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Nano-Bioremediation: An Innovative Approach for Remedying Heavy Metals using Fungi

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Abstract

Increasing attention has been received by almost all the toxic heavy metals worldwide as they have caused massive environmental pollution. If living organisms remain exposed to these metals for long time, they can have deleterious health impact on them. However, microbes perform a critical part in the bio-transformation of toxic metals in some less hazardous forms. An insight into the mechanism of metal build-up at molecular level has enormous biotechnological implications for remedifying metal-contaminated sites. Fungi are considered to have an enormous prospect for metal bioleaching and are extensively utilized as the most potential and capable group for the metal resistance. Fungi are recognized to defy and detox metals by numerous mechanisms, by virtue of this property these are a drive to future appliance for metal remediation from soil, and thus bioremediation is measured as a budding technology. One of the approaches that revealed huge potential is established on the biological synthesis of nanoparticles utilizing micro-organisms such as fungi. It is assumed that they cause decline of these metal ions which occurs through enzymatic route, thus create the prospect of establishing a rational fungal-based approach for nanomaterials synthesis with different chemical compositions, which at present is not possible by any other method. Thus, time demands to use such innovative remediation techniques for sustainable environment. The main objective of this review was to analyse the technique of bioremediation as well as nanobioremediation of heavy metals utilizing fungi. Besides, we have also summarized the role of myco-synthesized nanoparticles in different bioprospective applications.

Keywords: Nanotechnology; Heavy metals; Mycoremediation; Nanoparticles; Nanobioremediation; Fungi

Introduction

Owing to rapid industrialization, perilous agricultural practices and increased anthropogenic activities, environmental pollution has moved to unprecedented level in the past few decades. The contaminants that are of prime importance due to their ecological and public health concerns are toxic heavy metals, hydrocarbons, pesticides, nuclear residues and greenhouse gases [1]. Metal contamination of soil is among the great consequences of industrialization [2]. The presence of these persistent toxic and non-biodegradable metals in our surroundings has always been a matter of huge concern [3]. The associated anthropogenic causes have often resulted in environmental pollution. Heavy metals such as Cd, Cu, Hg, Ni, Cr, Zn, Pb etc are significant components of industrial wastes and are leaked in our environment, subsequently polluting the ecosystem [4]. Heavy metal toxicity could prevail for a longer time period in nature, some can even convert less toxic to more toxic forms within certain environment, e.g., mercury, where the normal physiological processes can be damaged and harm human life eventually due to bioaccumulation in food chain. Some metals like Cd and Hg are extremely toxic even at minor concentrations of 0.001 to 0.1 mg/L [5].

Heavy metals, in broad-spectrum use an inhibitory function on micro-organisms, hence blocking critical functional groups, displace important metal ions, modify the active configuration of organic molecules and lastly retort to form toxic compound in cells [6]. Some toxic metals, even at lower concentrations, are very crucial for microbes as they offer essential co-factors for enzymes and metallo-proteins [7]. These heavy metals, being non-biodegradable pollutants, can be modified through absorption, complexation, methylation, and by variations in valency state. These modifications have a great impact on the movability and bioavailability of toxic heavy metals [8]. Usually, the sites that are polluted by these metals prove to be a good source of metal tolerant microbes [9]. Fortunately, the reactivity of metals is significantly affected by microorganisms. Hence, micro-organisms are employed to detoxify some metals and thus avert further metal pollution, a process called bioremediation [10]. Arena of nanotechnology has revolutionized the field of bioremediation to overcome the problems of environmental pollutions. Approaches applied for the monitoring and treatment of contaminants includes control of pollutants, sensing the pollutants and remediation by nanoparticles. Nanoparticles cover the treatment of surface water, soil, groundwater and industrial wastewater contaminated by toxic metal ions, radionuclides, organic and inorganic solutes and also reduce aromatic recalcitrant compounds from soil and air pollution. There is also a scope of enhancing the remediation potential of nanoparticles by manipulating size and geometry. They have given a new hope towards positive sustainable approach for environment and human welfare [11]. However, the occurrence of heavy metals or

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their compounds in the surrounding environment consequently leads to the morphological, cytological and physiological modifications of the microbial consortium. This exerts a critical pressure on the microorganisms and affects their enzymatic activity as well [12]. Microorganisms such as fungi and bacteria procure nutrients through the production of enzymes extracellularly from complex organic compounds. Such enzymes result in hydrolysis of polymers to smaller sub units that are taken up by the cell [13]. It is outlined that owing to heavy metal contamination which affected soil fertility negatively, there was decrease in soil microorganisms along with the suppression in soil enzyme activities [14].

Enzymatic reactions are suppressed by toxic heavy metals in three separate ways [15]:

(a) Complexation of the substrate

(b) Amalgamation with protein-active groups on the enzyme

(c) Reaction with the enzyme-substrate complex.

It is revealed that heavy metals resulted in the inactivation of extracellular enzymes. Mechanistic details showed that some amino acids in enzymes were attached to metals and hence indirectly decreasing the count of micro-organisms responsible for the enzyme production [16]. Soil enzymes and soil microbes share a very close association, and enzymes secreted by some microbes play a part in the movement of soil ecosystems and energy together [17]. Micro-organisms can easily change toxic heavy metals to less-toxic or non-toxic forms. Micro-organisms are recognized to have two-way efficient defense i.e., production of deteriorative enzymes for target containments and resistance against relevant heavy metals. As metal contamination is a grave environmental issue with extreme health impacts, its remediation is fundamental. A number of remediation processes are known such as biosorption, bioremediation, biotransformation, bioaccumulation, bio mineralization and bioleaching which prove to be sustainable and environment friendly [1].

