolytica, and *Plectonema boryanum* have been applied for the formation of gold nanoparticles (Sehgal et al. 2018). Gold nanoparticles (AuNPs) are mainly applied in the field of drug delivery for cancer, diabetes mellitus, and heart-related diseases. It also finds application in medicine, mainly diagnosis and therapy of cancer and as anti-arthritic and malarial agents (Prasad et al. 2018). AuNPs are synthesized by *Klebsiella pneumoniae*, which is pathogenic bacteria, and their antimicrobial activity is assessed against pathogenic bacteria, i.e., *E. coli*, *Staphylococcus epidermidis*, *S. aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* (Prema et al. 2016). AuNPs of various shapes i.e. rectangular, square, cubic, and triangular shape with an average diameter of 60 nm were synthesized using *Rhodomonas capsulata*, which was later on applied for the treatment of human colon, lung, prostate, heart, and breast cancer (Menon et al. 2017). Pourrali et al. (2017) synthesized gold nanoparticles (AuNPs) using bacteria *Bacillus cereus* and fungus *Fusarium oxysporum* in vitro and their confirmation was done by UV-Vis, TEM, and XRD. Further, AuNPs were also used for the treatment of human fibroblast cell line CIRC-HLF (Pourrali et al. 2017). Srinath et al. (2017) reported the synthesis of biocompatible gold nanoparticles using *Brevibacillus formosus* and assessed their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Srinath et al. 2017).

AuNPs were synthesized by using bacterium *Ps. fluorescens 417*, and properties confirmed by UV-Vis, FTIR, and TEM and finally showed their antimicrobial potential against *Ps. aeruginosa*, *Bacillus subtilis*, *E. coli*, *S. aureus*, and *Klebsiella pneumoniae* (Syed et al. 2016). Nangia et al. (2009) synthesized AuNPs using bacterium *Stenotrophomonas maltophilia (AuRed02)* and characterized them by electrophoresis, zeta potential, and FTIR (Nangia et al. 2009). Sharma et al. (2012) biosynthesized AuNPs using bacterium *Marinobacter pelagius* and analyzed them with TEM, UV-Vis, and dynamic light scattering (Sharma et al. 2012).

Besides bacteria, there are few reports in literature for the biosynthesis of AuNPs and AgNPs using microalgae (Agarwal et al. 2019). These microalgae are rich in proteins, flavonoids, and several other enzymes which are absent or present in very less content than the bacteria. Moreover, microalgae being a eukaryotic, have different modes of synthesis of both metallic and metal oxide nanoparticles. Sintubin et al. synthesized both Au and AgNPs using dried powders of *Spirogyra* spp. (Sintubin et al. 2011).

7.5.2 Silver (Ag) Nanoparticles

As we have already discussed above, AgNPs have various applications, especially in the field of medicine and drug delivery. They exhibit almost all applications shown by AuNPs. AgNPs are applied in food packaging in order to prevent their spoilage by foodborne pathogens. They are also used as antimicrobial agents for plant crop protection. All these applications of AgNPs are possible due to their economical and biocompatible nature (Hoseinnejad et al. 2017) (Siddiqi et al. 2018). In pharmaceutical products, AgNPs could be used as antimicrobials, agents for

targeted drug delivery, and anti-biofilm agents. While in agriculture, it could be used for plant disease management. Further, it could also be used for environmental cleanup like water electrolysis, treatment of wastewater, degradation of dyes and pesticides, detection of pathogens and contaminants as a biosensor, etc. Silver nanoparticles showed antimicrobial potential against *B. cereus*, *B. subtilis*, *S. aureus*, *Corynebacterium rubrum*, *E. coli*, *Vibrio parahaemolyticus*, *K. pneumoniae*, *Salmonella typhi*, *Ps. aeruginosa*, *Citobacter koseri*, and *Salmonella typhimurium* (Thomas et al. 2015). Gahlawat and Choudhury (2019) have already reported earlier the biosynthesis of AgNPs from various pathogenic and nonpathogenic bacteria (Gahlawat and Choudhury 2019).

In the field of research, Kumar and Ghosh (2016) studied that, in history, silver has been used as an antibiotic in human health care (Kumar and Ghosh 2016). For many centuries, the antimicrobial property of silver has been well known to cultures around the world. Nalenthiran et al. (2009) reported the synthesis of AgNPs from *Bacillus* sp. (*Brevibacillus borstelensis*_MTCC10642) by exposing them with AgNO₃ solution and further characterizing them with advanced instruments. The size of AgNPs was 5–15 nm, formed intracellularly in the periplasmic space of the bacteria (Nalenthiran et al. 2009). Suman et al. (2014) reported the synthesis of AgNPs from *Agrococcus* sp. and characterized them with TEM, UV-Vis, and FTIR. Silver nanoparticles were prepared using the bacterium *Escherichia coli* in liquid broth medium (Suman et al. 2014). Silver nanoparticles showed antimicrobial potential against *Lactobacillus fermentum*, *Streptomyces* sp., *Bacillus cereus*, *Brevibacterium casei*, *Staphylococcus aureus*, *Bacillus licheniformis*, *Enterobacteria*, and *Ureibacillus thermosphaerius* (Chintamani et al. 2018). Fang et al. (2019) synthesized AgNPs using bacteria *Bacillus amyloliquefaciens*, *Bacillus subtilis*, and *Stenotrophomonas* sp. *BHU-S7*, whereas gold nanoparticles were prepared using bacterium *Deinococcus radiodurans* (Fang et al. 2019).

Karthika et al. (2015) synthesized AgNPs from bacterium *Serratia marcescens*, whose characterization was done by sophisticated instruments. Here, the AgNPs exhibited antibacterial capability against *E. coli*, *Staphylococcus* sp., and *Pseudomonas* sp. (Karthika et al. 2015). El-Saadony et al. (2019) synthesized AgNPs from *Bacillus pseudomycooides* MT32 and showed antifungal properties against a group of *Aspergillus* spp. (*Asp. flavus*, *Asp. niger*, and *Asp. terreus*), *Penicillium notatum*, *Rhizoctonias olina*, *Fusarium solani*, *Fusarium oxysporum*, *Trichoderma viride*, *Verticillium dahlia*, and *Pythium spinosum*. Characterization and analysis of silver nanoparticles were done using TEM, XRD, EDS, DLS, zeta potential, and UV-Vis (El-Saadony et al. 2019). Further, AgNPs synthesized from bacterium *Haemophilus influenzae* showed antimicrobial potential against various pathogenic bacteria like *Ps. aeruginosa*, *Klebsiella* spp., *Streptococcus* spp., *Serratia* spp., *S. aureus*, *E. coli*, and yeast (*Candida albicans*) using the agar well diffusion method. Characterization of AgNPs was confirmed using atomic force microscopy (Gahlawat and Choudhury 2019). Yadav and Fulekar (2018) reported the synthesis of AgNPs from bacterium *Pseudomonas* sp. ARS-22 and characterized them using various microscopic and spectroscopic instruments (Archana et al. 2015).

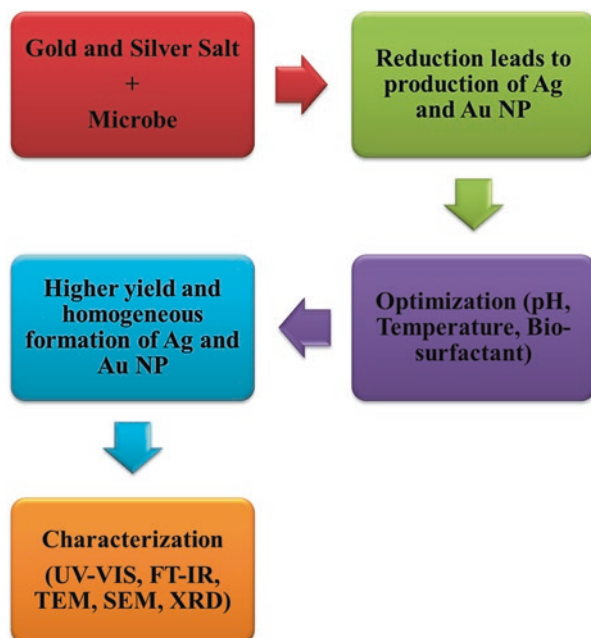


Fig. 7.4 Flowchart for the biosynthesis of AuNPs and AgNPs from microorganisms

Further, Kumar and Ghosh (2016) reported the synthesis of AgNPs from bacterium *Brevibacillus centrosporus* DSM8445T, *B. levickii* LKG22481, *B. invocatus* NCIMB13772T, *B. panacihumi* DCY35, and *B. choshinensis* DSM8552T, and the confirmation of AgNPs was done by sophisticated instruments (Kumar and Ghosh 2016). The schematic representation of the biosynthesis of AgNPs and AuNPs is shown in Fig. 7.4.

