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Role of Various Techniques in Bioremediation of Contaminated Water: A Review

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ABSTRACT

Industrialization, urbanization and modernization have caused enormous harm to the environment. The toxic pollutants generated through industries and other activities are deteriorating the quality of soil, air and water day by day leading to a serious threat to all living beings on the earth. So, to improve the quality of environment various physico-chemical methods of remediation were used but they had various limitations such as high energy requirements, time constraints, high operational and maintenance cost. Nanotechnology has been emerged as a boon for mankind. It has found application along with bioremediation to remove contaminants from water. Although bioremediation methods have enormous potential as a green method but they require long treatment time and sometimes high concentration of contaminants in water may prove toxic to microorganisms. To overcome these limitations, nanoparticles are used along with living microbiomes to clean the contaminants from water which is called nano bioremediation. The present paper reviews use of metal nanoparticles in the treatment of contaminated groundwater, dye wastewater and domestic wastewater through bioremediation, application of nanoparticles in increasing the efficiency of microorganisms and how limitations of various methods can be overcome by combining nanoparticles with bioremediation.

Key words : *Nano bioremediation, Nanoparticles, Microorganisms, Contaminated water, Pollutants.*

Introduction

Dissipation of organic compounds is increasing day by day due to extending industrialization, urbanization and modernization. It has escalated organic contamination and releasing of toxic waste into the environment. These toxic pollutants are deteriorating the quality of soil, air and water day by day leading to a serious threat to all living beings on the earth. Toxic pollutants such as heavy metals, nuclear wastes, pesticides, drugs, explosives, green-house gases, hydrocarbons etc are harmful for ecosystem as well as human health. According to a study by NRDC (Natural Resources Defence Council, 2022) approximately 80% of the world's wastewater is thrown almost untreated, into the environment, polluting rivers, lakes and oceans. Runoff of water from municipalities and industries causes accumulation of heavy metals and metalloids in water bodies and soil. These contaminants in water impose serious problems to whole ecosystem. Due to pollutants in water dissolved oxygen concentration decreases affecting aquatic life negatively (Kahlon *et al.,* 2018). Owing to the effect of biological magnification heavy metals may exist in the food chain causing harmful effects on the whole ecosystem (Yuvan *et al.,* 2021). Unsafe water kills more people each year than war and all other forms of violence combined. (Water for life decade, 2014). On the other hand, our potable water sources are limited, i.e., less than 1%

of the earth's freshwater is accessible to us (Bratt Israel, 2010). According to The United Nations World Water Development Report 2018 in the present situation, the global demand for freshwater is expected to be one-third greater, than present demand by 2050 (UNESCO world water assessment program, 2018).

The substantial environmental damage due to contaminants caused the efforts to be done to improve the quality of environment by wastewater treatment. Although many physicochemical methods such as precipitation, electrochemical treatment, electrocoagulation, and adsorption (Fomina and Gadd, 2014) are in use but they had various limitations such as high energy requirements, time constraints, high operational and maintenance cost. Also, many of these techniques generate toxic byproducts. Thus, finding out eco-friendly wastewater management techniques became need of the hour. It gave rise to the emergence of bioremediation as an eco-friendly technique which utilizes living microorganisms for cleaning environment without harming the nature. These days various technologies such as nanotechnology, spectroscopy, chromatography, biochemical engineering and genetic engineering are used along with bioremediation to clean the environment.

This review covers use of various techniques in bioremediation of contaminated groundwater, dye wastewater and domestic wastewater and how the combination of various techniques is more efficient in treatment of waste water.

Research methodology and literature review

The sources of this study are the Scopus and Web of Science databases because of being largest databases for academic papers. We used research papers of last five years. At some places the work carried out by international organizations is taken from websites their.

Bioremediation of ground water and marine water

Mosmeri *et al.* (2019) investigated the use of CaO_2 nanoparticles in the benzene-contaminated groundwater through the continuous flow sand-packed plexiglass reactors. Two processes, bioremediation and Modified Fenton (MF) reaction were used to study the efficiency of nanoparticles in benzene (50 mg/l) removal from groundwater. In case of MF reaction 75% of initial benzene was removed after 100 days while in bioremediation benzene was removed completely within 90 days. CaO_2 releases oxygen, which stimulates the growth of biodegrading microorganisms. However, the composition of microbial community got changed in the presence of CaO_2. The OH free radical is produced by the reaction of CaO_2 with water, it destroys the aromatic structure of contaminants. So, bioremediation was found useful for low contaminant concentrations. High contaminant concentrations require more amount of CaO_2 which may demolish the microbial community.