The physical and synthetic techniques for remediation are tedious and costly; henceforth a biological solution gives an elective arrangement of the issue. The procedure by which the contaminants are converted into less harmful forms utilizing various biological agents is called bioremediation [18]. The different areas of molecular biology, microbiology, biochemistry, analytical chemistry, environmental and chemical engineering, among others, have led to many advances in the science of bioremediation [3]. Microorganisms have inherent ability of decomposing wastes and are capable to survive under harsh conditions. Microbes are ubiquitous that dominate in metal-contaminated soil and can by far transform heavy metals into less-toxic forms [19]. The use of microbial structures for metal and metalloid biorecovery and bioprocessing has received greater consideration lately, with renewable energy supplies and sustainable environmental concepts becoming new trends in many industries. Demand for clean and efficient energy production and usage rely on a range of raw materials, of which metals are of essential and strategic importance [20]. Microorganisms eradicate the toxic heavy metals from the soil by means of chemicals for their growth and development. They are able to dissolve heavy metals and reduce or oxidize transition metals. Immobilizing, oxidizing, volatizing, binding and transformation of toxic metals are the various methods by which microorganisms regenerate the ecosystem. With the help of designer microbe approach, and the knowledge of mechanism monitoring activity and growth of microbes in the polluted sites, their metabolic abilities and response to ecological fluctuations, bioremediation can thrive in a particular place [21]. Bioremediation methods more often employ immobilization procedures. Immobilization may be defined as restricting the mobility of the microbial cells or their enzymes with a concurrent conservation of their catalytic functions and viability [22]. Immobilization significantly reduces the cost and improves the effectiveness of bioremediation processes. This method enhances the process of bioremediation in many ways, such as advanced efficacy of pollutant degradation, numerous uses of biocatalysts, reduced cost and better tolerance to high pollutant concentrations [23]. There is a wide diversity of microorganisms (fungi, yeast, algae, bacteria, etc.) which are proficient in uptaking contaminants. Among microorganisms, fungi are acknowledged for their greater ability to produce ample variety of extracellular proteins, enzymes and organic acids etc which ultimately helps in sequestration of metals [24].

Fungi possess the ecological and biochemical capability to decompose environmental toxins and to reduce the threats associated with metals, metalloids and radionuclides, by chemical modifications or by influencing chemical bioavailability. Moreover, the capability of fungi to form extensive mycelial networks, their independence from utilizing pollutants as a growth substrate and the low specificity of their catabolic enzymes make these fungi suitable for the process of bio-remediation [25]. Fungi are omnipresent members of sub-aerial and sub-soil environments, and become a principal consortium in metal-dominant or metal-contaminated habitats [26]. Fungi have developed outstanding bioremediation mechanism that is well known to degrade wide range of toxic substances and compounds, processes known as mycodeterioration [27]. Recent research has shown that the strains isolated from polluted areas have significant potentiality to tolerate such noxious situations. Their biomass may be used as an efficient biosorbent for reduction, elimination and detoxification of industrial effluents. However, these effluents contain increased amount of toxic metals which may enter into animal and human population through the food chain, resulting in many metabolic disorders in the affected person [28]. Hence, it is necessary to exterminate the toxic metals from soil and wastewater using low price technology such as bioremediation [2].

There are many factors which make fungi a better choice for bioremediation than other microorganisms:

a) Contrary to bacteria, fungi do not need continuous water phases for their active dispersal. The hyphae sprout across water-air interfaces and grow into soil pores. Fungal mycelium also assists in the mobility and transport of hydrophobic organic pollutants and nutrients between spatially separated source and sink region [29].

b) Fungi co-metabolize various environmental substances and hence are not dependent on the utilization of such substrates as energy and carbon sources. Toxin degrading enzymes of fungi include numerous extracellular oxidoreductases, chiefly intended to decay ligno-cellulose, in addition to cell-bound enzymes, which allow fungi to act on large number of contaminants [30].

c) Mobilization, immobilization, sorption to cell walls and uptake into fungal cells are the main interactions which take place between fungi and metals, metalloids, radionuclides. After incorporation into cells, such compounds transform chemically and are stored in various portions of the cell or translocated along fungal mycelium [31].

d) The utilization of filamentous fungi is beneficial in such cases for which translocation of crucial factors (water, nutrients, the contaminant itself, etc) is necessary for the detoxification or transformation of environmental compounds [32].

e) Fungal sequestration must be considered for that group of contaminants which are not degraded by bacteria efficiently, including "classical" pollutants like dioxins and heavy metals and substances found in environmental matrices (water, sediments and soil) [33].

f) Fungus is most appropriate for the removal of organic pollutants, metals, volatile organic compounds from air, and the treatment of concentrated organic contaminants in surface soils utilizing enzymes of extracellular origin as an alternative of whole fungal organisms [34].

g) There is inclination towards monitored 'natural attenuation' for the recovery of polluted land, which includes energy and cost-efficient bioremediation schemes. This natural attenuation of soil involves low degree of mechanical intervention that may favour the establishment of filamentous fungi [35].

