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Biosynthesis of Nanoparticles using Microorganisms and their Applications

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Abstract: **Materials behave in a different way when they are in their nano sizes. Nanomaterials encompass a high surface-to-volume ratio, a high adsorption capacity, high sensitivity, and reactivity, hence these can be efficiently used for textile wastewater treatment. Numerous studies have shown that nanoparticles can effectively eliminate various pollutants of domestic and industrial effluent. Nanoparticles are conventionally synthesized by physical and chemical methods but these methods use toxic chemicals, complicated procedures, and expensive materials. Presently, enzymatic processes are the most preferred method as they are economically cheap and environmentally sustainable. Microorganisms, break down the metal salts into metal nanoparticles, which can be isolated and used for textile dye degradation. This review epitomizes the various biosynthesized metal and metal oxide nanoparticles by different microorganisms and if they are efficient to degrade diverse textile dyes.**

Index Terms: **Biosynthesis, Metal oxides, Microorganisms, Nanoparticles, Textile dye.**

I. Introduction

Removal of textile dyes from wastewater is a complex and tedious task. The untreated effluent with chemical dyes is mostly disposed of directly into the water streams. It makes the waterbodies unsuitable for domestic and agricultural purposes. Even chemicals also leach out into the surrounding groundwater systems. Textile dyes containing water are treated by physical, chemical, and biological methods. Effluents containing textile dyes are treated with chemical oxidation, coagulation, flocculation, and membrane filtration. Other conventional methods include photochemical degradation, aerobic and anaerobic biological degradation. All these processes can treat textile wastewater although, none of them ensures the complete elimination of toxic dyes from effluent (Dizge et al. 2008).

In the past few years, nanotechnology has grabbed special attention from the scientific community for wastewater treatment. Certain research suggests that nanoparticles can be applied in water purifying technologies, effluent treatment, contaminants absorption or adsorption. Nanomaterials are found to be more reliable as they are smaller, stronger, faster and safer. Their properties are also different from their bulk material. Metal nanoparticles (MtNPs) are continuously being studied to treat textile dyes from wastewater, especially for the decolourisation of the tinted effluent from textile industries. The application of nanoparticles for dye degradation depends on their size, shape, type, and structure. Also, various physical and chemical routes can control the rate of reactivity between nanoparticles and dyes.

Nanoparticles can be synthesized physically, chemically, biologically or by hybrid methods. Conventional physical and chemical methods require toxic chemicals and pollutants in the form of by-products which is an unneglected concern. Although these are more commonly used in the synthesis of nanoparticles as they carry the risk of environmental deterioration. On the other hand, the biologically synthesized nanoparticles are cost-effective and non-toxic. They do not require the use of chemically produced organic solvents, stabilizers, or harmful thermal heat. The processes involved are generally simpler as compared to those which are used during chemical synthesis. Hence, eventually, if the appropriate techniques are utilized properly, biologically synthesized nanoparticles can lower the overall production cost by almost one-tenth as compared to chemically synthesized nanoparticles (Ovais et al. 2018).

Therefore, scientists and researchers are working towards the development of low-cost, non-polluting, safer, reliable, and sustainable methods for nanoparticle synthesis to expand their environmental applications. This goal can be achieved by using microorganisms to synthesize nanoparticles. Various studies have shown the successful formation of metal nanoparticles by microorganisms via metal-ion salts.

This review provides a complete canvas of different methods for the biological synthesis of nanoparticles by the various microorganism, their characterization, and methods to check their degradation potential to treat textile dyes containing wastewater.