7.6 Algal-Mediated Synthesis of Metallic Nanoparticles

Algae like fungi are eukaryotic organisms, which are widely utilized for the formation of both metal and metal oxide NPs, though their mode of synthesis of nanoparticles is more or less similar to prokaryotes. Several approaches have been made which are available in literature for the microalga-mediated formation of metal and metal oxide nanoparticles from their respective aqueous salt solutions (Kuppusamy et al. 2016). But the synthesis of nanoparticles by algae has numerous drawbacks as it is highly time taking, require more space for harvesting and tedious task (Agarwal et al. 2019). Simultaneously, there are several advantages also like photosynthetic property, ability to convert solar energy and CO₂ into biomass, and accumulation of nutrients in the form of C, N, and P. All these properties have direct impact on the

morphological properties of nanocrystals. Microalgae could synthesize NPs in four different ways:

1. Direct utilization of biomolecules extracted after the breakage of the algae
2. Using supernatant obtained from microalgae culture media
3. Directly exposing the salts with the microalgae cells
4. Using living cells of microalgae

Merin et al. and several others have synthesized AgNPs from microalgae from the family of Haptophyta, Chlorophyta, and Ochrophyta (Mohseniazar et al. 2011). Mahdieh et al. reported synthesis of AgNPs by using living biomass of *Spirulina platensis* (Mahdieh et al. 2012), while Luangpipat et al. incubated gold chloride with *Chlorella vulgaris* under optimal conditions and reported the synthesis AuNPs, which was confirmed by TEM (Luangpipat et al. 2011). Microalgae due to their richness in various biomolecules like sulfated polysaccharides and proteins and pigments were used for the synthesis of Ag, Pd, Cd, and NPs, by either whole cells, supernatant, or dried biomass. Microalgae, mainly exploited so far for the above NPs, are cyanobacteria, *Chlorella* spp., *Lyngbya majuscula*, *Spirulina platensis*, and other *Chlorophyta* spp. (Patel et al. 2014).

7.7 Microbial-Mediated Synthesis of Metal Oxide Nanoparticles (MONPs)

7.7.1 Bacteria-Mediated Synthesis of Metal Oxide Nanoparticle

Metal oxide NPs have changed the face of industries due to its application in research, medicine, ceramics, space, defense, wastewater treatment, and composite preparation (Patel et al. 2014). Some of the most commonly used metal oxide nanoparticles are iron oxide nanoparticles, silica nanoparticles, and alumina nanoparticles. These nanoparticles are mostly synthesized from a commercial precursor using chemical and physical approaches. The most common chemical approaches employ sol-gel, coprecipitation, hydrothermal and solvothermal decomposition (Bilton et al. 2012), while chemical vapor deposition and laser ablation are common examples of the physical method. Because it is highly energy-intensive, both of these approaches are not only expensive, but also hazardous due to applications of various chemicals in the synthesis. So the biosynthesis of metal oxide NPs is an economical, biocompatible, and green method (Jiang et al. 2018). Among the biological approaches, various photoproducts and microbes have also been used. Plant products like leaf, flower, fruit, bark, and stem have various proteins, phytochemicals, flavonoids, terpenoids, and enzymes that act as a reducing agent, while microbes like bacteria, fungi, yeast, and algae have numerous biomolecules (proteins and enzymes and organic acids) that act as both reducing and

capping agent in addition to transforming the precursor into a biocompatible product (Shah et al. 2015a).

The mechanism of synthesis of metal oxides from the precursor material by microorganisms is similar to single metals. Generally, microorganisms can synthesize metal oxide NPs either intra- or extracellularly. In the previous process, metal precursors are taken up from the medium by the microbes and transform them into respective metal oxides using their machinery, whereas in the extracellular process, the metal precursors are present in the medium, where the microbes secrete their microbial products. The secreted microbial products interact with the metal precursors and get transformed into metal oxide nanoparticles (Mohd Yusof et al. 2019). Out of these two processes, extracellular mechanisms are considered advantageous, as the metal oxide nanoparticles form outside, so the recovery or downstream processing will be easier and economical in comparison to the intracellular process. Moreover, in the extracellular process, there are fewer chances of contamination.

Metal oxide nanoparticles are very effective in inhibiting the growth of various GP and GN bacteria and they have emerged as the most promising candidate as antimicrobial agents. The most common metal oxides include ZnO, TiO₂, CuO, Fe₂O₃, and MgO. The biological material used for the synthesis of NPs like bacteria, fungi, yeast, and plant extract lies on the principles of green chemistry and is compatible with the use of microorganisms (Mohd Yusof et al. 2019). The biologically synthesized NPs have various applications such as drug delivery, biolabeling, coating of medical products, and treatment of cancer (Yadav and Fulekar 2018). Opposite to physicochemical methods, biosynthesized NPs are nontoxic, making them suitable for biomedical applications. Moreover, the oxidized form of synthesized NPs is even more useful due to their physicochemical properties. Like metal NPs, bacteria could also synthesize metal oxide NPs by means of intracellular or extracellular process. Based on several works, it was found that among bacteria, NADH-dependent nitrate reductase (NDNR) enzyme activity plays a key role in the conversion of metallic ions to nanoparticles. The general synthesis methodology for the biosynthesis of nanoparticles using bacteria is shown in Fig. 7.5.

7.7.2 Iron Oxide Nanoparticles (IONPs): A Special Class

The unique physicochemical properties of FeO nanoparticles have fueled their demand not only from research and industrial viewpoints, but perhaps these are one of the most preferred nanomaterials in biotechnology and microbiological domains (Gu et al. 2006; Tamer et al. 2009; Chang et al. 2008; Dong et al. 2011; Meng et al. 2011). The common link with hemoglobin present in the blood has recently fascinated research towards them in modified forms, whereby these entities could be prepared using facile approaches. On the basis of microbial stain and respective nanoparticle concentration, NPs of FeO could either stimulate or suppress microbial growth. The superparamagnetic attributes of these particles make them befitting heat coupling sources, forming the basis of their suitability in targeted drug delivery