Fungi and yeast accumulate micro-nutrients like Cu, Mn, Zn and non-nutrient metals such as, Hg, Ni, U, Cd and Cr in amounts greater than the nutritional requirement. The prospective of fungal biomass as biosorbent has been acknowledged for removing the toxic heavy metals and radionuclides from contaminated waste materials. Fungal cell walls and their mechanisms have most important part in the sequestration of heavy metals due to the occurrence of various functional groups i.e., hydroxyl, carboxyl, sulphydryl, phosphate and amino groups which assist them to bind the toxic heavy metals [36]. Fungal biomass can uptake substantial amount of toxic metals from aqueous solution through adsorption or associated process, even with lack of physiological pH, temperature and availability of nutrients [37]. Diverse species of *Aspergillus* have been described as proficient chromium and nickel reducers [38]. Several filamentous fungal species have also been found to be helpful for the biological treatment of the sludge under controlled conditions during operation. Fungi belonging to the genera *Rhizopus* and *Penicillium* have been recognized as possible biomass for removing toxic metals from solutions [39]. It is reported that *Aspergillus oryzae* can help in removing cadmium and copper ions from aqueous solutions [40].

Likewise, It described potential biosorption for Cr and Cd by two filamentous fungi, Rhizopus sp. and *Aspergillus* sp., isolated from metal-polluted agricultural soil [12]. It is assumed that fungi cause decline of the metals that occurs by an enzymatic pathway, thus create the prospect of creating a lucid, fungal-based technique for the formation of nanoparticles over a variety of chemical compositions which simultaneously offers sequestration of heavy metals [41]. Hence, these nanoparticles may be used for biological remediation, which will not only have reduced noxious effect on microbes, but will also improve the activity of microbes of the specific waste material. The science dealing with these nanoparticles is termed as nanotechnology. Nanotechnology is a highly advanced field in science and technology, emerging as a novel trend that will lead in redesigning the future technologies, which is going to change every facet of our lives [42].

Nano Bioremediation

Bioremediation provides an excellent clean-up approach for numerous types of waste, but it has some limitations as well. For instance, bioremediation may not offer a viable strategy at sites with elevated concentrations of pollutants that are toxic to most microbes. These consist of heavy metals and their salts. Further, the innovation in science and technology has improved the standard of living which ultimately contributes to the increase in toxic waste material. Therefore, the removal of contaminants by utilizing current technology is not efficient and effective in cleaning up the ecosystem [43]. To facilitate survival in environments that contain higher concentrations of metals, living organisms have adapted by developing different mechanisms to deal with them. These mechanisms may encompass changing the characteristics of the toxic metal, thus rendering it less-toxic and result in the synthesis of nanoparticles of the concerning metal. Hence, formation of nanoparticles is regarded as the "by-product" of a resistance mechanism against a specific metal, and this can be used as substitute way of producing them [42].

It is explained that the morphology, particle size distribution, specific surface area, surface charge and crystallographic characterization are the important characteristics that help understand the behaviour of the nanoparticles [43]. There are many reasons for different nanomaterials (NMs) to be used in bioremediation; for instance, when the matter is brought to nanoscale, surface area per unit mass of a material increases; thus, a larger amount of the material can come into contact with surrounding materials and this affects the reactivity. NMs show quantum effect; therefore, less activation energy is required to make the chemical reactions feasible Nano bioremediation is an emerging technique proving to be helpful in many fields (Figure 1) [44].

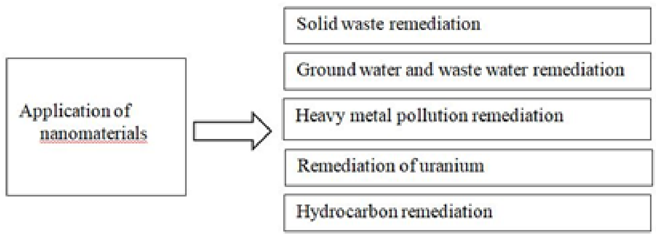

Figure 1: Application of nanomaterials in bioremediation

The capability of NMs to reduce pollution production is in evolution phase and could potentially catalyse the most revolutionary changes in the field of environment in the coming years [45]. Metal nanoparticles have the capability to absorb maximum amount of contaminants and pollutants due to large surface area and high surface energy. They catalyse the reactions in faster rate in comparison to bulk material, thus reducing energy consumption during degradation or helps in stopping produce of pollutants. The nanosized form of particles makes them easy to get into the contaminants, hence promoting in situ remediation rather than ex situ remediation [46]. The capability of the metal nanoparticles to be coated with different ligands and control of surface area to volume ratio by changing the shape of the metal nanoparticles enables the design of sensors with high selectivity, sensitivity, and specificity [47].

Myco-synthesis of metal nanoparticles

Due to the extraordinary optical, photo-electrochemical, electronic and chemical properties of these nanomaterials, there is a massive interest in their design [48]. In different aspects like the fabrication of nanoscale matter and utilizing or understanding their unusual optoelectronic and physicochemical properties, notable advances have been observed. Advanced developments in the association of nanoscale structures and predefined superstructures imply that nanotechnology is going to play a highly decisive role in several important technologies of the new era [11]. It is earning huge significance in certain potential areas like biomedical sciences, catalysis, mechanics, optics, magnetic and energy science. Over different chemical composition and elevated monodispersity, the design or synthesis of such nanomaterials is still demanding in material science. Numerous industrialized techniques that generally utilize atomistic, molecular or particulate processing were used in liquid medium or in vacuum. Most of these approaches were capital intensive and also incompetent in energy and materials use. Thus, there is an ever-increasing requirement to build up clean, non-toxic and environmentally benign production measures [49].

