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Sustainable Nano-Bioremediation Approaches for the Treatment of Polluted Soils

Irrinki Hemalatha*, Dakamari Harika* and Manoj Kumar Karnena†**
*Department of Mathematics, DADI Institute of Engineering and Technology, Visakhapatnam, India
**Department of Environmental Science, GITAM Institute of Science, GITAM (Deemed to be) University, Visakhapatnam, India
†Corresponding author: Manoj Kumar Karnena; manojkumarenviron@gmail.com

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ABSTRACT

Due to the widespread adoption of conventional approaches for the remediation of contaminated soils, these techniques have become more well-known in the literature. However, these methods have both advantages and disadvantages. Integrating traditional degradation technologies with Nano-technology might be the right solution for removing toxicants from the environment to overcome these problems. Nano bioremediation is a new technique that has gained prominence in recent years among many researchers worldwide. These techniques aim to remove the contaminants' concentration and minimize their impacts on the environment. The integrated approaches benefit bioremediation and nanotechnology to remove the pollutants more efficiently within less time in an eco-friendly manner than individual processes. The current review provides insights into nanotechnology and different kinds of nanomaterials that have been reported in eliminating pollutants from the environment. Further, the mechanism and challenges with nano bioremediation were explained in detail.

INTRODUCTION

Nanotechnology in recent years has been gaining much prominence among many researchers owing to its advantages in allied fields of science. Feynman introduced nanotechnology concepts in 1952, and these technologies have become one of the top research areas in science and technology (Yadav et al. 2017). Rapid modernization and industrialization increased the unsustainable pollution load on the environment (Singh et al. 2020). Noxious pollutants in the background are growing at an alarming rate and degrading the quality of the ecosystem, which leads to the deterioration of human health. In India, increasing pollution is the third most global trend, as reported by the outlook of Global Agenda 2015 (Councils 2015, Vara & Karnena 2021); thus, many research groups have proposed many techniques for remediating the contaminated site with pollutants in a larger scale. Nevertheless, these methods have certain limitations and have not gained much importance in their widespread application as they are expensive in operation, and maintenance, require high energy, etc. The application of nanotechnology was increased in several sectors of our day-to-day life over the last few decades, moreover, in environmental remediation. The existing literature on nanotechnology clearly shows that it will enhance the capacities of remediation technologies and many challenges competently (Chauhan et al. 2022). In recent times, remediation technologies with sustainability have gained much importance. It mainly focuses on reducing the toxic pollutant concentrations to safe levels without adding additional environmental impacts. Recent advancements are combined with these technologies to form a single system, which can be a proper solution to remediate the contaminant site economically within a lesser period in an efficient manner. Amongst the restoration methods, bioremediation is one such method that is competent and useful in remediating contaminated sites in an eco-friendly and economical way. Microorganisms are used in bioremediation techniques to remove the pollutants in soils and water (Galdames et al. 2020, Ramezani et al. 2021, Singh et al. 2020). Over the physicochemical methods, bioremediation has several advantages like cost-effectiveness, specificity, minimal energy requirement, etc. These methods have disadvantages, i.e., degrading the toxic compounds or recalcitrant chemicals in the soils takes months to a year. These technologies are limited if the contaminated sites have excess concentrations of harmful pollutants (Kahraman et al. 2022, Konni et al. 2021); However, integrating these methods with other methods might be an excellent solution to overcome these problems. Nano-bioremediation is a distinctive method that has gained prominence among several researchers in recent years; Nano-remediation has the added advantages of nanotechnology in combination with the benefits of bioremediation methods.

The current review provides insights into nanotechnology and different types of nanomaterials that have been reported in eliminating pollutants from the environment, followed by a detailed account of techniques in nano-bioremediation and their applications.

DEFINITION OF NANOTECHNOLOGY AND NANOPARTICLES

The utilization of nanoparticles might be seen in all the science fields, for instance, agriculture, textiles, medicines, engineering, etc. "National Nanotechnology Initiative (NNI) of USA defined nanotechnology as the understanding and controlling matter at dimensions between approximately 1–100 nanometres, where unique phenomena enable novel nanotechnology applications." Nanotechnology includes measuring, imaging, modeling, and manipulating the matter at the nanoscale (Fig. 1). In recent years, the utilization of nanotechnology in removing pollutants from the environment gained much attention from many researchers due to the size of the particles having high surface-volume ratios; due to these salient features, it gained flexibility for the application in both ex-situ and in-situ conditions.