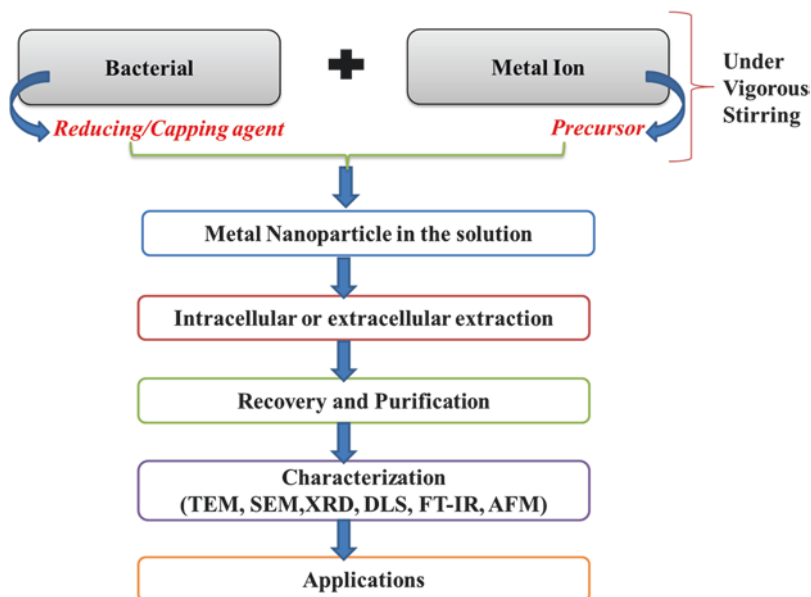


Fig. 7.5 Generalized nanoparticle synthesis methodology using bacteria

to injurious or damaged cell or organ systems and improving the efficacy of modified drug delivery through hypothermic coupling (Khanna et al. 2018; Xue et al. 2018). The heat coupling efficacy of FeO NPs is already in robust consideration towards improving the antimicrobial efficacy of AgNPs (Senthil and Ramesh 2012). Similarly, the magnetically responsive ability of FeO NPs has rapidly increased their implication in biosensing (separation of pathogenic and nonpathogenic microbial strains), immobilization of select microbial population(s), and process intensification of biotechnological processes. Till date, FeO NPs are produced by several Fe-reducing bacterial species such as *Thiobacillus thiooxidans*, *T. ferrooxidans*, *Gallionella*, *Shewanella*, and magnetotactic bacterium, with existence in several phases, such as magnetite ($\gamma\text{-Fe}_3\text{O}_4$), hematite ($\beta\text{-Fe}_2\text{O}_3$), and maghemite ($\alpha\text{-Fe}_2\text{O}_3$) (Ali et al. 2016). Two broad mechanisms for synthesizing FeO NPs include either intervention of magnetotactic bacteria or through exposure of Fe precursors to non-magnetotactic bacteria. The two approaches are discussed ahead with their working distinctions.

7.7.2.1 Magnetotactic Bacteria: Magnetite Nanoparticles

Magnetotactic bacteria comprise a peculiar category of bacteria, owing to their capability of intracellular generation of magnetite NPs in specialized organelles, called magnetosomes. These structures encode specialized globular protein, ferritin, which, in turn, comprises 24 distinctive subunits (Sakaguchi et al. 1993; Philipse

and Maas 2002). This encapsulation aids in the storage of Fe in a nontoxic and soluble form, inside the magnetosomes, which could be extracted from bacteria through mechanical or chemical means (extracted magnetosomes could be ruptured to harvest magnetite NPs). The magnetite NPs are arranged in a crystalline manner within the magnetotactic bacteria with the entire population having equal size and shape. A characteristic/unique aspect of these magnetite NPs inside the magnetotactic bacteria is their ability to align them along the earth's magnetic field (Blakemore 1975). As harvested magnetic NPs are being used in diverse fields of cutting-edge, substantiating drug delivery, life sciences, MRI, CT scan, electronically equipped memory storage devices, ink printing, and several others. The most common bacterial species studied for the generation of magnetite are *Magnetospirillum magnetotacticum*, generating two distinct kinds of NPs, the first being magnetic (Fe_3O_4) NPs (having chain like structure), while the second being greigite (Fe_3S_4) NPs (Xie et al. 2009). Rarely, synthesis of both nanostructures is also reported from the same microbial species (but definitely under different culturing conditions). Popular species, among other bacteria well known for intracellular formation of magnetite (Fe_3O_4) NPs, include *Aquaspirillum magnetotacticum*, *Magnetospirillum candidatus*, *Magnetoglobus multicellularis*, and magnetotactic bacterium MV-1.

Magnetosomes are capable of forming robust crystalline as well as noncrystalline nanocrystal morphologies, having diverse shapes ranging from hexagonal to octahedral and faceted cuboctahedral shape and octahedral symmetry with either tied down or collected morphology within the phospholipid bilayers. It is pertinent to discuss some of the characteristic formations of magnetic NPs by bacterial stains, with the first being *Desulfovibrio magneticus* (RS-1), an anaerobic bacteria capable of reducing sulfur and intracellular accumulation of magnetite NPs, with sizes of most accumulated magnetite crystals remaining in the order of 30 nm (all particles being superparamagnetic in nature). In an interesting study, Klaus-Joerger demonstrated the possible replacement of Fe in biosynthesized magnetite NPs by Co, Cr, and Ni (all having an unpaired electron that contributes to spin-based characteristic magnetic sensitivity) in *Thermoanaerobacter ethanolicus*, a heat-enduring Fe-reducing bacterium. These substitutions produced octahedral magnetite NPs of <12-nm sizes in significant quantities, coexisting with poorly crystalline magnetite phase near the cell surface. Elblbesy and coworkers documented the formation of magnetic NPs from the *Magnetospirillum* strain AMB-1 to produce 47-nm-sized magnetite NPs, further working on the investigation of magnetic behavior as a function of varying incubation temperatures. Almost working on the same principle, Philipse and Maas reported the synthesis of magnetite crystals in single-domain, folded-chain, and flux-closure ring morphologies in *Magnetospirillum magnetotacticum* through regulation of bacterium locomotion via varying the externally applied magnetic field (Philipse and Maas 2002). Organisms living in Fe-rich surroundings harness their energy through a reduction of mFe^{+3} , with the partial reduction resulting in magnetosome having a peculiar protein-constituted phospholipid bilayer as its membrane. Several studies have screened the ability of diverse bacterial species isolated from Fe ore mining sites for their magnetic property conferring ability at laboratory conditions. In one such attempt, the *Thiobacillus thioparus*

strain was identified through ribotyping, with microbial growth and magnetite production being optimized at varying pH, temperature, and substrate concentrations (Katayama and Kuraishi 1978). Moreover, magnetic NPs were purified via growth and lysis of bacteria and the magnetic properties were screened using empirical observations under the influence of magnetic field. With an interest to pursue their specific biological impact, the harvested NPs were monitored for their suitability in SDS-polyacrylamide gel electrophoresis (PAGE) in conjunction to those of coprecipitated magnetic NPs as well as particles coated with bacterial protein. The observations revealed that purified particles were synthesized using isolated bacterial strains having a protein coating, visualized on the stained polyacrylamide gel. The fluorescence property of the solution under magnetic field and aggregation of the particle along the edge of the wells in the absence of protein coating displayed the presence of monodispersive magnetic NPs in the preparation. Another attempt focused on the utilization of magnetically insensitive anaerobic bacterium GS-15, to reduce nonmagnetic brown disordered ferric oxide into a magnetic black solid particle, with the synthesized crystals residing outside the cell in a nonaligned hierarchy (Elcey et al. 2014).

7.7.2.2 IONP Synthesis by Non-Magnetotactic Bacteria

Several studies have reported the synthesis of FeO NPs using non-magnetotactic bacteria, through culturing in peculiar environments at specific pH and temperature. One such attempt reports the biological synthesis of 73.30-nm-sized FeO NPs through *Lactobacillus rhamnosus* strain, followed by characterization using atomic force microscopy (AFM) and FTIR spectroscopy (Mohammed et al. 2016). Another attempt reports the utility of *P. islandicum* strain in getting rid of heavy metal manifested as pollution through a transformation of Fe, Cu, Cr, and U to their oxides at high temperatures (Tchounwou et al. 2012). Study results like these provide highly crucial information about the optimization of microbial species to attain optimum biological degradation as the genes coding for concerned biochemical conversions could be selected and engineered for a higher expression to maximize the detoxification efficacy. Many investigations report better efficiency of biodegradation using symbiotic cultures compared to single microbes, inferring a probable synergistic mode of functioning by microorganisms. These possibilities could be further optimized for enhancing the performance as requirement of distinctive nutrients from culture media would allow better growth of a bacterial and fungal consortium compared to bacteria or fungi alone.