Most commonly used approaches for the production of all of these nano-particles include wet-chemical approaches that are low cost as well as high volume. However, the potential use of such procedures is largely limited in bio-medical applications due to the need of harmful solvents and also due to chemical contamination [50]. Thus, a green and non-toxic procedure is essential for the preparation of these metal nano-particles in wide variety of industries. This can be obtained possibly only by organic approaches. Accordingly, researchers in the synthesis of nanoparticles have moved towards biology for insight. It is well recognized that numerous creatures can offer inorganic materials by intra and extra-cellularly as well [26]. Different biotechnological applications including the remediation of poisonous metals use different micro-organisms like fungi and bacteria, such micro-organisms are now established as potential eco-friendly nanofactories [50]. Depending on the usage of microbes in the bio-synthesis of nanomaterials, some processes created by nature for the manufacturing of inorganic materials on nano and microlength scales contribute to the advancement in comparatively novel and mostly unmapped research area [51]. Use of microbes such as fungi, yeast, actinomycetes and bacteria has been explained for the manufacturing of nano-particles [52]. Recently, fungi have emerged as the most significant route for the bio-synthesis of nanoparticles (Table 1). Fungi have more advantages than other living organisms in many ways (Figure 2).

Fungal species	**Nanoparticles**	**References**
Fusarium oxysporum	CdS	Rai *et al.* (2009)
Aspergillus fumigatus	Ag	Bhainsa *et al.*(2006)
Neurospora crassa	Pt	Sanghi and Verma (2009)
Verticillium sp.	Au	Ramanathan *et al.* (2013)
Penicillium fellutanum	Ag	Venkataraman *et al.*, (2011)
Coriolus versicolor	Ag, Au	Sukumaran (2012)
Aspergillus flavus	Ag, TiO_2	Vigneshwaran (2007)
Aspergillus niger	Ag, Au	Gade *et al.* (2008)
Lichen fungi	Bioactive nanoparticles	Shahi (2003)
Candida albicans	Au	Chauhan *et al.* (2011)
Fusarium semitectum	Au	Venkataraman *et al.*, (2011)
Colletotrichum sp.	Au	Shankar et al. (2003)
Trichoderma asperellum	Ag	Venkataraman *et al.*, (2011)
Trichoderma viride	Ag	Venkataraman *et al.*, (2011)
Trichothecium sp.	Au	Fayaz *et al.* (2010)
Phaenerochaete chrysosporium	Ag	Vigneshwaran *et al.* (2006)
Fusarium solani	CdS	Ingle *et al.* (2009)
Phoma glomerata	Au	Birla *et al.* (2009)

Table 1: Potential fungal isolates used for the biological synthesis of metal nanoparticles