These technologies generally deal with the nanoparticles having a dimension/range of 1-100 nm, and these particles form functionalized network systems that might be used to perform a function; thus, these features enabled these technological applications to be more suitable in the different scientific fields like purification of water, medicinal, packing industries, etc. The nano-sized particles might be developed in various shapes like spherical, rods, triangular, cubes, etc., and based on their forms (Fig 2), these particles are called "nanospheres, nano-rods", etc. (Wu et al. 2016); nanomaterials structures can be arranged based on their dimensions; most of the nanomaterials are with zero-dimensional (e.g. Fullerances); one-dimensional (e.g. nanowires); or two-dimensional (e.g. nanodisks) (Darwesh et al. 2021).

SYNTHESIS OF NANOPARTICLES AND THEIR CHARACTERIZATION

Nanoparticle synthesis is done by two approaches that are "top-down and bottom-up". In the top-down approach, larger particles are broken down into nanoscale-sized particles with the help of ball milling, laser ablation techniques, etc. In contrast, smaller atoms are mongrelized in the bottom-up approach and form congregates further. These substances will form nanoparticles (Karnena et al. 2020). Chemical reduction, co-precipitation, etc., are examples of the bottom-up approach method. The nanoparticle synthesis methods might be classified as "physical, chemical and biological methods". Fig. 3 depicts the different processes for the synthesis of nanoparticles that fall into "physical, chemical and biological methods". After synthesizing nanoparticles, there is a need

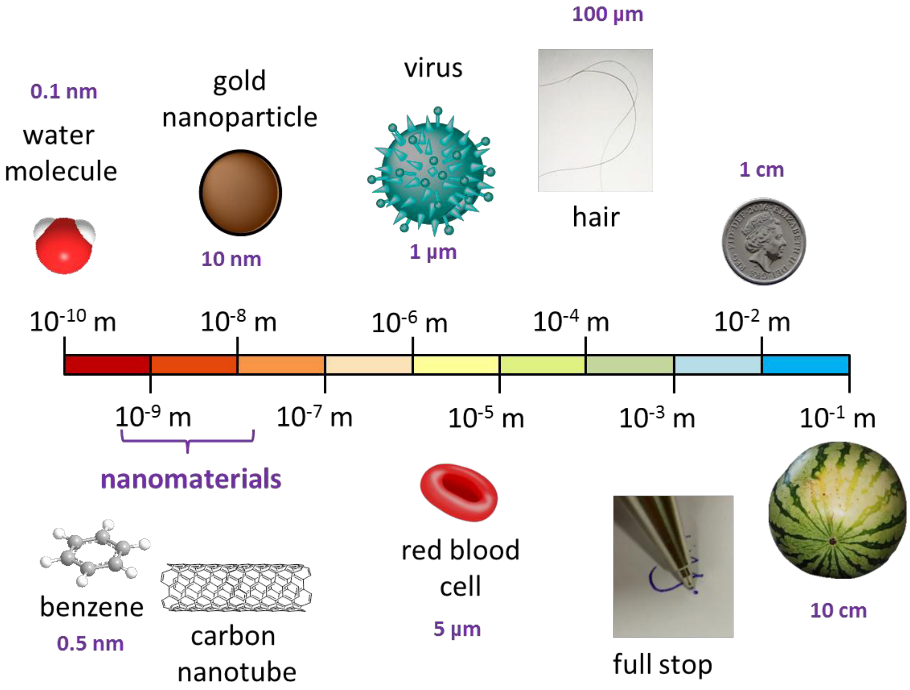


Fig 1: Nanotechnology and Nanoscale science.

to identify their physiology, morphology nature, etc. The nanoparticle characterization might be done via numerous instrumental techniques (Souza et al. 2020, Zhou et al. 2022, Singh et al. 2020).

ENVIRONMENTAL REMEDIATION

Novel technologies are required to decrease environmental pollutants as pollution loads are alarming. Even though other technologies are available for remediating the pollutants, nanotechnology becomes more prominent as it has more significant advantages than the other technologies in removing the contaminants with greater efficacy in a lesser duration of time, which is more economically viable. The different types of nanomaterials were utilized to remove the pollutants from the environmental systems; The nanomaterials are classified as “nanofibers, nanoshells, nanoclusters, nanotubes nanocomposites” based on their composition and size. Nanomaterials showed their efficiency in removing the noxious pollutants from the soil, water, and sediments. Nanofibers eliminated the toxic impurities and showed their efficiency in treatment (Fig. 4). Qi et al. (2014) reported