II. Biosynthesis of Nanoparticles by microorganisms

In the past two decades, scientists have been showing interest in the interaction of biological species and inorganic molecules. On the other hand, microorganisms have gained vast consideration, as they play a pivotal role in modern sciences, and researchers all around the globe have approved their technological importance. Through different experiments and data, it is proven that many microorganisms can produce inorganic materials. Among the different species of microorganisms, prokaryotic bacteria and fungus is being widely studied for metal nanoparticle biosynthesis, due to the fast growth and easy cultivation of these microorganisms. They have high yield and require specific conditions such as temperature, nutrients, incubation time, light exposure (in some cases), pH, and oxygenation which can be controlled in laboratories. Primarily enzymes secreted by microorganisms, act as biocatalysts for the breakdown of food particles, and are also they can hydrolyze metals. Therefore, enzymes reduce metal ions and produce nanoparticles which are further applied in various fields. There is a broad range of microorganisms that interact with metal ions in different ways. Although many microorganisms have been studied to produce different metals their full potential and diverseness are still unknown. Hence, there is a possibility in near future that microorganisms will be utilized for the green synthesis of metal and metal oxide nanoparticles for on-site applications.

A. Nanoparticle synthesis by bacteria

Numerous studies have reported the formation of nanoparticles by bacteria. They can synthesize the nanoparticles by excreting enzymes on their surface extracellularly or by letting molecules inside the cells to convert metallic molecules into nanoparticles intracellularly. Some of the commonly found bacteria like *Escherichia coli, Pseudomonasaeruginosa, Pseudomonas stutzeri, Salmonella typhus, Staphylococcus currens, Plectonemaboryanum, Vibrio cholerae* etc. have successfully exploited in the nanoparticle formation (Klaus et al. 1999; Nayantara and Kaur 2018). Kalimuthu et al. (2008)isolated *Bacillus licheniformis* from the sewage of municipal wastes. These bacteria were capable of synthesizing silver nanoparticles. The nanoparticle formed generally had a 50 nm average size however for achieving a smaller particle size of around 7nm, electrochemically active biofilm (EAB) was used against a solution of silver nitrate with electron donor as sodium acetate (Kalathil et al. 2011). One study suggests that nicotinamide adenine dinucleotide phosphate (NADPH) reduces silver nitrates to form nano silver particles (Hietzschold et al. 2019). Earlier studies by Du et al. (2007) used Escherichia coli DH5α, Srinath and Rai (2015) used *Enterobacter aerogenes*, Dehnad et al. (2015) used *Arthrobacter nitroguajacolicus*for gold nanoparticle synthesis with single-step process at room temperature. These bacteria reduced Au^3 to Au^0in the presence of NADH and NADH-dependent enzymes. Similarly, different studies have successfully synthesized nanoparticles by using bacteria which are mentioned in the following Table 1.

Table I. List of Bacteria biosynthesizing metallic and metal oxide nanoparticles

Bacterium	Synthesised Nanoparticle	Size	Shape	Temperature	Reference
Proteus vulgaris ATCC-29905	Iron oxide	19.23 nm and 30.51 nm	Spherical	37°C	Majeed et al. 2021
Escherichia coli	Iron oxide	27.7 nm	Spherical	37°C	Hassan Mahmood 2019
Halomonas elongate	Copper (II) oxide	57-79 nm	Rectangular	28°C	Rad et al. 2018
Klebsiella pneumoniae	Silver	40-100 nm	Spherical and Pseudo-spherical	37°C	Sayyid Zghair 2021
Pseudomonas aeruginosa	Silver	30–70 nm	Spherical	27°C	Yang et al. 2020
Serratia nematodiphila	Cadmium sulfide	12 nm	Spherical	35°C	Malarkodi et al. 2013
Bacillus mycoides	Titanium dioxide	40-60 nm	Spherical	37°C	Órdenes-Aenishanslins et al. 2014
Rhodopseudomonas capsulate	Gold	10-20 nm	Spherical	RT	He et al. 2007
*Geobacillus*sp**.**	Gold	5-50 nm	Quasi-hexagonal	65°C	Correa-Llantén et al. 2013
Enterobacter aerogenes	Zinc phosphate	30-35 nm	Worm-like	37°C	Sadeghi-Aghbash et al. 2020

B. Nanoparticle synthesis by fungi

Fungi are often labelled as nano-factories as they can easily produce nanoparticles of desired sizes and can be cultured in bulk (Khan et al. 2017). Numerous tested studies preferred microbial fungi as nanoparticle producers as they can secrete a vast variety of proteins and enzymes other than bacteria. These enzymes are more sustainable, cost-friendly, fast and simpler than using chemicals or other means for nanoparticle synthesis (Alghuthaymi et al. 2015).