To reduce the negative impact of nanoparticles on microorganisms, Gholami *et al.* (2019) investigated the use of encapsulated magnesium peroxide nanoparticles for removal of toluene and naphthalene from ground water in permeable reactive barrier. They studied both biotic and abiotic contaminant remediation processes, and natural microbial species present in groundwater were used. The encapsulation process not only increased the stability of nanoparticles but also reduced their negative impact on water microbes. It was found that naphthalene was completely removed within 20 days and toluene was completely removed after 30 days. It was also found that microorganisms *P. putida* and *P. mendocina* which were able to remove toluene and naphthalene respectively were stimulated by addition of MgO_2 nanoparticles.

The mechanism of heavy metal reduction was studied by Qurbani *et al.* (2022). Authors isolated *Aeromona sobria* from soil which was highly polluted with heavy metals and reported that *A. sobria* KQ21 was useful in reducing heavy metal toxicity by different mechanisms. It was observed that after 72 hours of incubation *A. sobria* KQ21 was able to reduce the concentration of copper by 54.89% (0.549 mM), nickel by 62.33% (0.623 mM) and zinc by 36.41% (0.364 mM). It was revealed by transmission electron microscopic studies that copper and nickel metals were reduced by bioaccumulation mechanism and in case of zinc the mechanism was biosorption.

Oil, petroleum gas on combustion produces polycyclic aromatic hydrocarbons which accumulate in environment and pollute ground water sources also. Agrawal *et al.* (2018) reported the use of white rot fungi *G. lucidum* for the degradation of polycyclic aromatic hydrocarbons which are formed by incomplete combustion of oil, petroleum gas, wood, municipal and urban waste. Their release in the environment causes harmful effect on human and other animals. It was found that in mineral salt broth *G. lucidum* degraded 99.65% of phenanthrene and 99.58% of pyrene.

Instead of using microorganism directly, Chaprao *et al.* (2018) reported the use of biosurfactant produced by microorganism in the bioremediation of oil contaminated environment. This biosurfactant was produced by Bacillus methylotrophicus UCP 1616 bacteria. It was isolated from seawater of port area and cultivated with industrial waste containing corn steep liquor and sugar molasses. Interestingly this biosurfactant worked well with porous surface also. Although in previous studies also the use of biosurfactants in removal of motor oil from coral reef has been reported by Luna *et al.* (2016). In the present study the motor oil removal rate was around 70% when contaminated porous surface was shaken with biosurfactant for 5 minutes. Thus, the biosurfactant produced from *B. methylotrophicus* can also be used to remove hydrophobic pollutants from highly sensitive coral reefs.

El-Sheekh *et al.* (2021) reported the use of iron nanoparticles synthesized from aqueous extract of brown seaweeds *P. fascia*, *C. sinuosa*, and *P. pavonica*. Bioreduction and stabilization of Fe_3O_4-NPs was caused by proteins and lipids found in algae extracts. Iron nanoparticles synthesized by using *P. pavonica* extract (4 mg ml^{-1}) were best in nitrogen and phosphorous removal 89.8% and 93.96% respectively. Iron nanoparticles also exhibited antialgal activity, which was confirmed by tracing optical density and Chlorophyll-A in seawater sample. Optical density and Chlorophyll-A was reduced by more than 90% after 10 days with 2 mg ml^{-1} concentration of algae extracts.

Bioremediation of industrial waste water

Industrial waste water is a major consequence of industrialization and modernization. Treatment of industrial waste water is a big challenge for scientists. Many studies have been carried out to address this issue.