Maghemite Nanoparticles (γ -Fe₂O₃)

Microbial species like *Actinobacter* (an aerobic bacterium) have been optimized to produce superparamagnetic Fe₂O₃ NPs at extracellular locations, using FeCl₃ and FeSO₄ under ambient culture conditions. Exploring the untapped potential of

biologically abundant and environmentally safer technologies to utilize inorganic metals and their varying stoichiometry compounds has been on the rise over the past few years. The justified reason for this is the renewable nature of energy input needed to catalyze the bioconversions and catalysis processes. It seems quite overwhelming when the microbes residing in marine and terrestrial habitats are cultured for development into biologically altered forms that could selectively act on harmful synthetic dyes and poisonous heavy metals and their compounds, recalcitrants. These technologies reflect the untapped potential of natural biomaterials or biore-sources to clean the environment and regulate the net balance of several useful metals and nonmetals, via precipitation, decomposition, and degradation. For instance, *Bacillus* species encode a significant population of enzymes and proteins aiding in the synthesis of metal oxide NPs, such as that of hexagonal and protein-capped crystalline Fe_2O_3 NPs by *Bacillus cereus* using FeCl_2 as a precursor. Capping with microbial proteins during synthesis ensures a thorough stability of as synthesized NPs using the same material as reducing and capping agents (Fang et al. 2019). So no separate capping agent needs to be added and NPs or nanomaterials made in this manner find astounding significance in drug delivery applications (where cytotoxicity has to be controlled).

The use of a thermophilic bacterium, *P. islandicum*, is immensely significant in this regard, through which amorphous Fe (III) oxyhydroxide is reduced to magnetically receptive iron oxides at 65 °C. Similarly, the enzyme extract of *Lactobacillus casei*, upon incubation with FeSO_4 solution (1 mM) at 5.6 pH, 37 °C, and in 5% CO_2 -containing environment for 3 weeks, provided spherical NPs of 15 nm (evaluated using transmission electron microscopy) size. Preliminary formation of NPs was noted by the transparent to black color changes of the culture medium, after which confirmatory screening was made using electron microscopy and X-ray diffraction. Approaches like these offer benign methodology for FeO NP synthesis, with simple, efficient, and economic methodology, owing to which the as-harvested product could be readily utilized for drug delivery and pharmaceutical applications (Torabian et al. 2018). Similarly, *Bacillus subtilis* strains (isolated from rhizosphere-rich soil) are reported for the extracellular synthesis of Fe_3O_4 NPs, using a supernatant fraction of their culture medium (to write details). It would be interesting to note here that same yield of NPs is not possible with supernatant and pellet as the population of biological catalysts differs, thereby affecting the NP formation activity (Sundaram et al. 2012).

A peculiar aspect in FeO NP formation by magnetotactic bacteria involves the movement of Fe particles, leading to the formation of siderophores, which are the magnetosome vesicles facilitating Fe reduction from the +3 (ferric) to +2 (ferrous) state. After this biological reduction, controlled biomineralization of magnetite (in the last stage) happens. An important consideration here is the formation of siderophores, characteristic magnetic vesicles formed in magnetotactic bacteria, typically low-molecular-weight (0.5–1.5-kDa) Fe^{+3} -chelating molecules synthesized by most bacteria under Fe limiting conditions (Arakaki et al. 2008). In general, these siderophores are natively synthesized by magnetotactic bacteria.

Table 7.2 Various metal oxide nanoparticles synthesized using bacteria

	<i>Bacteria</i>	Size	Shape
ZnO	<i>Rhodococcus pyridinivorans</i> NT2	100–120	Hexagonal
ZnO	<i>Bacillus licheniformis</i> MTCC 9555	100	Hexagonal wurtzite
ZnO	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	3.9 ± 0.5	Sphere
ZnO	<i>Klebsiella pneumoniae</i>	20–25	Hexagonal pyramids
FeO	<i>Actinobacteria</i> sp.	10–50	Spherical
Fe ₂ O ₃	<i>Shewanella oneidensis</i> MR-1	30–40	Irregular or rhombohedral
Fe ₃ O ₄	QH-2	80–90	Rectangular
Fe ₃ O ₄	<i>Aeromonas hydrophila</i>	50–60	Spherical
TiO ₂	<i>Bacillus subtilis</i>	66–77	Spherical
TiO ₂	<i>Bacillus amyloliquefaciens</i>	22.11–97.28	Spherical
TiO ₂	<i>Lactobacillus</i> sp.	8–35	Spherical
TiO ₂	<i>Micrococcus lylae</i> , <i>Micrococcus aloeverae</i> , <i>Cellulosimicrobium</i> sp.	14–17	Spherical
TiO ₂	<i>Bacillus mycoides</i>	40–60	Spherical
BaTiO ₃	<i>Lactobacillus</i> sp.	20–80	Tetragonal
NiO	<i>Microbacterium</i> sp. MRS-1	100–500	Spherical flower

Table 7.2 presents an account of different metal oxide NPs synthesized using bacterial species, with the characteristic shape, size, and species. Yet again, it is interesting to note the NPs of similar size, shape, and extent, which consequently form the basis of their distinct applications (owing to size- and shape-dependent NP functional responses).

7.7.2.3 Fungus-Mediated Synthesis of FeO NPs

Recognized as eukaryotic organisms for their decomposition activities, fungi are ubiquitous in several ordinary lodgings and are one of the most well-known decomposer organisms. Biological diversity and robustness of living requirements for the fungi could be well judged from the supposedly 1.5 million species (reported on earth), even though only 70,000 of these are distinctly known. Digestion of extracellular food sources through secretion of specified enzymes to hydrolyze complex chemical compounds into simpler fractions that are finally absorbed and utilized as energy resources is a characteristic feature of these microorganisms. Latest data enabled through high-throughput screening methods estimate a prevalence of nearly 5.1 million fungal species (Wu et al. 2019). Higher tolerance of culture environment adversities (such as temperature and pH diversity) along with greater metal bioaccumulation abilities by the fungi renders them highly suitable compared to bacterial species (Archana et al. 2015). Apart from the higher culture fluctuation tolerance of fungi, the fascinating features for intense interest in fungal preparation of metal and metal oxide NPs are due to the simplicity of their scale-up process alongside an

efficient extracellular secretion of enzymes (Boroumand Moghaddam et al. 2015; Prasad 2016, 2017; Prasad et al. 2018; Aziz et al. 2016). Utilization of fungi for making NPs is substantially preceded through thin solid substrate fermentation technique, manifesting the optimization of high wall-binding and intracellular metal uptake attributes, owing to their fast growth (Salvadori et al. 2015). Till date, several fungal species, including *Verticillium luteoalbum*, *Fusarium oxysporum*, *Aspergillus oryzae*, *Alternataalternata*, *Trichoderma viride*, etc., have been cultured and optimized to yield NPs of different shapes and sizes. An effort worth in this direction by Gawande et al., in 2016, reports the biomimetic mineralization of fungi to synthesize nano- or meso-sized NPs through activity of intra- and extracellularly secreted enzymes (responsible for a range of geometry and sizes of synthesized NPs). Fast growth and easier handling of fungal populations within the laboratory are the key advantages for their preferential usage in such applications (Gawande et al. 2016). The sole constraint behind the use of fungi in nanoparticle preparation is their difficulty in scaling up, hindering the commercial development and application of synthesized nanomaterials. This constraint arises due to higher biomass fraction of fungi (compared to bacteria), and for needful enzymatic activity, the biological activity of cultured microbe has to be adequately expressed in the absence of which the product synthesis becomes impaired or affected (with respect to product quality). Among all the fungal species, *Verticillium* and *Fusarium* are most utilized for metal oxide NP synthesis, owing to their possession of versatile enzymes, proteins, and other metabolites (Ovais et al. 2018). One study reports the synthesis of FeO NPs at room temperature using ferrous and ferric salt mixtures as precursors (Mazrouaa et al. 2019). Success has also been obtained regarding the formation of crystalline magnetite NPs through cationic protein enabled hydrolysis of anionic Fe complexes at the extracellular sites. The obtained NPs exhibited a characteristic ferromagnetic transition with an almost insignificant magnetization extent at low temperature. In processes like this, it is very pertinent to take note of the electrostatic proximity of cationic proteins with anionic Fe precursors, and it becomes imperative to have an aggregation preventing capping activities of cationic proteins, which in turn promotes interaction with negatively charged species/molecules (Fig. 7.6).

A rigorous investigation by Latiffah and coworkers reports the formation of spherical FeO NPs using three distinct manglicolous fungal species, namely, *Trichoderma asperellum*, *Phialemoniopsis ocularis*, and *Fusarium incarnatum*, isolated from mangroves. The NPs were, respectively, 25 ± 3.94 , 13.13 ± 4.32 , and 30.56 ± 8.68 nm in sizes, inferring highest monodispersity with *Phialemoniopsis ocularis*, a consideration which could be further screened with respect to the biological catalytic action of *Phialemoniopsis ocularis* (Latiffah et al. 2010).