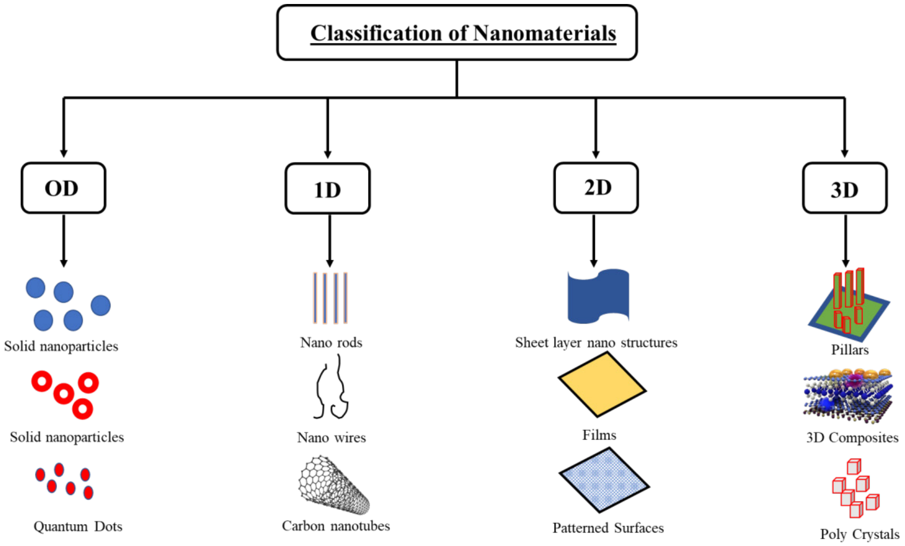


Fig 2: Nanomaterials based on the Size.

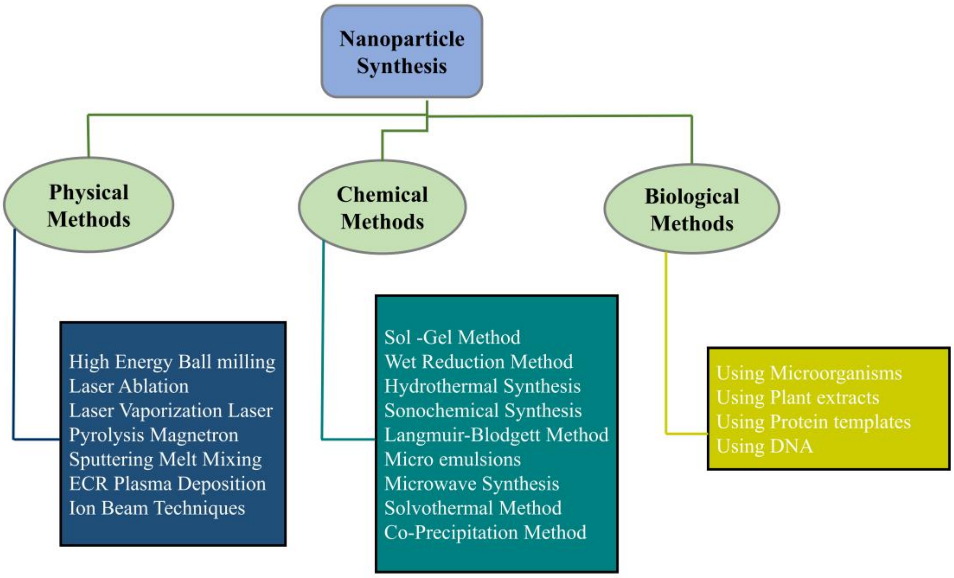
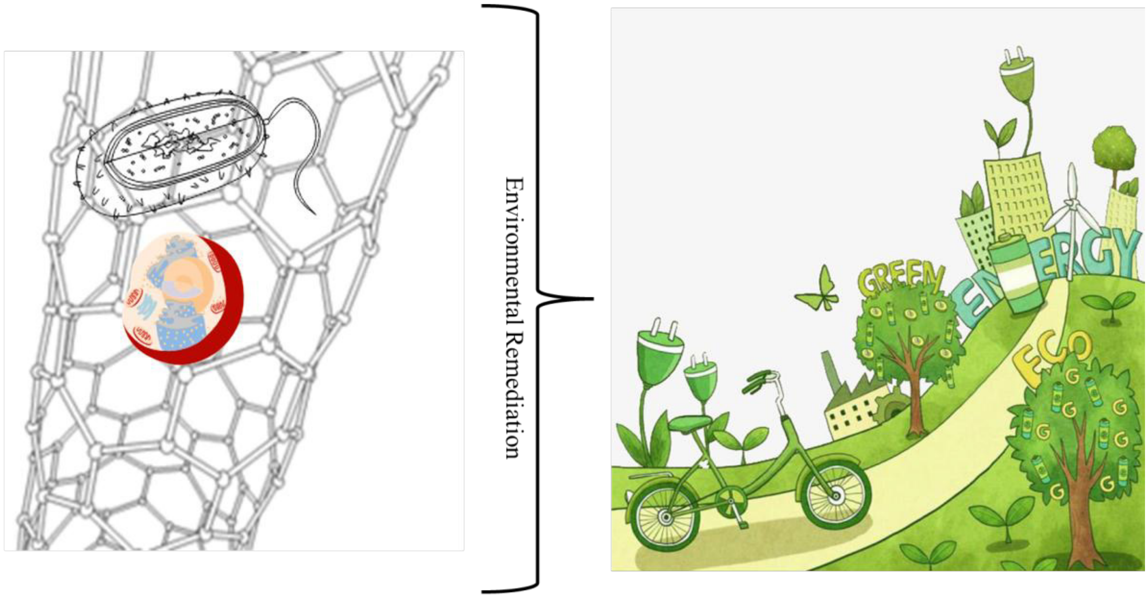