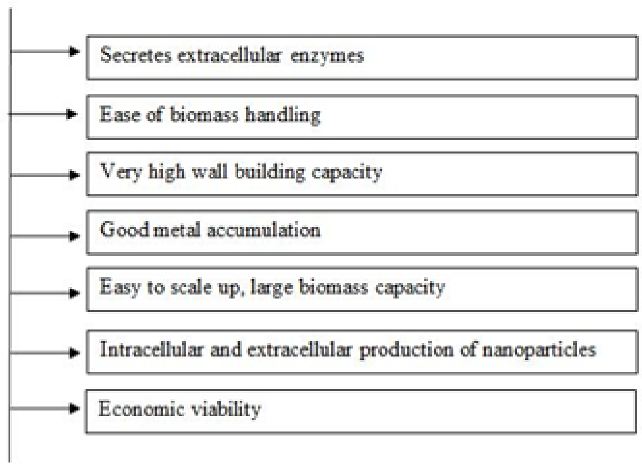

Figure 2: Advantages of fungi for NP production

Fungal mycelia can tolerate high pressure, perturbation and other harsh conditions inside a bio-reactor or reaction chambers than bacteria and other plant materials. Further, fungi are easy to handle and easy for fabrication as well. Fungi grow very fast and there are more extra-cellular secretions composed of reductive proteins that are easy to handle in downstream processing. Also, the nano-particles are produced outside the cell and are deprived of needless cellular components and thus can be used directly in various applications [53]. As *Fusarium oxysporum* and *Verticillium* sp. fungal biomasses were exposed to aq. $AgNO_3$ solution, they produced extracellular and intracellular silver nano-particles respectively [54]. The pace of intracellular particle formation and their size as well, can be controlled to some extent by governing the important reaction parameters like concentration of substrate, exposure time to substrate, temperature and pH [55]. Different attempts were done to control the size and shape of gold nanoparticles that were formed by micro-organisms by changing different growth parameters [56]. Bioreduction of $AuCl_4$ was done by *Verticillium* sp. and ultimately resulted in the creation of gold nanoparticles with good mono dispersity and well-defined dimensions [57]. These observed results have acknowledged that $AuCl_4$ ions were trapped onto the fungal cell surface by their electrostatic interaction with positively charged ends (such as lysine residues) of enzymes which are present in the cell wall of the mycelia. The Au ions were then concentrated by the enzymes within cell wall and resulted in the aggregation and generation of gold nano-particles [58]. However, they were unable to explore the exact mechanism for the production of gold nano-particles. From their study, it can be concluded that fungi could be a better source for large scale production of such nano-particles than plants and bacteria. Since, it is well known that fungi fabricate huge amounts of proteins and thus could have remarkably better yield of nano-particles in the biosynthetic approach. To get the accurate mechanism of nano-particle formation, an in vitro method was used. In this method, the bio-reduction of $AuCl_4$ ions to get gold nanoparticles was done successfully by species specific NADH-dependent reductase, produced by the *Fusarium oxysporum*. For the first time, a novel fungal/enzyme-based in vitro method was reported for the formation of nano-materials. *Fusarium oxysporum* due to its specific properties is also utilized for the manufacturing of extremely stable silver hydrosol [59,60]. Further, it was reported that acidophilic fungus *Verticillium* sp. has huge potential of synthesizing gold and silver nanoparticles upon their incubation with Ag+ and $AuCl_4$ ions respectively [58]. However, a new biological approach was reported for the formation of intra and extra-cellular silver nano-particles by the fungi, *Verticillium* sp. and *Fusarium oxysporum* respectively. This has established an interesting possibility in which the nano-particles can be captured within the biomass as film or formed in solution, both having exciting commercial potential [54]. Also, It is reported that *Aspergillus flavus* lead to the aggregation of Ag nano-particles onto its cell wall surface as incubated with $AgNO_3$ solution [61].

An advanced research on the fabrication of silver nanoparticles (AgNPs) was done wherein they utilized *Aspergillus fumigatus* to synthesize extracellular Ag nano-particles with 5-25 nm size. It has been reported that fungus *Trichoderma reesei* have revealed to fabricate extracellular AgNPs [62,63]. They were capable to fabricate AgNPs utilizing this fungus after 3 days (72 hours), that was remarkably slow than *Fusarium oxysporum* and *A. fumigatus*. But the usage of *T. reesei* has an edge than other fungi in the fabrication of metal nano-particles. As a relatively well studied organism, it could be modified to yield large amount of enzyme, up to 100 g/L which may assist to augment the production rate of nanoparticles in near future. This research by Vahabi and Mansoori was granted a patent in the year 2013 under patent number: US8394421B2. The King Saud University of Saudi Arabia was also granted a patent in the year 2017 for explaining the method of synthesis of silver nanoparticles using fungi (Patent number: US9701552B1).

Despite extracellular production of nano-particles has superiority like lower cost and simpler downstream processing as well intracellular production of nano-particles is equally important [64]. In case of bio-remediation, some heavy metals ions like Pt and Cu are demanded to be eliminated from polluted sites. By utilizing fungi that has the potential to fabricate intracellular nano-particles, it could be too easy to eliminate the fungi along with its amassed metal pollutants from the polluted sample. A remarkable study was conducted on *Coriolus versicolor* (white rot fungus) into the accumulation and production of intracellular silver nanoparticles (AgNPs). The researchers manipulated the reaction parameters and detected that *C. versicolor* had the potential to synthesize silver nanoparticles extracellularly and intracellularly. Hence, the nanoparticle production process is not static and could be modified in accordance to distinctive requirements [65,66]. Over recent times, the importance of gold nano-particles (AuNPs) is increasing but, inspite of this; there are fewer instances of their biological synthesis by fungi than those composed of silver. The minute size of Au nano-particles makes them more reactive, contrasting to the gold in bulk form, making the AuNPs perfect for use as precursors and also as catalysts for electronic applications [67]. Very little is known about the bio-synthesis of Platinum nanoparticles (PtNPs), unlike the most commonly studied metal nanoparticles like AgNPs and AuNPs. An informative study exhibited the formation of PtNPs by fungus *Neurospora crassa*. The intracellular single PtNPs were reported to be synthesized by this fungus, with 4-35 nm diameter and spherical nano-agglomerates of 20-110 nm in diameter. The most interesting thing regarding this study is that they endeavoured to synthesize PtNPs utilizing *N. crassa* extract and then equated the outcome with the PtNPs produced from the *N. crassa* biomass. After the experiment, the sample from the extract included single-crystal nano-agglomerates. An additional survey [68,69] On the fungus *F. oxysporum*, also assured the formation of PtNPs. In case of PtNPs, they were produced both extracellularly and intracellularly, though the quantity synthesized intracellularly was considered to be statistically insignificant. The quantity of extracellular production of PtNPs was stated to be 5.66 mg^{-1} with temperature difference effecting synthesis rates of the PtNPs and even minor pH variation different from the average inhibiting the synthesis of PtNPs. The knowledge of these results is very important to comprehend the effects of environmental factors on nano-particle synthesis as they can assist us optimize the bio-synthesis of metal nanoparticles.

Magnetite (Fe_3O_4) is a very common oxide of iron that having magnetic properties and magnetite NPs (MaNPs) were revealed to be produced by the endophytic fungi *Verticillium* sp. and pathogenic fungus *F. oxysporum* [70]. The utilization of magnetite nanoparticles has been applied extensively in various biomedical applications like magnetic resonance imaging for position sensing and oscillation damping and also in non-medical applications, such as in magnetic recording devices [71,72]. The Bharde group synthesized MaNPs intracellularly, which also means an additional step for the purification of MaNPs if they are meant to be of commercial use. Like bacteria, fungi also have a key drawback when it comes to its safe handling. Some well recognized fungi like *F. oxysporum* are pathogenic in nature and thus can cause a safety hazard [73]. While as, some fungi such as *Trichoderma reesei* and *Trichoderma asperellum* synthesize AgNPs as they are exposed to Ag salts [63]. These fungi have demonstrated to be non-pathogenic in nature and are thus perfect for commercial use [74]. In fact, *T. reesei* have been extensively used already in different sectors such as paper, food, pharmaceuticals, animal feed and textile industries [75].