Many research efforts have explored the bioreducing ability of the fungus *Aspergillus niger* for making magnetite (Fe_3O_4) NPs, in view of this fungi's ability to catalyze the FeSO_4 and FeCl_3 decomposition to FeS and Fe_2O_3 , respectively. Upon exposure to ethanol-assisted supercritical conditions, the as-formed particles were stored at 300 °C and 850 PSI units of pressure. Subsequent analysis using phase structure and morphology aspects yielded spherical and pure Fe and Fe_3O_4 NPs having average sizes of 18 and 50 nanometers, respectively. Screening for

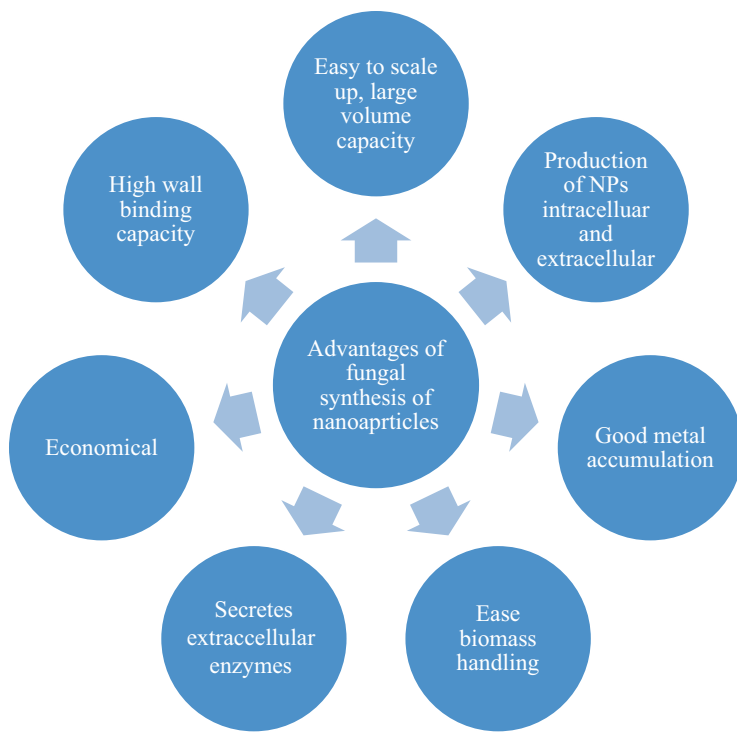


Fig. 7.6 Advantages of fungus-based synthesis of nanoparticles

magnetic properties revealed ferromagnetic behavior with saturation magnetization extent of 68 emu per gram for Fe_3O_4 . The analytical profile provided a novel paradigm shift to optimize biophysical methods for the large-scale synthesis of magnetic NPs optimized for biomedical applications (Abdeen et al. 2016).

Biosynthesis of Fe NPs is also reported using *Pleurotus* sp. in optimum culture media, where the fungus was allowed to grow in 2×10^{-4} M FeSO_4 solutions up to 72 h. UV-Vis absorbance peaks at 226 and 276 nanometers predicted the formation of NPs, while involvement of proteins in the NP formation (as component of culture medium) was ascertained through spectral analysis of broth containing culture medium at varying time intervals corresponding to 265 nm. Interactions of proteins with NPs were inferred through FTIR spectroscopy through a concomitant involvement of functional groups indicated by shift of functionalities of the treated cells. Morphology inspection through TEM revealed particle deposition at the inner as well as outer surface for completion of the synthesis process. The peculiar role of fungal proteins was confirmed by the absence of these depositions in control samples, where no *Pleurotus* sp. was furnished. This was also confirmed through X-ray fluorescence, which showed no Fe in control samples but prevailed in mycelium. The presence of few other elements was also noted, which could be due to

randomized compositional fluctuations of culture medium. A characteristic aspect of FeO NP synthesis by fungi is the formation of Fe transport molecules like hydroxamates that bind the complex molecules and transport them inside the cells (Mazumdar and Haloi 2011). Similarly, the potential of *Aspergillus japonicus* isolate AJP01 is also reported for extracellular synthesis of 60–70-nm FeO NPs through hydrolysis of a mixture of Fe cyanide complexes (acting as precursor), under ambient growth conditions. Screening the mechanism, it was noted that hydrolysis of these complexes released Fe^{+2} and Fe^{+3} , through involvement of protein facilitating coprecipitation and controlled nucleation, ultimately forming FeO NPs. Analysis using FTIR spectroscopy revealed the presence of proteins that confer stability to as-formed NPs, in agreement with the observations of an earlier preliminary investigation (Bhargava et al. 2011).

7.7.3 Microbially Mediated Synthesis of TiO_2 , ZnO , CuO , SiO_2 , and Al_2O_3 NPs

7.7.3.1 Titanium Dioxide (TiO_2) NPs

The significance of TiO_2 in multidimensional domains of electronics, optics, and environmental remediation is well known. In general, the nontoxic and biocompatible properties of TiO_2 impart its suitability for biomedical requirements such as bone or tissue engineering as well as in pharmaceutical industries. Majorly, TiO_2 is synthesized using chemical and biological reduction approaches such as sol-gel, hydrothermal, solvothermal, combustion, plant (extract), and microbially assisted biosynthesis. Microbes such as *Lactobacillus* (bacteria) and *Saccharomyces cerevisiae* (yeast) are used for TiO_2 NP synthesis. Few specific examples of TiO_2 NP synthesis using bacterial cultures are notified here. Firstly, Jayaseelan et al. and Kirthi et al. reported synthesis of TiO_2 NPs using $\text{TiO}(\text{OH})_2$ as a precursor and *Bacillus subtilis* as a reducing agent. The morphology of the synthesized TiO_2 was found to be spherical/oval while the size was in the 66–77-nm range (Kirthi et al. 2011b; Jayaseelan et al. 2012). Secondly, Malarkodi et al. (2013) reported the synthesis of TiO_2 NPs of size 100–500 nm, irregularly shaped, using the biomass of *Planomicrobium* spp. obtained from melted ice. The synthesized NPs exhibited antimicrobial properties against *B. subtilis* MTCC 3053 and *K. planticola* MTCC 2727 in agar disk diffusion method (Malarkodi et al. 2013).

Thirdly, a report by Khan and Fulekar focuses on the biological synthesis of TiO_2 using *Bacillus amyloliquefaciens* as a capping agent. The synthesized TiO_2 exhibited a spherical morphology in the 22–97-nm size range. The synthesized TiO_2 were used further in the photocatalytic degradation of Reactive Red 31 (RR31, a poisonous dye) using platinum-doped TiO_2 , developing the highest potential (90.98%) for RR31 degradation as compared to native TiO_2 (75.83%) (Khan and Fulekar 2016).

7.7.3.2 Zinc Oxide (ZnO) NPs

Owing to its simplified synthesis approach as well as its increasing suitability in the fields of pharmaceuticals, cosmetics rubber industry, biosensor development, optics, solar cells, and environmental remediation, ZnO NPs have emerged as one of the most sought-after metal oxide NPs (Khan et al. 2016, 2018). The specific interest has been to explore the antimicrobial, antibacterial, and antioxidant activities through the inherent nontoxicity of nanoscale forms (Bhuyan et al. 2015). Zinc (Zn) is evidently the second most abundant metal in the earth's crust (after Fe) and it is the only metal present in all six enzyme classes (*lyases, transferases, oxidoreductases, hydrolases, isomerases, and ligases*). Similar to Zn, ZnO is the most researched and studied metal oxide after TiO₂. Compared to other synthesis methods, the microbial approach offers much advantages emerging helpful for NP synthesis at low pH, temperature, and pressure. In an interesting attempt, Kalpana et al. examined the antibacterial activity of ZnO NPs on *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Kalpana et al. 2018). Further, Kundu et al. synthesized extracellular ZnO NPs of average diameter (100–120 nm) using *Rhodococcus pyridinivorans* NT2 and applied it for textile finishing and drug delivery in colon carcinoma (Kundu, Hazra) (Kundu et al. 2014). Jayseenlean et al. suggested a novel biological route for the synthesis of spherical, oval-shaped ZnO NPs of size 57.7 nm using bacterium *Aeromonas hydrophila*. The synthesized ZnO NPs were characterized by all the major sophisticated instruments and its potential was assessed against pathogenic bacteria and fungi (Jayaseelan et al. 2012). Taran et al. (2018) reported a simple, eco-friendly method for the synthesis of ZnO and TiO₂ NPs using bacterium *Halomonas elongata* IBRC-M 10214. The morphological confirmation of NPs by microscopy revealed that the particles were spherical shaped whose average diameter was 104.63 ± 27.75 for TiO₂ NPs and 18.11 ± 8.93 nm for ZnO NPs. Both of the NPs were assessed for their antibacterial activity against multidrug-resistant bacteria (MDRB), i.e., *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 43300 (Taran et al. 2018).