Fig. 3: Process used for nanoparticle synthesis (Redrawn with permission from Singh et al. 2020).

that electrospun nanofibres made up of nylon 6 removed the estrogen from the aqueous medium. It can be used for seven more cycles for removing the other pollutants. Barrocas et al. (2017) reported that nanofibers made up of titanium could degrade the phenols up to 96%. Nanoshells are spherical and have a dielectric core with thin metallic shells; primarily, silver nanoshells are utilized to degrade the organic dye in industrial effluents. Nano-based materials like nanocomposites and nanoclusters also showed their efficacy in treating environmental pollutants. Hussain et al. (2017) reported that nanocomposites degraded the nonylphenols with 96.2% efficiency with a dosage of 40 $mg.L^{-1}$ in 2 h. Sarkar et al. (2018) reported that nanocomposites or nanostructures made of graphite/silica have greater efficacy in eliminating heavy metals. For the remediation of noxious pollutants, nanoparticles are significant as they can be used in both ex-situ and in-situ conditions; In the ex-situ remediation, soil and water that are contaminated with hazardous pollutants are bought to the remediating units and remediated with the nanoparticles to remove its toxicity; In contrast, in situ remediations, the nanomaterials are introduced directly to the sites or injected to PRBs (Permeable reactive barriers) of the contaminated sites or as it treats the toxicants and eliminates them from the areas (Qian et al. 2020). Zero valent iron oxide nanomaterials have shown more extraordinary abilities to reduce pollutants and effectively remediate the soil and groundwater via direct or PRBs injection (Oh et al. 2001). For instance, zerovalent iron of nanoscale was injected into the soils of metal fabrications in Czech Republic industrial areas; It achieved more than 50% removal efficiencies in treating the sites contaminated with the ethylenes and chlorides within 180 days (Raja & Husen 2020, Shahi et al. 2021). Ahn et al. (2016), reported that the aquifers polluted with trichloroethylene are treated with the zerovalent iron nanoparticles and achieved removal efficiencies up to 96 % without releasing any secondary pollutants like chlorinated compounds; and found that these nanoparticles can be utilized many times after five months (Singh et al. 2020).

Sakulchaicharoen et al. (2010) found that nanoparticles have a greater tendency to accumulate, which may lead to fast oxidation. To overcome this problem, the nanoparticles must be coated with stabilizers which increase the stability and reduce the accumulation of nanoparticles; Coating the surfaces of the nanoparticles increases the adsorption capacities and reduce nanoparticle accumulation. Rashid et al. (2017) showed that nanoparticles coated with humic acid would remove the phosphate content efficiently from the water. Ekka et al. (2019) reported that silica nanoparticles coated with titanium degrade the safranin dyes up to 93 % within optimal conditions. Guo et al. (2016) showed that gold nanoparticles could be used for six cycles in removing pollutants from the environment with an efficacy of 90%. Table 1 shows the nanomaterials that have been widely utilized for eliminating contaminants.