To produce biosynthesized iron nanoparticles, Srivastava and Mukhopadhyay (2014) took three manglicolous fungi. The ferrous nanomaterials were characterized as spherical and the particles' diameter lay between around 25 nm, 13 nm and 30 nm for *Trichoderma asperellum*, *Phialemoniopsisocularis* and *Fusarium incarnatum* respectively. The formation of particles was endorsed by Transmission Electron Microscopy (TEM) and Field Emission Scanning Electron Microscopy (FESEM). Affirmation of iron nanoparticles was substantiated by X-Ray

Diffraction (XRD) pattern and Energy-Dispersive X-Ray analysis (EDX). Table 2 represents the fungi synthesized nanoparticles and their characterization.

Table II List of fungus biosynthesizing metal nanoparticles

Fungus	Synthesised Nanoparticle	Size	Shape	Temperature	Reference
Aspergillus versicolor	Silver	5-30 nm	Spherical	35°C	Elgorban et al. 2016
*Aspergillus oryzae*TFR9	Iron	10 nm-24.6 nm	Spherical	28°C	Tarafdar and Raliya 2013
Pochoniachlamydosporium, Aspergillus fumigatus	Magnesium	20 - 60 nm 40-80 nm	ND	25°C	Kaul et al. 2012
Candida albicans	Zinc oxide	15-25 nm	Quasi-spherical	30°C	Shamsuzzaman et al. 2017
Penicillium chrysogenum	Gold	5-100 nm	Spherical, Triangular, Rod	27–29°C	Sheikhloo and Salouti 2011
Alternaria alternata	Iron	9 nm	Cubic	28°C	Mohamed et al. 2015
Aspergillus niger	Copper	5-100 nm	Spherical	30°C	Noor et al. 2020
Rhizopus oryaze	Magnesium oxide	20.38±9.9 nm	Spherical	35°C	Hassan et al. 2021
Fusarium oxisporum	Gold	22 nm	ND	21°C	Thakker et al. 2013
Rhizopus stolonifera	Iron	ND	ND	35°C	Adeleye et al. 2020

III. Textile dye degradation by metal and metal oxide nanoparticles synthesized by microorganisms

It is not unknown that humanity is facing a global environmental crisis today. Not only world is gradually getting deployed of natural resources, but the existing resources are also losing their purity to pollution. Water is categorised as a renewable resource; however, one cannot exploit it limitlessly. The textile sector is infamous for consuming gallons of water during manufacturing and their disposal of effluents containing toxic synthetic dyes, bleaching agents and other chemicals into the water bodies. The textile industry uses reactive, synthetic and disperse dyes for their colour which cover almost 50% of the total world demand for dyes. While dyeing the fabrics only a certain amount of dye is fixed on the fabric while the rest of the unused dye is directly given out in waste streams. This unused dye is the main culprit of Textile pollution. A single industry is aware of the dyes being used in the process on a daily basis, so if they have primary treatment of the chemical dyes, the overall treatment of the collective wastewater is easier. However, if industries do not treat their effluent and get them out into streams directly then different types of dyes get mixed which makes wastewater treatment difficult, costlier and more time-consuming. Many of the known conventional methods for treating textile wastewater have limitations. Some of the advanced treatments are costly or require long contact time which hinders the application of treatment plants in small industries. The dyes also consist of heavy metals such as lead, cadmium, chromium etc. which inhibit the growth of microorganisms, which ultimately leads to their denaturation and weakening of the bioremediation capacity of microbes.