Ikegami *et al.* (2020) reported an interesting study. They investigated the use of sugarcane molasses obtained from sugar factory as a cheaper byproduct in reducing Chromium, a harmful contaminant generated by various industries. By using Column Chromatography, they separated different constituents of sugar molasses and reported that the constituent containing polyphenolic compound removed Cr(VI) without bacteria while the constituent containing sugar content causes the growth of microbes used in Cr(VI) reduction. Thus, sugarcane molasses can be used as an economic source for bioremediation. Molasses act as a Carbon source for promoting Cr(VI) reducing microbial growth and presence of polyphenol is an additive which also reduces Cr(VI) without adversely affecting bacterial growth.

Banerjee *et al.* (2019) reported isolation and use of a Bacillus strain TCL in bioremediation. It was found to be chromium, cadmium and nickel tolerating strain having remarkable stress responses. It completely reduced Cr(VI) (200 mg/l) within 16 hours under heterotrophic condition and could tolerate up to 2000 mg/l Cr(VI). It can work efficiently at temperature ranging from 15-45 °C, 5-9 pH and in presence of other metals such as Cd, Cu, Mo, Ni and Pb, oxyanions and metabolic inhibitors.

Sher *et al.* (2020) isolated bacterium *Micrococcus luteus* strain AS2 from industrial waste water and studied its bioremediation effect against arsenite and arsenate at 37 °C and pH 7. Arsenite was adsorped in bacterial cell due to interaction between arsenite and functional groups present on bacterial cell as revealed by FTIR analysis. Scanning electron microscopy confirmed no morphological change in bacterial cell under arsenite stress. The bioremediation efficiency was found to be 72% after 2 hours and 99% after 10 hours. Apart from arsenic the bacterial strain showed resistance against lead, cadmium, chromium, mercury, nickel and zinc also.

Use of enzymes produced by bacteria in bioremediation was first time reported by Jardine *et al.* (2018). Authors reported that bacteria cultured from hot spring of South Africa can be used for producing enzymes useful for wastewater bioremediation. The bacteria cultured were of the genus Bacillus and were able to produce amylase and protease. Enzymes were screened using liquid chromatography - Tandem mass spectrometry (Lc-Ms/Ms) which was cost-effective. 56 bacterial isolated were cultured and the enzymes produced were able to remove polyaromatic hydrocarbons and dye pollutants, antibiotic residues, contaminants from food industry, in solubilisation of phosphates and reduction of heavy metals, chromate and lead.

Darwesh *et al.* (2023) reported the use of the cell-free extract of a novel copper-resistant fungus strain, *F. oxysporum* OSF18 in biosynthesis of copper oxide-nano particles (CuO-NPs). The biosynthesized CuO-NPs were having the size in range of 21–47 nm. When they were immobilized in alginate beads, they were found highly efficient in disinfecting not only microbes (99.995%) from raw textile industrial wastewater but also found effective in removing heavy metals (93, 55, and 30 % for Pb, Cr, and Ni, respectively) and dyes (90%) from raw textile industrial wastewater.

Lang *et al.* (2013) identified oxygen-insensitive bacterial Azoreductase, cloned it and over expressed in *E. coli.* The recombinant BrAzo was found to be highly effective in decolourizing a broad range of synthetic dyes. Methyl orange was degraded completely in 3 hours and it continued over 9 cycles. It was a very interesting study as azoreductases enzymes can reduce azo bonds specifically under aerobic/anaerobic conditions (Carliell *et al.*, 1996). Reductive cleavage of N-N bond leads to the formation of aromatic amines, sometimes more toxic than dye itself. Although aromatic amines are easily degradable under aerobic conditions (Saratale *et al.*, 2011) but aerobic conditions may adversely affect the microbial growth. Thus, in this process decolorization by Azoreductase producing oxygen-insensitive bacteria was followed by treatment with oxidizing enzymes producing cells to detoxify aromatic amines.

Dye wastewater containing metal complex is a big threat for environment as it is resistant to degradation. Cheng *et al.* (2019) reported an eco-friendly and low-cost technique for decolorization and degradation of a metal complex dye-Naphthol Green B (NGB) using a marine bacterium (*Pseudoalteromonas* sp CF10-13). The major structure in NGB molecule is naphthalene sulfonate which was converted into lesser toxic benzamide. It was concluded that decolorization and degradation took place through extracellular electron transfer and secretion of redox mediators in soluble extracellular metabolites. The bacterium was reported to work at wide ranges of dye concentration, salinity and under anaerobic conditions. Interestingly during this process black stable iron-sulphur nanoparticles were synthesized avoiding the hazards of H_2S releasing and ferric ion accumulation making the process eco-friendlier.