Integration of microbes with nano materials

Pollution Free Environment

Fig. 4: Integration of microbes with nanomaterials for environmental remediation.

NANOBIOREMEDIAITON

Nanobioremediation is a combined technology comprised of nanotechnology and bioremediation; this integration helps remediate the pollutants with greater efficacy in a lesser time in an eco-friendly manner. Drawbacks of the individual technologies for the treatment of contaminants can be overcome by these integrated approaches to achieve better results in remediation. For example, combining zerovalent iron oxide nanoparticles with the strains of the microbes remediated

Table 1: Some widely used nanomaterials for removing pollutants (Singh et al. 2020).

S.no	Nanoparticles used	Pollutant	Observations	**References**
1.	Activated Carbon	Copper and sulfates	The hydrophilicity of activated carbon nanoparticles increased by the surface area of nanoparticles and enhanced the removal efficiencies.	Hosseini et al. (2018)
2.	Aluminum	Arsenate	At optimum pH and temperatures, aluminum nanoparticles adsorb arsenate efficiently.	Prabhakar and Samadder (2018)
3.	Caesium oxide	Cadmium, Lead, Hexavalent Chromium	These nanoparticles effectively remove the heavy metal ions at pH 5-7.	Contreras et al. (2015)
4.	Copper oxide	Arsenic	The copper oxide nanoparticles adsorbed arsenic metal ions from the aqueous solutions and proved that these nanoparticles could be applied for potential applications.	Reddy et al. (2013)
5.	Hematite	Carbatrol	Adsorption efficacy increased with time; 90% of removal was observed after 120 minutes.	Rajendran and Sen (2018)
6.	Iron/Nickel (Bimetallic)	Tetracycline	During aging, the efficacy of bimetallic nanoparticles is decreased in degrading the tetracycline with time. The aging product in these experiments is magnetite/maghemite.	Dong et al. (2018)
7.	Maghemite-PC-MAs	Polyaromatic Aromatic Hydrocarbons and metal toxicants	Removal efficiencies were found to be more significant, 85%.	Guo et al. (2016)
8.	Manganese oxide	Estradiol	Estrogens in the soil were removed with an efficacy of 88% with these nanoparticles; a decrease in the velocity of injecting nanoparticles into the contaminated soils enhanced the degradation capacities.	Han et al. (2017)
9.	Modified Cetyltrimethylammonium bromide	Hexavalent Chromium	Iron-modified Cetyltrimethylammonium bromide nanoparticles removed hexavalent chromium efficiently.	Elfeky et al. (2017)
10.	Palladium	Pentachlorobiphenyl	PCBs in the soils were removed by the palladium nanoparticles doped with the CO_2 fluid (supercritical) at 200 atm.	Wang and Chiu (2009)
11.	Polystyrene	Estrone	Nanoparticles of polystyrene were low compared to nanofiltration, and removal efficiency was only 40%.	Akanyeti et al. (2017)
12.	Silver doped graphene oxide	Phenols, Atrazines, Bis-phenols	The silver-doped graphene oxide nanoparticles degraded the toxic organic compound under visible light during photocatalytic degradation, promoted oxidative degradation, and reduced contaminants.	Bhunia and Jana (2014)
13.	Titanium dioxide	Cadmium	Cadmium severely affects the lungs are removed effectively by titanium dioxide nanoparticles.	Zand et al. (2020)
14.	Titanium oxide	Endocrine-disrupting hormones	It was observed that the titanium oxide nanoparticles degrade the Endocrine-disrupting hormones; however, the larger size of the EDC particles are not degraded fastly.	Czech and Rubinowska (2013)
15.	Zero Valent Iron	Copper, Lead, Antimony	These experiments observed the selective removal efficacy of metals like copper, antimony, and lead in the soils.	Boente et al. (2018)
16.	Zerovalent iron	Lead	Plumbism is a disease caused by lead, and the zero-valent nanoparticles remove these contaminants.	Cao et al. (2018)
17.	Zerovalent iron	Cadmium	Cadmium severely affects the lungs are removed effectively by zero-valent nanoparticles.	Cao et al. (2018)
18.	Zinc oxide	Benzophenones	Endocrine-disrupting hormones (benzophenones) are degraded efficiently by zinc oxide nanoparticles.	Rajesha et al. (2017)

the pollutants more efficiently, and Iron oxide nanoparticles obliterated the aliphatic hydrocarbons.