Method of fungal nanoparticle formation

The two common methodologies used for the biosynthesis of nano-particles involve extracellular mechanism and intracellular mechanism. In extracellular mechanism, the test strains (culture) were grown inside a suitable medium and then incubated in an orbital shaker at 37°C and 150 rpm. After incubation is done, the broth is centrifuged and the obtained supernatant is utilized for the bio-synthesis of nano-particles. The supernatant obtained is then added to a distinct reaction container having suitable metal ions concentration and incubated for about 72 hours. The change in the colour of reaction indicates the existence of nano-particles in the solution. The bio-reduction of metal ions within the solution is observed by analysing the aqueous solution and measuring the absorption spectrum utilizing a UV-Vis spectrophotometer. The uniformity and morphology of these nano-particles is studied by Scanning electron microscopy (SEM) and X-ray diffraction (XRD). However, the interaction of protein with metal nano-particles (AgNPs) is checked by Fourier transform infrared spectroscopy (FTIR) [76] (Figure 3).

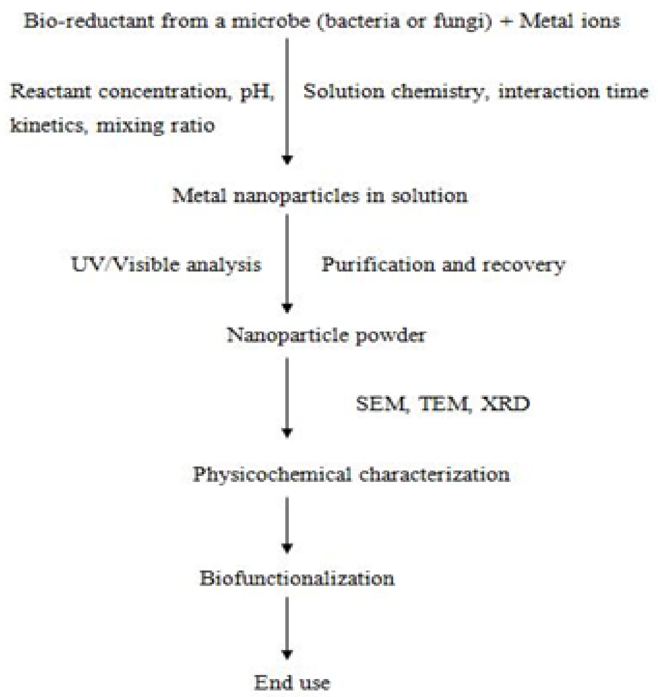

Figure 3: Flowchart outlining the biological synthesis of nanoparticles

In intracellular mechanism, the culture is allowed to grow in appropriate liquid medium and incubated on shaker at optimum temperature. After incubation is done, the flask is allowed to be in steady condition so that the biomass is settled down. The supernatant is then removed and the cells are washed with sterile double distilled water. Again, the flask is allowed to stand for 30 minutes to settle down the biomass and the supernatant is dumped again. The above step is repeated many times. The biomass is centrifugated for 10 min and thus separated from the sterilized double distilled water. The moist biomass is exposed to about 50 ml of sterilized aq. metal solutions of different dilutions and incubated using shaker at appropriate temperature until visible colour change is seen. The colour transforms from pale yellow to brownish which suggests the formation of Ag nano-particles. But if colour changes from pale yellow to pinkish, it suggests the synthesis of Au nano-particles and change from whitish yellow to yellow confirm the synthesis of Manganese and Zinc nano-particles [77]. The morphology of nanoparticles is generally determined by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) [26].