7.7.3.3 Copper Oxide (CuO) NPs

One of the most important transition metal oxides, CuO promises interesting applications in terms of its size-tunable and captivating properties, forming the basis of its inclusion in highly critical temperature gas sensors, superconductors, and catalytic, optical, photoconductive and photoelectronic, electrical, and energy storage purposes. It has been used as an antimicrobial agent recently against various bacterial species (Yadav et al. 2017).

7.7.3.4 Silica (SiO₂) NPs

A green technique of SiO₂ NP formation, using a thermophilic bacterium (BKH1) as a biological template, is reported using an inorganic precursor (magnesium trisilicate) in an organic (tetraethyl orthosilicate) reducing agent (Show et al. 2015).

This novel method of bacterially synthesized SiO₂ NPs is eco-friendly and is workable at ambient temperature, thereby not requiring drastic energy inputs. This method avoids the complex protocol of multistep synthesis and is therefore much cost-effective. A notable aspect is the functional activity of thermophilic bacterium (BKH1) at high temperatures, pertaining to which identification of genes conferring survival and needed bioactivities at high temperatures could be an interesting framework for strengthening the impact of similar studies on other microbes. Such activities also create an interest in enzyme/protein stability against denaturation at high temperatures.

The synthesis of silicon/silica nanoparticle composites by the bacterium *Actinobacteria* sp. is reported by Marikani et al., by exposing the bacterium to K₂SiF₆ precursor under ambient conditions. The formation of silica NPs is due to the secretion of several reductases and oxidizing enzymes. Further, few scientists have also reported on the synthesis of silica NPs from *F. oxysporum* isolated from tomato wilt (Marikani et al. 2016). Silica NPs were synthesized from rice husk ash (RSA) by *F. oxysporum* in malt-glucose (MG) and malt-glucose-yeast-peptone (MGYP) media. The confirmation of size, shape, and purity was analyzed by sophisticated instruments. It was concluded from the results that solubilization of silica was not directly associated with their production of organic acids. The results showed that the production of organic acids was not directly related to the solubilization of silica. It was found that solubility and stability of silica are due to the extracellular proteins released into the medium during the exponential growth phase. Out of both the media, MG media were found more suitable for growth as well as for the formation of semicrystalline, quasi-spherical SiO₂ NPs of size 2–8 nm (Pineda-Vásquez et al. 2014).

7.7.4 Virus-Based Synthesis of Metal Oxide Nanoparticles

Plant viruses have been used widely as templates for organic–inorganic hybrid synthesis. However, by simply adjusting the pH, fine-tuning of hybrid nanoparticle structures, especially the control of inorganic particle size and the location where silication occurs (i.e., outside and/or inside of the capsid), remains a challenge. By using the templating effect of the cowpea chlorotic mottle virus (CCMV) protein cage, it was shown that the silication at the outer or inner surface of the protein capsid, and the resulting structures of silica/virus hybrid nanoparticles, can be finely tuned by adjusting the pH (Liu et al. 2017). At pH 4.0, only small silica particles (2.5 nm in diameter) were formed inside the protein cages, whereas at pH 6.0, mainly silication occurred in the protein cages, resulting in monodisperse silica nanoparticles with 14-nm diameter. At pH 7.5, silica deposition was found on both the surfaces, i.e., inner and outer surfaces of the protein cage under aqueous conditions. In these reaction circumstances, multicomponent hybrid virus/nanoparticle systems, such as CCMVAu/silica and Au/silica nanoparticles, were prepared stepwise. After removal of the CCMV template in thermal degradation, a single gold nanoparticle can be encapsulated within a hollow silica shell to simulate the

structure of a baby rattle in which unattached solid particles are in the hollow particles. The Au/silica core-hollow shell nanoparticles can further be used as a stable catalyst. These synthetic methods are expected to provide a versatile method for preparing core-shell nanomaterials with well-designed structures and functions (Liu et al. 2017).

A sol-gel procedure has been developed to integrate bionanoparticles, such as cowpea mosaic virus, turnip yellow mosaic virus, tobacco mosaic virus, and ferritin into silica while maintaining the particle integrity and morphology. The structures of the resulting materials were characterized by TEM, small-angle X-ray scattering (SAXS), and N adsorption-desorption analysis. The results obtained show that the shape and surface morphology of the nanoparticles are largely retained after the incorporation of silica. After removal of the bionanoparticles by calcination, a mesoporous silica having monodisperse pores with well-defined shape and surface morphology of the bionanoparticles was replicated inside the silica (Niu et al. 2010).

7.7.5 *Alga-Mediated Synthesis of Metal Oxide Nanoparticles*

7.7.5.1 Alumina Nanoparticles (Al_2O_3) by Algae

Algae are a good source of biomolecules among all other aquatic organisms; this is so because algae contain proteins, carbohydrates, fats, nucleic acids, pigments, and secondary metabolites such as alkaloids, some aromatic compounds, peptides, macrolides, and terpenes (Siddiqi and Husen 2016a). They can act as reducing agents that help in the preparation of nanoparticles from metal salts without producing any toxic by-product. Once the algal biomolecules are identified, the nanoparticles of the desired shape or size may be fabricated. The antimicrobial activity of the thus synthesized metal and metal oxide nanoparticles against several gram-positive and Gram-negative bacterial strains and fungi has been investigated (Shannon and Abu-Ghannam 2016).

The dimensions of synthesized alumina NPs depend on the pH, temperature, incubation time, and concentration of the solution. A new biological methodology is proposed for the production of ceramic α -aluminum oxide nanoparticles using an extract of the algae *Sargassum ilicifolium*. The algal extract works as a stabilizer as well as a bioreducing agent. The UV-Vis analysis shows the presence of an absorption peak at 227 nm, which confirmed the formation of the aluminum oxide nanoparticles. FTIR analysis has indicated that bioreduction of aluminum ions and stabilization of nanoparticles may be caused by interactions between aluminum and the biofunctional groups of the algal extract. The XRD pattern has confirmed that after calcination at ~ 1200 °C, the Al_2O_3 nanoparticles with 35-nm diameter were alpha crystalline in nature and have rhombohedral structure. TEM analysis showed that the alumina nanoparticles were well dispersed and spherical in shape with an average size of 20 ± 2.1 nm, while EDX spectroscopy has confirmed high purity of the alumina nanopowder, as sample contained only aluminum (46.31%) and oxygen (53.69%) (Koopi and Buazar 2018; Siddiqi and Husen 2016b).

7.8 Various Conventional Approaches for Wastewater Treatment

With the continuous advancement of technology, several methods have made huge impact on wastewater treatment. Although there are several conventional wastewater treatment methods, like coagulation, precipitation, electrolysis, filtration, adsorption, adsorption (Azimi et al. 2017), etc., most of them are less efficient, expensive, and energy-intensive. Out of these, the most efficient and economical method is adsorption and the process can become cheaper by using waste materials derived from agriculture, industry, domestic, and poultry. The adsorbents developed from such materials have different groups on their surface that act as adsorption sites. These active sites have a role in the removal of multivariate pollutants from the wastewater.