Mechanism of Nanobioremediation

The characteristics of the nanomaterials lead to enhancing the degradation of the contaminants in the soil. Due to their larger surface areas, the nanomaterials react faster with pollutants. The particle size of the nanomaterials allowed these materials to enter into the pores of the sediments in the soil, enhancing the contact with the contaminants-the nanoparticles exhibit Brownian movements due to gravity. The flow of the ground might be sufficient to transport the particles. Nanoparticles remain in the soil and facilitate the in-situ treatment. Once the nanoparticles contact the pollutant, they degrade them by various mechanisms. The microbial integration with the nanoparticles enables the adsorption and degradation of the contaminants at a greater level. The enzymes of the microbes even dissolve the pollutants to make them available to the nanoparticles for degradation. Figure 5 depicts the pictorial representation of the pollutant degradation in the contaminated soil.

Further from the literature, it is understood that chlorinated aliphatic hydrocarbons are removed by Koenig et al. 2016 by integrating technologies and maintaining appropriate dosages; further suggesting that the minerals can produce zerovalent nanomaterials utilized for the remediation. Polybrominated diphenyl ethers are degraded by the species of Sphingomonas and zerovalent iron oxide nanoparticles consisting of a reductive oxidative approach. Kim et al. (2012) reported that zerovalent iron oxide nanoparticles lessened the polybrominated diphenyl ethers to lower degree compounds (less toxic). The by-products released during this process will further degrade the microbes more easily. Zerovalent iron oxide nanoparticles consisting of alginate beads under normal conditions removed the hexavalent chromium up to 91 %. The alginate beads removed the chromium when they were used in column experiments in comparison to the free beads; this may be attributed to the increase of column size enhanced the reactive sites; nevertheless, in real-time applications, the removal efficiencies of the hexavalent chromium decreased due to the presence of colloidal particles. The authors suggested that iron nanoparticles degraded the plumes of the ammunition and might be an effective option to treat these toxicants and enhance the argumentation process. Compared to the individual approach for remediating the integration process, i.e., zerovalent iron oxide with white-rot fungi was more effective.

Hydrogen liberated during the by-product degradations will donate electrons to the microbes responsible for the biotransformation of toxicants; the reasons to produce hydrogen gas and donate electrons to the microbes for the degradation were reported by several researchers in detail (Liu et al. 2005, Zhu et al. 2019). Xiu et al. (2010), showed that chlorinated compounds could be reduced by using zerovalent iron oxide nanoparticles with greater efficacy with integrating bacteria that use cathode depolarization. The nanoparticles that are nano metallic (Carboxymethylcellulose doped palladium/iron oxide) combined with species of Sphingomonas for the reduction of hexachloride hexanes revealed that the nanoparticles are stabilized with the carboxymethylcellulose and produced some bio stimulatory effects on the microbial growth. Shin & Cha (2008) reported that the microbial cells' bio stimulatory effects are mainly due to the iron nanoparticles; other zerovalent iron oxide nanoparticles enhance

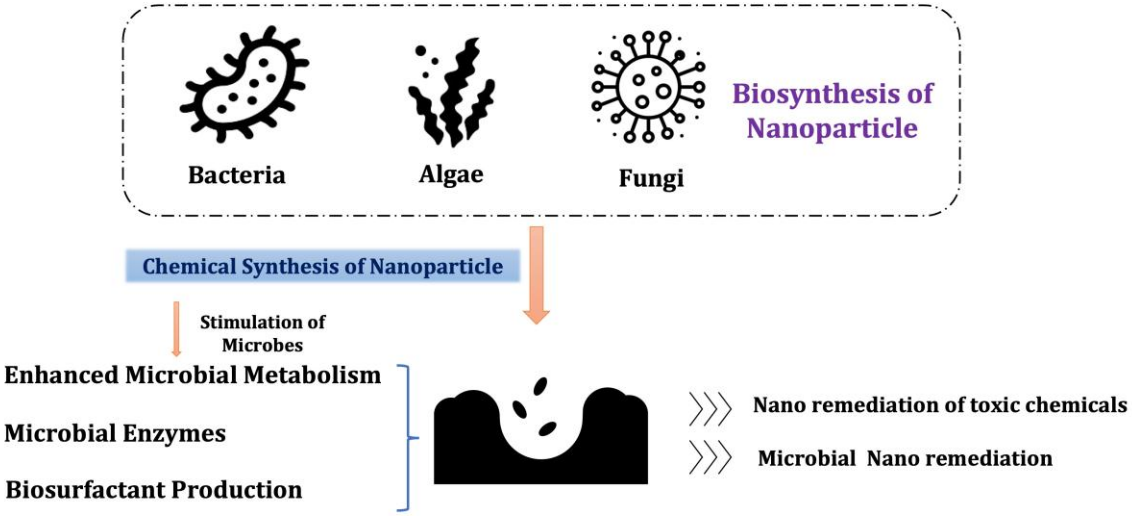


Fig. 5: Mechanisms of Nano-bioremediation in treating polluted soils.

the microbial reductions even at fluctuating temperatures; however, this may be disadvantageous in the case of nitrate reduction.