In an initial protein evaluation of Ag nano-particle formation by *Fusarium oxysporum* (5-15 nm), researchers recommended that one of the proteins involved in the reduction of Ag ions, followed by the formation of Ag nano-particles is NADH-dependent reductase. The authors recommend that this reductase probably is not available in every fungus because Ag nano-particles are not produced intra or extracellularly in presence of fungi *Fusarium moniliforme* [78]. In another study, many *Fusarium oxysporum* strains were utilized to synthesize extracellular metal nano-particles in 20-50 nm size. A mechanism of Ag nano-particle formation was proposed in this work. Through the UV-Visible, fluorescence spectra and analysis of enzymatic activity, it was confirmed that decrease of the metal ions took place by a nitrate-dependent reductase and an extracellular shuttle quinone [79]. For the mechanism of production researchers confirmed that Ag nano-particles formed by *F. oxysporum* were stabilized by proteins in fungi [80]. By TEM-electron spectroscopic imaging (ESI) analysis , N and S atoms were detected in the region of AgNPs (1.6 nm) signifying the probable association of these atoms with nanoparticles. The results in silver nanoparticle production with *F. oxysporum*, suggested that nitrate reductase is responsible for the formation of the nano-particles [79,81]. It was confirmed by commercial nitrate reductase discs that nitrate reductase is present in fungal filtrate. Hence, it is inferred that the enzyme NADH-dependent reductase is linked with reduction of Ag ions to Ag nanoparticles in case of fungi. Nitrate reductase was also found in *F. moniliforme*, however, anthraquinone was not present. This outcome was very significant because it showed that not only was the reductase essential but that an electron shuttle was also vital for metal ion reduction, verified the above findings and produced in vitro nanoparticles of silver (10-25 nm) stabilized by a capping peptide utilizing nitrate reductase enzyme purified from *F. oxysporum*, phytochelatin and 4-hydroxyquinoline in presence of a co-factor (NADPH) [79,82]. Without the presence of any of these components (enzyme or phytochelatin or 4-hydroxyquinoline or NADPH), silver nano-particles are not synthesized, suggesting that the presence of all of these molecules is important in the synthesis of metal nanoparticles [46]. Das et al. reported extracellular production of Au nano-particles by *Rhizopus oryzae* and characterized them by FTIR. The spectra after $AuCl_4$ addition in the fungal culture exhibited the presence of amide I, II, and III groups and the absence of carboxyl groups present in mycelia, suggesting the involvement of polypeptides/proteins in reduction of Au ions. The shifting of IR peaks from 1.034 cm^{-1} to 1.025 cm^{-1} proposed the participation of phosphate bonds in this reduction. Thus, gold nanoparticles are synthesized by surface-bound protein molecules that behave as reducing and stabilizing agents. It was observed the extracellular formation of gold nanoparticles of 10-20 nm by the bacterium *Rhodopseudomonas capsulata* and suggested that these nanoparticles were synthesised via an NADH-Dependant Reductase, an enzyme that has been shown in the past to be important in metal biosynthesis (Figure 4) [83].

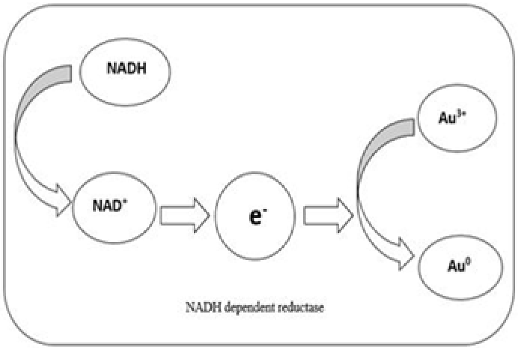

Figure 4: A suggested mechanism for the reduction of gold ions into AuNPs

Characterization of nanoparticles

UV-Visible spectroscopy: The magnitude of peak, wavelength and spectral bandwidth associated with nanoparticles are dependent on size, shape and material composition [84]. Advantages of UV-Visible spectroscopy include quick analysis, high precision and accuracy, suitable for a wide variety of chemical compounds and quantitative and qualitative assessment. The biggest limitation is that it is non-selective for compounds that absorb at the same wavelength.

Transmission Electron Microscopes (TEM): The crystalline sample in TEM interacts with electron beam generally by diffraction instead of absorption. Exfoliation, intercalation and orientation of nanoparticles can also be visualized using a TEM micrograph other than distribution and dispersion [85]. Advantages of TEM are comprehensive, high quality and powerful magnification of element and compound assemblies. However, there are some limitations as well which include difficult sample preparation, artifacts from sample preparation are definitely large and expensive.

Scanning Electron Microscopy (SEM): SEM images the surface of the sample by scanning it with a high energy beam of electrons. When the ray of electrons strikes the surface of the specimen, it interacts with the atoms of sample, signals of secondary electrons, back scattered electrons and X-rays are created that comprise information about sample's surface topography, composition etc [85]. Advantages of SEM include 2-D imaging, easy sample preparation and facility of digital data forms, but improper sample preparation can create muddle between artifacts and actual data.

X-ray diffraction (XRD): X-ray diffraction is used to provide information about crystallite size, crystallinity, orientation of the crystallites and phase composition. It also aids in molecular modeling to govern the structure of the material [85]. Advantages of XRD are simplicity of sample preparation, quick measurement and determine sample purity. Its disadvantages include necessity of homogenous and powdered material, peak overlays lead to indistinct data.

Fourier Transform Infrared Spectroscopy (FTIR): Fourier transform infrared spectroscopy (FTIR) gives data of proteins and other complexes that interact with metal ions. The identification of functional groups helps to determine the reducing agent and the capping agent responsible for synthesis and stability of nanoparticles [86]. FTIR can identify and detect changes in protein secondary structures, but overlapping peaks make it difficult to distinguish and quantify better results with solid components.