Metal nano substances possess various properties which have allowed researchers to exploit them for numerous environmental applications. Nanoparticles are being used in remediation, environmental sensing, pollution control, wastewater treatment, and energy storage (Pathakoti et al., 2018). Metallic nanoparticles are gaining tremendous impetus in textile dye degradation due to their catalytic nature. It has been also reported that biosynthesized nanoparticles particularly from microorganisms are effective in treating toxic dyes containing wastewater used in textile industries.

Each nanoparticle has a varied shape and unique nature and therefore has different mechanisms to treat dyes. In one of the studies by Ghaedi et al. (2012) the activated carbon-loaded silver nanoparticles were used against methylene blue. Around 71mg of methylene blue was treated by adsorption. Another study by Baruah et al. (2018) used gold nanoparticles to treat Methyl Orange and Rhodamine B by reductive degradation. The process suggests that the sunlight excites the electrons in gold atoms from the low-energy band to the high-energy valence conduction band and these electrons are transferred to the dye molecule which leads to their degradation and subsequent mineralization. The zero-valent copper nanoparticles loaded on filter paper-chitosan-titanium oxide catalytically degraded Bromocresol Green, Rhodamine B, Methyl Orange, and Eriochrome Black T in the presence of $NaBH_4$ by reducing RhB with $NaBH_4$. This resulted in high decolorization efficiency (Alani et al., 2021). Various biosynthesized nanoparticles for degradation of textile dyes are discussed as follows-

A. Ferrous and Ferrous oxide

Ferrous and ferrous oxide nanoparticles are also known as magnetic nanoparticles. They even show super paramagnetism in smaller nanoparticles. An iron nanoparticle is more reactive than its bulk materials and hence highly unstable. Iron combines with oxygen to form iron oxide. Iron oxide nanoparticles are more popularly used in various environmental applications.

Hammad and Asaad (2021) compared biologically and chemically synthesized ferrous nanoparticles in terms of their morphologies and efficiency. Biologically synthesized ferrous oxide nanoparticles were obtained from water extract of *Chlorella vulgaris* microalga.

Both types of nanoparticles were tested for their capabilities in adsorbing dose of 0.1g of methylene blue dye. It was observed that the removal percentage in case of biosynthesized iron nanoparticles was 99% after 60 minutes and for chemically synthesized iron nanoparticles was 94% after 90 minutes. The formed nanoparticles were characterized by Brunauer–Emmett–Teller (BET) and X-Ray Diffraction (XRD). The size, as well as shape of these particles, were affirmed with High-resolution Transmission Electron Microscopy (TEM) where it was found that biologically synthesized nanoparticles were slightly smaller than chemically synthesized nanoparticles. It was suggested that both nanoparticles had a similar recovery rate hence, both can be reused for dye removal applications.

In another research, ferrous nanoparticles were produced using *Penicillium oxalicum* (fungi) which was isolated from *Tecomellaundulata* (Desert Teak). Biosynthesized ferrous nanoparticles were confirmed as nanoparticles under UV-VIS absorption spectrum had adsorption at 300 nm and Fourier Transform Infrared Spectroscopy (FTIR) spectrum showed absorbance peaks on 34307 mm to 4668mm. Scanning Electron Microscope (SEM) images depicted that those particles were rounded having a size of around 140 nm and atomic force microscope (AFM) showed a width of 100nm and height of 3.9 nm. The ferrous nanoparticles combined with H_2O_2 decolorized methylene blue dye for up to 99.17% in six hours (Mathur et al. 2021).

B. Zinc oxide

In a comparative study by Siddique et al. (2021) efficacy of biosynthetically formed zinc oxide and chemically synthesized zinc oxide were tested. Their study proposed that biogenic zinc oxide is a potential eco-friendly solution for harmful chemical dyes present in textile wastewater. The bacterial species *Pseudochrobactrum*sp. C5 synthesized zinc oxide nanoparticles biologically. The particle ranging from 90nm to 110nm were able to decolorize methanol blue and reactive black-5 for more than 90%. These nanoparticles also managed to enhance the physio-chemical properties of effluent with a high amount of reactive black- 5 and reactive red- 120 dyes.