Out of these adsorption is the most reliable technique by nanoparticles due to their economical nature and surface modification by microorganisms (Khan et al. 2019). Generally, nanoparticles adsorb inorganic pollutants on their surface. One more advantage with adsorption method is that the nanoparticles can be treated with 0.1 M NaOH for removal of all the surface-attached pollutants, mainly heavy metals. This helps in the reuse of nanoparticles, whereas if nanoparticles are IONPs, then it can be easily recovered after the reaction is complete, making the whole process economical.

7.9 Applications of Nanoparticles for Wastewater Treatment

7.9.1 *Typical Composition of Wastewaters*

Before moving on to the mechanisms and efficacies of nanoparticle-enabled wastewater treatment technology, it is quite logical to know about the chemical diversity of wastewater streams in terms of its composition. Multiple research attempts on wastewater streams from different regions of the globe list six major deleterious components, namely, suspended solids, biodegradable organics, pathogens, nutrients, heavy metals, and soluble inorganic salts (Abdel-Raouf et al. 2012). All these constituents pose a significant risk to the pollution level of wastewaters and could be present either in native form or added at various stages of domestic water discharge. For example, the nutrients carbohydrates, fats, and proteins could be present from the source itself (may be due to excretion products of humans and animals), whereas heavy metals could be added to the wastewater stream if it has access to industries. Similarly, the organic constituents act as existence support towards the environmental microbial species, which continuously grow, propagate, and increase their metabolic activities while residing on it (Lareen et al. 2016). Consequently, the microbial load increases, and with waste material as dietary input, these organisms exhibit toxic responses and are easily the opportunistic carriers of communicable infections (Figs. 7.7 and 7.8).

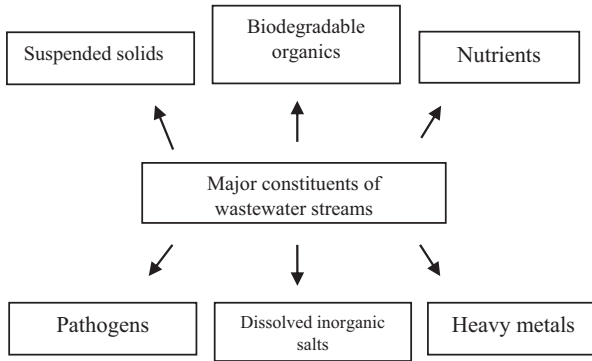


Fig. 7.7 Major polluting constituents in a wastewater stream

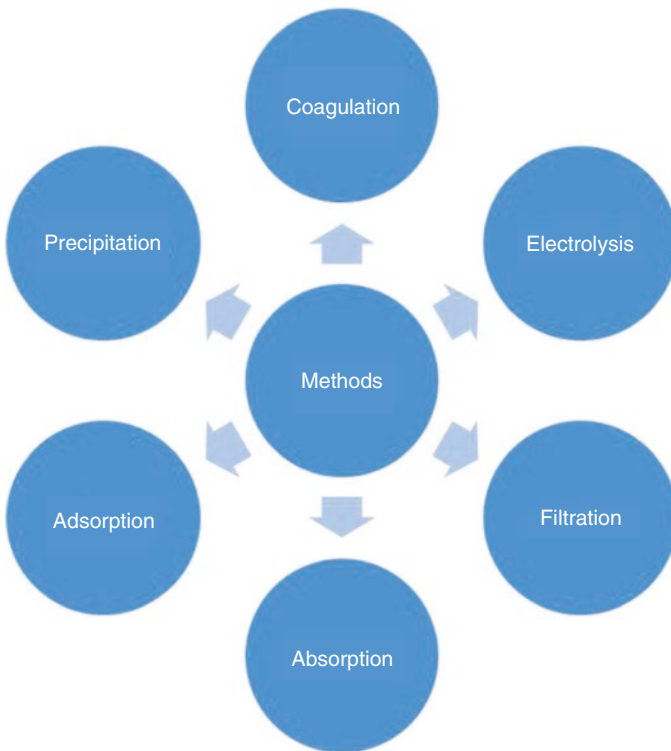


Fig. 7.8 Different methods of wastewater treatment

The nitrogen in the discharged fats, proteins, and carbohydrates in the wastewater streams catalyzes the microbial activities through the eutrophication phenomenon, as a consequence of which the toxicity is enhanced (Puyol et al. 2017). The oxidative proximities of proteins, carbohydrates, and fats (summed as biological

oxygen demand, BOD) deplete the environmental oxygen whereby serious loss to biodiversity could be driven. Like various other pollutants, inorganically dissolved impurities (such as calcium, sodium, and sulphate) could be added to wastewaters from household activities. These materials make the wastewater unfit for use owing to which its chemical load moderation is mandatory before final discharge as waste streams. Among the major organic materials (OM), fibers were found to prevail in the majority, followed by proteins and sugars, respectively accounting for 20.64, 12.38, and 10.65% of the total organic content (TOC). A certain percentage of endocrine-disrupting chemicals (mostly polyaromatic hydrocarbons [PAHs] and phthalates) were also found to contribute towards the pollutant load of wastewaters (Puyol et al. 2017). Together, volatile fatty acids, soluble proteins, and sugars contribute to nearly 30% of the total chemical oxygen demand (COD) of wastewaters, with the majority of proteins and sugars being $>0.001 \mu\text{m}$.

Several stages of treatment are employed to reduce the chemical load of wastewater streams, such as removal of impurities and solid materials through sedimentation, froth floatation, or salt-driven precipitation. Each successive stage is intended to reduce the toxic content through treatment procedures implicit towards the chemical diversity of impurities. The microbial decontamination of wastewaters has gained immense popularity in the past few years attributed to powerful chemical breakdown actions of microbial enzymes (Singh et al. 2016b). The advantage with this technology is that microbes can feed on the wastewater as their nutrient base and there is no requirement of maintaining their survival through external controls. The stage is now right to know about the specific bacterial species and strains for specific kinds of wastewater impurities.

Hyperthermophilic microorganisms can help in the migration of metal contaminants by reducing them to less mobile forms. Thermal radioactive or metal-containing industrial wastes may be treated potentially in bioreactors using microorganisms similar to the metabolism of *P. islandicum* or their enzymes (Kashefi and Lovley 2000). Many mesophilic microorganisms having the ability to use Fe (III) as a terminal electron acceptor can also reduce various metals and metalloids other than Fe (III) (Lovley 2013). Fe (III)-reducing bacteria and archaea were found capable to precipitate gold by reducing Au (III) to Au (0) (Kashefi et al. 2001). The reaction appeared to be enzymatically catalyzed, which was dependent on temperature and the presence of hydrogen (as a specific electron donor). Many Fe (III)-reducing microorganisms were able to reduce the forms of oxidized metals, including radionuclides such as uranium (VI) (Wu et al. 2006) and technetium (VII) and trace metals including arsenic (V), chromium (VI), cobalt (III), manganese (IV), and selenium (VI). Many of these metals and metalloids act as environmental pollutants. Thus, Fe (III)-reducing microorganisms can be used to remove metals from water and waste streams and to immobilize metals in the subsurface environments.

Microbial reduction of metals may play a major role in the formation of metal deposits, which may be especially important in hot environments containing metal-rich waters (Drewniak and Skłodowska 2013). Common products of bacterial iron-reduced nanosized magnetic particles enable early disease detection and accurate

prognosis and personalized treatment, monitoring efficacy of prescribed therapy, or study of cellular interaction in a certain biological environment (Chircov et al. 2019). These particles might be used in different radionuclide therapies, drug delivery, magnetic resonance imaging (MRI) (Yadav and Fulekar 2018), diagnostics, immunoassays, molecular biology, DNA and RNA purification, cell separation and purification, cell adhesion studies, hyperthermia, and magnetic ferrofluids for magnetocaloric pumps.