Bio-prospective applications of metal nanoparticles: Nanotechnology, an emerging technology can achieve the remediation of toxic heavy metals and trace elements. On comparing with conventional methods, a number of nanoparticles or nanomaterials were found to be very efficient for the elimination of a range of harmful metals from the environment. Nano-bioremediation is the augmentation of microbial activity by nanoparticles to eliminate toxic pollutants [87]. Nano-based technology not only lessens the costs of cleaning up polluted sites at a huge scale, but also decrease the progression time as well. Bio-fabrication of nanoparticles or bifunctional macromolecules used as tools to create or manipulate nano-objects is called "Bionanotechnology" or "nanotechnology through biotechnology". It is reported that extensive physiological diversity, genetic manipulability, small size and restricted culturability allow microbial cells to be suitable producers of nano-structures including natural products such as magnetosomes and polymers, protein constructs or engineered proteins such as tailored metal particles and virus-like proteins (VLP) [88]. Metal chelating polymers need harmful solvents for their fabrication and ultra-filtration as well for their separation. This can be attained by establishing metal binding materials which could be improved by changing the surrounding environment factors such as temperature, pH etc. According to one of the above materials is nano-scale tailored bio-polymers, that are produced via genetic and protein engineering of micro-organisms with controlled size at molecular level. This innovative technique could be a potential tool to handle the increasing dilemma of heavy metal and organic pollutants in the surrounding environment [89]. One of the most prominent applications of nanoparticles in the field of environment is bioremediation and treatment of water through different mechanisms mainly by adsorption of toxic chemicals, heavy metals and other pollutants, removal of pathogens and transformation of toxic into nontoxic or less toxic form [90].

Scientists and researchers are succeeding towards making pollution-free environment via synthesizing special nanostructures. Using silver nano-catalysts leads to inhibit or decline the by-products generated in production of propylene oxide, a common compound used in plastics, paints, detergents, brake fluid, etc. It has been found that most of the iron and iron-containing nanostructures are used as catalyst for the removal of toxicants from organic dyes to clean the ground water through photo-degradation method. In this process, nanoparticles scatter in overall water and degrade the organic dyes. This procedure is cost effective for water purification which is then to be pumped out of the ground. At ambient temperature, nano-crystals can break down the volatile organic compound from air. These are mostly composed of manganese oxide doped with gold nanoparticles. Silver nanoparticles synthesized from *Rhizopus oryzae* fungal species have been used for waste water treatment and adsorption of pesticides [91]. Silver nanoparticles synthesized from fungi are also used in many environmental applications like air disinfection, waste water treatment, ground water treatment and surface disinfections [92].

It is reported that nanoparticles have novel properties such as magnetic, electrical, thermal, optical, chemical and physical than their bulk counterparts [93]. These key features could be utilized for next generation catalysts, biosensors, electronics and antimicrobials [94]. Metallic nanoparticles being an important group of materials are widely studied and have exhibited great diversity in its uses. Their role in drug delivery, magnetic resonance imaging, catalysis, environmental sensing, textile engineering, food sectors and plant disease managements is well known. Several precious metals may be easily recovered from large heap of wastes containing metal salts. This process of producing nanoparticles by a redox process may be employed to produce pure metals. It should also be noted that metal biorecovery utilising microbial approaches can result in production of novel biominerals, which may be of nanoscale dimensions. This offers added value because of the added physicochemical properties that nanoparticles possess [20]. The fungi may therefore be used in metallurgical operations to sequester metal from ores. It can save time and money. Since some of the metal ions are toxic to many microbes, they can be used as a prophylactic to inhibit their growth [95]. There exist important correlations between nanoparticles synthesizing approach and their possible uses. It was reported in numerous studies that silver nanoparticles (Ag NPs) show antibacterial properties [96]. It has been reported that Ag and Au nano-particles have been very potent in suppressing the growth of both Gram-negative and Gram-positive bacteria [97]. In recent times, with the increase in antibiotic resistance and generation of only few new antibiotics, research has emphasised on these antibacterial nano-particles as promising new medical devices. For example, Ag nano-particles were used highly as optical sensors for the fabrication of small molecule adsorbates [98]. While as, Pt nano-particles based catalysts were found to show high reaction for the electro-oxidation of formic acid [99]. It is thus clear that metal nano-particles have great potency in a number of different industries. The necessity to synthesise such nano-particles in a reliable and green approach is becoming more crucial and critical.

Conclusion

From the preceding review, it is evident that fungi have remarkable potential towards the removal of toxic heavy metals and exhibit various resistance strategies towards removing them. The fungi must be widely explored for the bio-remediative ability and though, only a few studies were done in the said area, more inclusive and complete studies are to be carried out. On the other side, fungi are reported for the manufacturing of nanoparticles, it can be projected that in future a bio-synthetic mode of nano-particle synthesis will be largely accepted and fungi can become potential bio-factories for attaining the enormous demand of nano-particles for its various applications. Nanotechnology is revolutionizing every facet of our life. The unique characteristics of nano-particles have made them the particle of our choice in many fields including remediation of environmental pollutants. Environment friendly fabrication of nano-particles coupled with remediation can go together a long way in promoting sustainability. The rapid development in the field of synthetic biology aimed to create predictable, standardised systems and with such new technologies directed towards the synthesis of metallic nano-particles, biogenic nanoparticle samples are likely to become more homogenous and more reproducible, therefore the environmental as well as health issues posed will be assessed more easily and reliably. However, the physiological capability of microbial populations to rectify environments of relevant heterogeneity, size and variability were not sufficiently checked. Successful and effective application of bioremediation approach should direct the heterogenous nature of many polluted waste sites as well as complication of using living organisms. There has been great achievement in overcoming some of the hindrances that have hampered successful application of bio-remediation in the field. Scientists have to make huge attempts to look for organisms which have superior bio-degradation kinetics for a variety of pollutants within broad environmental domains. Nano bioremediation using fungi might immensely contribute to sustainability as it proposes environmental advantages and is economical when equated to other such technologies. The range of applications of myco-synthesized nanoparticles has demonstrated high effectiveness in the degradation of toxins, which provides new opportunities to face environmental challenges, and thus need to be studied further.

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