Cultural filtrates of *Aspergillus niger* can produce ZnO nanoparticles, which can degrade up to 90% of Bismarck brown dye (Kalpana et al. 2018).

C. Copper

Noman et al. (2020) report on the formation of Cu nanomaterials by *Escherichia* sp. SINT7 is a Cu-resistant bacterial strain. Spherical crystalline-shaped nanoparticles having the size of 28.55 nm were tested against azo dyes at two different dye concentrations. The particles were left with the dyes for five hours in the presence of sunlight. At 25mgL^{-1}, copper nanoparticles decolored congo red, malachite green, direct blue-1, and reactive black-5 to around 97%, 90%, 88% and 83% respectively and at 100 mgL^{-1} dye concentration, the decolorization of the dyes was found to be 83%, 31%, 62% and 77% respectively. These copper nanoparticles were also able to improve the quality of textile effluent in terms of physio-chemical parameters of wastewater.

Copper carbonate nanoparticles can be synthesized by fungal culture supernatant of *Neurospora crassa*. These biogenic copper carbonate nanoparticles degraded methyl red dye effectively. The biogenic copper carbonate nanoparticles were able to remove Cr(VI) (Liu et al. 2021).

D. Silver

Marinospirillum alkaliphilum bacteria were isolated from a textile industry effluent and were used to synthesize Ag nanomaterials. The Ag nanoparticles were cubic and the size of the particles ranged between 30–70nm. The biosynthesized silver nanoparticles effectively decolored 100% of disperse blue 183 (Nazari and Jookar Kashi 2021).

Bacillus marisflavi TEZ7 was able to synthesize Ag nanoparticles. Biogenic silver nanoparticles in the presence of sunlight degraded 100 mg/L concentrated direct blue-1, methyl red and reactive black-5 dyes, ranging from 54% to 97% in five hours. Biosynthetic silver nanoparticles reduced the pH, electrical conductivity, chlorides, and other physiochemical parameters, improving overall effluent quality. Also, six different secondary products were formed after the dye degradation out of which none of them were found to have toxic effects on rice plants according to the phototoxicity experiment (Ahmed et al. 2020).

In one study, the methyl violet dye collected from industry was tested against biosynthesized silver nanoparticles by *Bacillus amyloliquefaciens*. The microbially synthesized Ag nanoparticles rapidly degraded methyl violet to up to 85% within 20 minutes (Jishma et al. 2018).

E. Gold

Different researches support the degradation of textile dyes by gold nanoparticles. Hence, *Bacillus marisflavi* YCIS MN 5 was used to produce Au nanoparticles extracellularly. The biologically formed gold nanoparticles reduced congo red and methylene blue showcasing their outstanding catalytic activity against textile dyes (Nadaf and Kanase 2019).

F. Others

Bacillus amyloliquefaciens biosynthesized TiO_2 nanoparticles are also found to possess photocatalytic efficiency for the degeneration of colored pigments used in textile industries. It has been seen that Ag, Zn, La, and Pt doping can increase the catalytic activity of titanium dioxide nanomaterials. The highest potential of up to 90.98% was shown by platinum doped TiO_2 in RR31 degradation as compared to undoped which was 75.83% (Khan and Fulekar 2016).

SnO_2 nanoparticles were biosynthesized using the bacterium *Erwinia herbicola.* The nanoparticles of SnO_2 acted as photocatalysts and exhibited excellent degradation of organic dyes of methyl orange (94%), methylene blue (~93%) and Erichrome black T (~98%) in light (Srivastava and Mukhopadhyay 2014).

In a study, supernatant of culture broth of *Alcaligenes aquatilis* was used to produce nanocomposite particles of $Ag_2O/AgO-TiO_2$. These nanoparticles were rounded and measured 39.6 nm in size. $Ag_2O/AgO-TiO_2$ nanoparticles degraded around 96% of 100 ppm Reactive Blue (RB220) under visible light irradiation. The nanocomposites were also able to degrade Acid yellow 17 and Methyl orange which categorize under azo dyes (Kulal and Shetty 2021).