7.10 Specific Metal and Metal Oxide NPs Appropriate for Wastewater Treatment

The suitability of metal or metal oxide NPs for wastewater treatment is manifested in their antimicrobial and antioxidant characteristics since the wastewater streams are rich in oxygen-consuming pathogenic microbes. The NPs are ideal candidates for reducing the chemical complexity of oxygenated aggregations of wastes, facilitating their smoother discharge and disposal (Chen et al. 2015). Among metals, mostly used NPs are Au, Ag, Cu, and Fe, while in oxides, TiO₂, ZnO, silica, and nano zerovalent iron (NZVI) NPs are preferred (Ruttikay-Nedecky et al. 2017b). To a smaller extent, MgO, CuO, and Al₂O₃ conjugates are also employed. The sole mandate of utilizing bacteria-driven NPs for wastewater treatment relies on the interaction enhancing the ability of NPs. In this reference, it is quite interesting to note that not only NPs can reduce the toxicity of wastewater streams but, sometimes, even the bacterial strains as such or in genetically altered forms are added to wastewater streams. Bacteria possess a number of enzymes which work as catalysts to break down the toxic wastes comprising the wastewater stream. With such abilities, sometimes the supplemented bacterial populations themselves become the sources to break down the toxic metal complexes into their salts which are further reduced to their zerovalent states. In the recent past, several interesting studies have reported the addition of genetically modified organisms (GMOs) into toxic waste streams to reduce their recalcitrant and heavy metal loads. This section selectively discusses bacterially synthesized metal and metal oxide NPs and their potential application in wastewater treatment.

In a very interesting 2013 contribution, Yang et al. have compared the wastewater treatment efficacies of nanoscale ZnO, TiO₂, zerovalent iron, and AgNPs. They discussed that even though a common toxicity reduction mechanism of all these nanomaterials relies on the generation of reactive oxygen species (ROS), the extent and manner of such activities differ on an individual basis. The important factors affecting the antimicrobial activities of these functionally distinct nanomaterials in wastewaters are their chemical properties and stability (Das et al. 2018). Interestingly, the researchers have confirmed that oxygen is essential for ROS generation by nanoscale Ag and iron particles, whereas for ZnO and TiO₂, illumination is needed to elicit the ROS expression. Interestingly, though having similar sizes, ZnO and TiO₂ exhibited greater toxicity than nanoscale Ag and Fe, owing to their oxidizing vulnerability

and water solubility which result in the generation of toxicity-inducing metal ions. Regarding the toxicity of AgNPs, it is well reported that the toxic response is due to Ag^+ formation, which is very sensitively affected by the oxygen concentration of the surroundings.

Under aerobic conditions, the antimicrobial response of AgNPs is due to oxidative dissolution of AgNPs, concomitantly leading to gradual release of Ag ions and the production of ROS (Skandalis et al. 2017). The requirement of oxygen for the toxicity induction of AgNPs is further assured by no Ag^+ formation in the anaerobic environment, predicting a necessity of dissolved oxygen to facilitate oxidative dissolution of nanoscale Ag (Abdal Dayem et al. 2017).

Similarly, Fe has comparatively higher reactivity than Cu and Ag, owing to which nano zerovalent Fe (nZVI) can reduce several chemicals in wastewater streams, such as aromatic nitro compounds, chlorinated solvents, and oxidized heavy metals (Yang et al. 2019). In the absence of oxygen (solution phase), water could oxidize nano zerovalent Fe, forming Fe^{+2} and H_2 gas. However, water causes rapid surface oxidation of nano zerovalent Fe under aerobic conditions, after which dissolved oxygen oxidize ferrous state (Fe^{+2}) to ferric (Fe^{+3}) (Yang et al. 2019). So, in totality, the pH and DO concentration regulated the reduction efficacy of nano zerovalent Fe (Park and Dempsey 2005).

Opposed to Fe and Ag, the nanoscale ZnO and TiO_2 generate ROS after photon absorption (Park and Dempsey 2005). Under illumination, electrons excited by photon absorption diffuse towards the surface and react with O to form superoxide anions (O^{2-}) and consequentially generated holes diffuse to the surface and react with water to form hydroxyl radicals (OH^-) (Liao and Reitberger 2013). A notable aspect is that nanoscale TiO_2 is nearly water-insoluble, whereas ZnO can dissolve in water to a higher extent. So unlike TiO_2 , ZnO on being dissolved in water releases free Zn (as Zn^{+2}) and $\text{Zn}(\text{OH})_2$ which are dominant zinc species at neutral pH conditions and exert a toxic response towards aquatic organisms (Reed et al. 2012). Thus, ZnO and TiO_2 mediate their toxicity-reducing responses in wastewater treatment through the additive association of sunlight, whereas nanoscale Ag and Fe express their toxicities through mutual oxidation and reduction. It seems reasonable here that a particular wastewater composition might be more specifically suited for treatment by ZnO and TiO_2 than Ag and Fe since ionic associations and redox existences are also affected by process temperature and pH. It is of further interest to note here that heterogeneous composition of wastewater streams complicates their detoxification, where one particular material may not be effective to a significant extent. In such situations, combinative approaches like adding microorganisms (preferably in consortium modes) to the wastewater reservoirs or adding two or more antimicrobial agents in simultaneous mode seem to be more effective than conventional technologies. Readers are suggested to refer to the 2016 contribution of Lu et al., where the various problems of nanoscale Ag, Fe, and Zn usage are discussed at length along with their probable resolutions. For example, nanoscale Ag alone often leads to fouling and clogging issues in the aqueous environment, while when AgNPs are attached to filter materials, not only the overall cost of the remediation process moderates but the disinfection efficacy also improves by significant extents (Mourdikoudis

et al. 2018). Likewise, NZVI has been found effective in large-scale removal of contaminants (comprising nitroaromatic compounds, halogenated organic compounds, phenols, organic dyes, heavy metals, inorganic anions, nitrates, metalloids, and radio elements) through simultaneous attributes of reduction, oxidation, adsorption, and precipitation, but the complications of aggregation, oxidation, and separation difficulty restrict the long-range disinfection efficacy of nZVI. As a consequence, several modifications are implemented for better nZVI treatment efficacy that includes surface coating, doping with other metals, conjugation with supports, emulsification, and encapsulation in the matrix (Liang et al. 2014). Studies on these modifications suggest enhanced nZVI reactivity via doping with other metals. Similarly, surface coating and conjugation strategies forbid the aggregation and enhance the nZVI dispersion. The modifications of conjugations and matrix encapsulation ensure an optimum nZVI separation from degraded wastewaters, paving the way for their efficient removal from the treated wastewater streams (Ledakowicz et al. 2001). Similarly, the photocatalytic attributes of TiO_2 are the reasons for its extraordinary suitability in UV-radiation-driven particle separation. With its reasonable price and long-lasting photo-, chemical, and biological stability alongside a large energy band gap (3.2 eV), TiO_2 remains the most sustainable photocatalyst till date (Fujishima et al. 2000). Remarkable photocatalytic efficacy of this material facilitates ROS generation in reasonable time durations, facilitating an efficient removal of contaminants.

The prospect of nanomaterial recovery from the waste stream is definitely a concerning factor for their scale-up suitability in detoxification application (Shan et al. 2009). This challenge can be addressed by careful consideration of the source of these nanomaterials, which critically affects their shape, size, and aggregation stability. For instance, bottom-up approaches offers significantly higher control on their size and shape, owing to which the application stability and suitability would be higher. On the other hand, top-down approaches (mostly used in physical methods of nanomaterial synthesis) are elaborate, utilizing higher energy due to which the end products are less stable and, consequently, more vulnerable towards aggregation.

7.11 Conclusion

Microbial synthesis of nanoparticles has gained tremendous applications in wastewater treatment due to their high surface area to volume ratio, functionalization by biomolecules, etc. Microorganisms have various organic compounds like enzymes, acids, polysaccharides, etc. that act as a reducing agent for metallic nanoparticles, whereas some of biomolecules are also involved in the metal oxide synthesis of nanoparticles. These metallic and metal oxide nanoparticles have adsorption sites on their surface that help in the removal of pollutants from the wastewater. Nano adsorption has several advantages over other pollutant removal process. IONPs not only have diversity in the phases but also are economical, recyclable, and easily

available. Microbially synthesized nanoparticles have huge potential for the green, economical, and biocompatible source of nanoparticles that find applications in the field of environmental cleanup.

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