Darwesh *et al.* (2019) reported the use of enzyme along with metal nanoparticles. They isolated, purified and immobilized peroxidase onto modified Fe_3O_4 magnetic nanoparticles. The magnetic nanoparticles-immobilized peroxidase was unaffected by temperature and pH change in comparison to free enzyme form. Also, its activity was maintained for 90 days upon storage at 4 °C and 25 °C. it could be used upon recycling up to 100 cycles. The immobilized peroxidase was tested for decolorization of textile industry wastewater containing various azo dyes. It was found that water containing both green and red azo dyes was completely decolourized in 6 hours.

Instead of using metallic nanoparticles, Monsour *et al.* (2022) reported interesting findings. They used native red seaweed species *Pterocladia capillacea* in nanoparticle form to remove toxic dye Ismate Violet 2R (IV2R) from textile industry effluent. It was reported that at 30 °C temperature, nanoparticle biomass dosage of 0.2 gm, pH of 2 and initial dye concentration of 60 mg/L 87.2% dye was removed with a 30 min equilibrium time. Active sites of the adsorbents attracted the dye ions by electrostatic attraction or by complexation. The adsorption process was exothermic and spontaneous as revealed by thermodynamic parameters. With increasing dye concentration and decreasing temperature degree, the adsorption ability of nanoparticles increased. The nanoparticles were found to be non-toxic, eco-friendly, low-cost bio-adsorbent with high uptake capacity.

Similarly, Rizzi *et al.* (2017) reported a very interested study, where they used olive pomace in isolating and recovering Dispersed Red and Dispersed Orange industrial dyes from wastewater. They used visible spectroscopy to obtain the percentage of dyes removed from the wastewater. Olive pomace being porous material removes the dyes by adsorption process. It works at the temperature of 100 °C. With 5×10^{-5} M of dye concentration the Dispersed orange dye was removed up to 98% in 30 minutes by using 3.00 gm of olive pomace. When the quantity of olive pomace was reduced to 0.25 g the dye removal was 85% in 180 minutes. However, when 0.25 g powdered olive pomace was used 99% dye was removed within 40 minutes. The reason was larger surface area in case of powdered olive pomace. It was observed that both dispersed orange and dispersed red were almost completely removed from wastewater after a contact time of 10 minutes by using 1.00 g of powdered olive pomace. The powdered olive pomace could be reused with same efficiency and same contact time for 2 consecutive cycles. After 3^{rd} cycle the efficiency was same but contact time was increased from 10 to 20 minutes. After several cycles the further adsorption of dye was hindered, due to saturation of active sites involved in adsorption process. The adsorbed dye was recovered by placing 1.00 g dye-loaded powdered Olive Pomace in 40 ml of acetic acid. The dye released was observed by UV-Visible absorption spectroscopy. Higher removal and recovery efficiency was exhibited by dispersed orange in comparison to dispersed red; the recovery was 85% after each cycle for the latter. Absorption spectra revealed that adsorption-desorption process had no effect on the chemical-physical properties of dyes.

Bioremediation of municipal waste water

In municipal waste water, copper is the most common contaminant. Although it is required in many metabolic processes of prokaryotes and eukaryotes. Its increased concentration is harmful for organisms. Zvab *et al.* (2021) reported an interesting study for cupric ion removal from municipal waste water. He investigated the use of a wild type strain of *Chlamydomonas reinhardtii* in removal of Cu^{+2} ion from municipal wastewater. Interestingly the microalga reduced the Cu^{+2} ions into copper nanoparticles. Heavily copper polluted wastewater (10 mg/l) was treated successfully with simultaneous biosynthesis of copper nanoparticles by using *C. reinhardtii*.