According to the literature available (Li et al. 2010), nanoparticle toxicity for microbes is extensively researched. The dosage plays a vital role in the integrated system to degrade the toxicants. Koenig et al. (2016) said that during the degradation of chlorinated aliphatic hydrocarbons by the zerovalent iron oxide nanoparticles, the bacterial cells become lethal. In contrast, the nanoparticles positively impacted the organochlorine respiring bacteria with a high dosage. The disadvantage of the toxicity of nanoparticles to microbial cells might be decreased by modifying nanoparticles via coating of nanoparticles, entrapment, or stabilization. Comparing the bactericidal effects of nanoparticles zerovalent iron oxide with polystyrene/poly aspartate and NOM (natural organic matter) with zerovalent iron oxide, it was found that modification of surfaces of nanoparticles reduced the toxicity with a dosage of 500 mg.L^{-1} (Li et al. 2010). An et al. 2010 stated that the improvement of iron oxide nanoparticles reduced nanoparticles' toxicity to microbes modified by the chitosan or sodium oleate during the nitrate reduction. Phenrat et al. (2009) said that nanoparticle oxidation is done with the aging of nanoparticles. To avoid directly contacting the microbes' cells, coating the nanoparticles will enhance the Dehalococcoides genes responsible for the dichlorination and improve the reduction capacities of trichloroethane (Xiu et al. 2010).

Le et al. (2015) used an integrated approach (integration of *B. xenovorans* and Iron/Palladium nanoparticles) to degrade the chlorinated poly biphenyls and found these methods are cost-effective; further reported that the toxicity of nanoparticles to the E.Coli during the degradation is chlorinated poly biphenyls is very low and not lethal to cells of the bacteria. Němeček et al. (2016 reported that zerovalent iron oxide nanoparticles consisting of microbes injected into the contaminated sites having hexavalent chromium degraded the heavy metal with the efficacy of 99%; Furthermore, microbes are oxidized by the iron nanoparticles and enhanced the degradation rate and decreased utilization of the nanoparticles dosage.

Integration MCNTs ("Multi-walled carbon nanotubes") with bioremediation techniques reduced the toxicants at a greater level. Yan et al. (2013) immobilized the strains of Shewanella oneidensis with beads of alginate made up of calcium doped with nanotubes and reduced the hexavalent chromium to trivalent chromium; further, the study revealed that integrated methods degraded the toxicants rapidly in comparison to the individual process; this may be attributed to the transfer of electrons by the carbon nanotubes. Pang et al. 2011 used Pseudomonas aeruginosa to reduce hexavalent chromium; immobilized the strains of microbes in the matrix of carbon nanotubes and alginates of sodium polyvinyl alcohols. The study revealed that the microbial cells immobilized by the modified carbon nanotubes reduced the hexavalent chromium nine-time effectively compared to pristine nanoparticles.

Chidambaram et al. 2010 conducted a study by integrating the Palladium nanoparticles Clostridium pasteurianum by the in-situ synthesis, the bacterial cells reduced the Palladium (II) ions to the Palladium nanoparticles, and these nanoparticles are retained in the cells of the bacteria to bio-palladium nanoparticles; the biobased palladium nanoparticles reduced the hexavalent chromium to trivalent chromium. Adikesavan & Nilanjana 2016 conducted a study on magnesium oxide nanoparticles integrated with the yeast candida species for treating the aqueous medium of Cefdinir and revealed that bio-based nanoparticles take lesser time for degrading the contaminants than individual microbial cells; the permeability of microbial cells enhanced, and pollutants are trapped by the cells of microbes and enhanced the degradation rate in comparison to particular treatment methods. Table 2 shows the various nano-bioremediation ways that have been widely used to treat pollutants.