IV. Challenges and limitations of MtNPs (Metal nanoparticles) synthesis by microorganisms for textile dye removal

Biosynthesized nanomaterials have come out as an excellent medium for textile dye degradation as they have a unique shape, size, and physical and chemical properties. Microorganisms produce enzymes that are able to form metal nanoparticles as they reduce metal ions to respective nanoparticles as they possess a metal-resistance mechanism. Hence, to produce nanoparticles from a microbial source, one should select the microbial species carefully. The selected candidates should have a high growth rate and high enzyme production. They should be able to survive in high metallic concentrations. Another challenging factor is controlling the morphology of the biogenic metal nanocomposites. Certain studies support that microbially synthesized nanoparticles have biological capping agents which act as a protective covering to the particles offering higher stability. Morphological constrain can also be achieved by changing the concentration and type of biomolecules used, their contact time, the temperature, pH, or other factors like the type of metalic salt and its concentration. It has also been found that molecules present in the growth media of microorganisms tend to involve in the production of nanoparticles, hence one cannot know the full potential of microorganisms to produce desired metal nanoparticles.

Various studies have proved that photocatalysis is an important process in dye effluent treatment. Apparently, biologically synthesized nanoparticles in combination with light has improved degradation intensity and take lesser time when compared to degradation occurring by the same particles without light. This happens as the irradiation excites valence electrons to the conduction band. Most of the studies supporting photocatalysis are *in-vitro*, hencemore studies should be encouraged to apply them on-site. Also, one should be cautious about the toxicity of possible secondary or tertiary bi-products resulting from the degradation of dyes.

The biogenic Ag nanoparticles are considered toxic if left in the wastewater stream without treatment. In a study, over half of the number of *Artemia* (Brine shrimp) was killed using 1 ppm of silver nanoparticles within 24 hours which proved that nanoparticles in such a small concentration can be highly toxic to some species (Wypij et al. 2018).

V. Conclusion and Future Approach

Various studies have successfully used microbiologically synthesized nanoparticles for textile dye degradation. Commonly observed that bacterial and fungal species can synthesize metal and metal oxide nanoparticles through the extracellular and intracellular processes. Different researches also suggest that with different pH, temperature, concentration, enzymes, catalysts, etc. one can enhance the shape, size, and functionality of the nanoparticles. These nanoparticles are tested against different textile dyes which are found to be cancerous, toxic, non-biodegradable, and mutagenic in nature. Different studies have found that textile dye decolorization can be seen for up to more than 90% in just 5 minutes after the dye is in contact with nanoparticles. Nanoparticles can significantly remove dyes from the effluent by dye degradation, decolorization, absorption, or adsorption. Not only dye decolorization but it is also found in certain studies that these nanoparticles have been helpful in reducing COD, TDS, EC and pH hence, improving the overall quality of wastewater. Biosynthesized nanomaterials have been examined and are proven to be effective for the removal of dyes *in-vitro*. Biosynthesized ensures wide scope for textile dye removal however, further studies should be done to apply this technique on-site.

No standard procedure was provided for the synthesis of biogenic nanoparticles. There have been an ample number of studies that have successfully used various microbes for the formation of metal and metal oxide nanoparticles. Hence, an elaborated study for the synthesis of desired nanoparticles from microorganisms would be very important. Different microorganisms secrete different enzymes, have different natures, need a different environment and therefore particle synthesis may vary with the selection of microorganism and the kind of nanoparticle one desires to synthesize. Overall, treating textile dyes with biologically synthesized metal nanoparticles is effective, cheap, sustainable, and eco-friendly. With the degradation of toxic dyes, these particles improve the quality of effluents. Certain nanoparticles such as silver, copper, etc, can also eliminate pathogenic microorganisms from waste effluents. Hence, considering the tremendous potential of microbiologically synthesized nanoparticles, in reducing the environmental pollution they should be produced in large quantities for the treatment of waste effluents from the industries.

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