Turakhia *et al.* (2018) used iron nanoparticles for the treatment of municipal waste water. They reported the synthesis of zero-valent iron nanoparticles using *Spinacia oleracea* leaves extract for treatment of municipal wastewater in terms of BOD and COD. The biosynthesized nanoparticles were able to reduce BOD from 56 mg/l to 22 mg/l and COD from 405 mg/l to 85 mg/l in sewage wastewater. Even, after 15 days the efficiency of nanoparticles for BOD was found to be 60.31% and for COD 73.82%.

Darwesh *et al.* (2021) reported the synthesis of iron nanoparticles using F-23 isolate of fungus *Fusarium oxysporum.* Authors collected 10 samples from soil contaminated with iron. These samples were enriched by using iron chloride. By using myco-synthesized iron nanoparticles the total bacterial count of municipal wastewater was decreased by 89.23% and total fungal count was decreased by 100%. The iron nanoparticles were also useful in removing heavy metals from municipal wastewater, i.e. lead by 95% and cadmium, chromium, nickel and zinc between 20 – 50%.

Bioremediation of dairy effluent

Untreated discharge from various industries is main reason for water pollution. One of them is untreated dairy effluent, which causes increase in biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC) causing adverse effect on the whole ecosystem. He *et al.* (2017) reported the treatment of dairy effluent by preparing Fe_3O_4/biochar nano-composites and upon this nano-composite photosynthetic bacteria (PSB) were immobilized. Fe_3O_4/biochar nano-composites enhanced PSB's capacity of bioremediation markedly. The prepared biomass removed 83.1% of COD, 87.5% of NH^{4+} and 92.1% of PO_3^{4-} from the wastewater. It was found that composites were recyclable and their bioremediation capability remained effective even after five cycles. After the end of each cycle, it was easy to recover due to its magnetic properties.

In 2021 Salama *et al.* (2022) reported bioremediation of dairy effluent using Iron nanoparticles in terms of BOD_5, COD and TOC reduction. Magnetic Fe_3O_4 nanoparticles were synthesized using *Eichhornia crassipes* extract. Maximizing BOD_5 reduction using a biological treatment was a major challenge. The use of *Proteus mirabilis, Pseudomonas batumici, Providencia vermicola* was investigated along with biosynthesized Fe_3O_4 NPs. It was observed that nanoparticles increased bacterial growth causing reduction in BOD_5, COD and TOC. *Providencia vermicola* had highest reduction efficiency, as BOD_5, COD and TOC reduction was improved up to 87.65%, 67.9% and 70.7% respectively with optimum concentration of 40 mg/l for Fe_3O_4 nanoparticles.

Salama *et al.* (2022) in 2022 first time reported the use of cerium oxide nanoparticles in removing nitrate and phosphate from dairy effluents. Wastewater and sludge were used as inoculum sources. It was found that CeO_2 NPs enhanced the microbial growth and hence nitrate and phosphate removal was accelerated. The optimal concentration of CeO_2 NPs was $1x10^{-10}$ ppm for sludge and $1x10^{-12}$ ppm. In case of using sludge as inoculum the removal efficiency of microbes was improved up to 89.01% for nitrate and 68.12% for phosphate in the presence of CeO_2 NPs. While in the case of wastewater, with CeO_2 NPs removal efficiency for nitrate was improved up to 83.30% and for phosphate 87.75% compared to control.

Future prospects

Nano bioremediation and other bioremediation techniques are eco-friendly, efficient and economical for waste water treatment. But their large-scale industrial application is still a big challenge because most of them are based on batch treatment of waste water. So, methods for their large-scale industrial application need to be explored.

Conclusion

In this paper, a mini literature review has been conducted to study the role of various techniques in bioremediation of contaminated water. The study revealed that nano bioremediation is eco-friendly and effective methodology for waste water treatment. Few microbes while removing metals from polluted water convert them into nano-particles. Various enzymes produced by microbes are more efficient in the treatment of waste water from dye industry and they are reusable after many cycles. Biosurfactants can be used effectively for treatment of porous surface such as coral reefs also. Interestingly seaweed, olive pomace and nano-composites were found very effective in the treatment of waste water obtained from various industries. In case of olive pomace, where adsorption-desorption method is used, the removed dyes as well as olive pomace can be recovered and reused for many cycles.

Conflict of Interests

The author has no relevant financial or non-financial interests to disclose.

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