Singh et al. 2020 stated that nano bioremediation had been applied to treat toxicants in two ways: first is the sequential technique in which the contaminants are first treated with the nanoparticles and later subjected to microbes for further degradation; second methods are combined method wherein the pollutants are treated with microbial and nanoparticles at the same time. Bokare et al. (2012), advanced a hybrid sequential approach with palladium/iron nanoparticles to degrade triclosan, an anti-agent used in cosmetics. In the first stage under the anaerobic conditions, 5mg/L of triclosan toxicants are remediated with the Palladium/Iron oxide nanoparticles to dechlorinate the triclosan to phenoxy phenol; later, the nanoparticles are removed from the integrated system and subjected to oxidation with the help of enzyme laccase obtained from the Trametes Versicolor which act as a redox mediator; the study revealed that triclosan could completely be transformed into non-toxic substances via the redox process. Kim et al. 2012 conducted similar investigations and degraded the polybrominated diphenyl ethers using zerovalent iron oxide nanoparticles integrated with the Sphingomonas bacterial species. He et al. 2009 reported sequential treatment methods for the pentachloro biphenyls with zerovalent nanoparticles with aerobic bacteria. Xiu et al. (2010) conducted a study with zerovalent iron oxide nanoparticles and microorganisms to remediate trichloroethane. Similarly, two different experiments were conducted under similar conditions, i.e., one with zerovalent iron oxide and the

Table 2: Treatment of noxious pollutants by nano-bioremediation (Singh et al., 2020).

S.no	Type of nanoparticle	Biological Agent	Family of Microbe	Toxicants removed	**References**
1.	CNTs	*Shewanella oneidensis*	Shewanellaceae	Hexavalent Chromium	Yan et al. (2013)
2.	Electrospun nanofibers	*Lysinibacillus*	Bacillaceae	Nickel	San et al. (2018)
3.	Electrospun nanofibers	*Lysinibacillus*	Bacillaceae	Chromium	San et al. (2018)
4.	Iron oxide	Species of *Sphingomonas*	Sphingomonadaceae	Carbazole	Li et al. (2013)
5.	Iron oxide	*Pseudomonas delafieldii*	Pseudomonadaceae	Dibenzothiophene	Shan et al. (2003)
6.	Iron oxide	Species of *Sphingomonas*	Sphingomonadaceae	Carbazole	Wang et al. (2007)
7.	Palladium	*Clostridium pasteurianum*	Clostridiaceae	Hexavalent Chromium	Chidambaram et al. (2010)
8.	Palladium	*Sphingomonas wittichii*	Sphingomonadaceae	Tetrachlorodibenzo-p-disney	Bokare et al. (2012)
9.	Palladium	*Burkholderia xenovorans*	Burkholderiaceae	PCBs	Le et al. (2015)
10.	Zerovalent iron	*Dehalococcoides*	Dehalococcoidaceae	Trichloroethane	Xiu et al. (2010)
11	Zerovalent iron	Species of *Paracoccus*	Rhodobacteraceae	Nitrates	Liu et al. (2014)
12.	Zerovalent iron alginate	*Acinetobacter junii*	Moraxellaceae	Hexavalent Chromium	Ravikumar et al. (2016)

other with species of Dehalococcoides individually (Singh et al. 2020). Even though many instances are available in the literature about nano-bioremediation, there is still a need to conduct many studies to move them from the bench-scale experiments to the industrial sectors to commercialize them.

Challenges with Nanobioremediation

During the in-situ remediation, the reactive compounds might harm the microorganisms and prevent degradation. The cost and the production of the nanoparticles on a larger scale are difficult. Further, the nanoparticles during the degradation might even react with the non-target compounds and form a cluster in the soils and stop the reactions and enzymes synthesis by the microbes. In the in-situ remediation, some nanoparticle inhibits the reactions and prevents nanoparticle dispersing of nanoparticles in the contaminated sites, reducing their effectiveness. The coating of nanoparticles can overcome this problem, but this process might add more costs to the treatment. Even such designs might affect the interactions of nanoparticles with the microbes and even causes toxicity to the cells of the microbes. Continued research on the area using nanoparticles might affect the people, wildlife, and plants near it. Thus, there is a need for conducting more research on these topics to overcome these problems.

CONCLUSIONS

Nano bioremediation was more promising and advantageous than conventional technologies currently used to remediate the toxicants present in contaminated soils. However, there is a paucity in the literature to gain in-depth knowledge about these technologies; thus, there is a need to conduct more studies on these integrated methods to bring more actions to develop and implement these technologies to full scale; furtherly the effect of environmental conditions on the nano bioremediation are also needed to understand these methods.

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