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Mini Review

Bioaugmentation and biostimulation: a potential strategy for environmental remediation

Abstract

As the world is heading towards rapid urbanization and technological advancements, more undesirable and unwanted activities by human is raising major environmental issues like global warming, imbalance in soil ecological processes leading to lower agricultural yield, drastic climate change, etc. Predominantly, among all, xenobiotic recalcitrant compounds are indiscriminately being disposed in the environment causing significant hazard owing to its high stability and complexity. However, there are several methods for disposing such materials but the most efficient and significant disposal strategy is said to be bioremediation. This biological approach of remediation can be executed through introduction of efficient microbial strains (bioaugmentation) or by addition of rate limiting nutrients to the soil (biostimulation) to enhance the remediation process significantly. Existing literatures provide a broad landscape of the efficiency of some of the microbial strains and a few of the rate limiting nutrients in remediation of the toxic, highly complex and resistant contaminants but lacks information regarding their degree of efficiency and eco-friendliness in comparison with those of other disposal strategies. This review extensively focuses on a comparative discussion on different strategies of bioremediation to remediate the environment from the toxic, hazardous waste with the goal of building a less-toxic, stable and a healthy environment.

Keywords: recalcitrant xenobiotic compound, bioremediation, bioaugmentation, biostimulation, sustainable environmental protection

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Introduction

With the rapid increase in population, urbanisation and industrialisation, currently, the environment is at a stake or on the verge of ecological damage.[1] In other words, the environment is endangered due to human activities that are continuously ruining it.[2] The ubiquitous contamination and pollution of the nature's elements such as the aquatic and terrestrial ecosystems by the industrial production of chemicals, excessive use of petroleum and its derivatives, polyethylene, pesticides and organic herbicides that are mostly used for protection against weed, insects, and fungal attacks are of serious concern.[3–6] The most prominent sources of these pollutants in the soil and water resources are the oil refineries, gas stations, seeping of water from the herbicide-pesticide treated agricultural lands, petrochemical and pharmaceutical industries. Most of these pollutants are recalcitrant in nature and persist in the environment for long periods of time.[7] Among all of the above, the most recalcitrant, non-biodegradable, severely toxic and most commonly used polymers are the polyethylene bags. This toxic and headstrong nature of polyethylene is mainly due to three factors - high molecular weight, complex structure and its hydrophobic nature.[8] Annually 140 million tonnes of synthetic polymers are produced at a growing rate of 12% per annum.[9,10] Each year, an estimated 500 billion to 1 trillion plastic bags are consumed worldwide.[11] The polyethylene bags or any other polyethylene based products are finally dumped into the landfills after their usage leading to severe environmental pollution since they are non-biodegradable under natural environmental conditions.[12,13]

The plastic wastes generated daily or annually worldwide are one of the serious threats to environmental pollution because of their semi-permanent stability in the environment. The semi-permanent existence of these plastics in the environment is mainly due to their high molecular weight, cross-linkages, high number of aromatic rings, halogen substitution in their structure[14] and also due to the absence of the functional groups recognizable by the microorganisms[15,16] etc. Similarly, rubber industries raise severe environmental issues due to different chemical usage at different stages of rubber processing.[17] India is the third largest natural rubber producing country of the world, next to Thailand and Indonesia, producing about 9 percent of the total output.[18] From about 200 hectares in 1902-03, the total area under rubber plantations increased to about 5.9 lakh hectares in 2003-04. Similarly, the production that was 80 tonnes in 1910 increased to about 76000 tonnes in 2003-04. Presently, India's rubber production has reached to 7 lakh tonnes annually.[19] Despite the numerous benefits that are rendered to the World by natural as well as synthetic rubber, the consequence of rubber and its derivatives has raised a serious problem due to its highly polluted effluents.[20] The most common environmental issue caused by the rubber industries is the discharge of huge amounts of wastewater containing chemicals, hazardous waste and thermal emission. In addition to this, other environmental issues of rubber processing sector are high concentration of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solids (SS), high concentration of ammonia and nitrogen compounds, high level of sulphate, high level of odour and many more.[17]

In case of polychlorinated biphenyls (PCBs), it is accidental spills or improper disposal that leads to their release into the environment.[21] The maximum PCB production represents a share of about 1.5 percent in world electronic hardware production.[22] While talking about the toxic pollutants, the most concerned area can be considered as that of the toxic and hazardous chemicals released by the industries every year. Each and every second 310 kg of toxic chemicals are released into the air, land and water by the industrial facilities all around the world. This amounts to approximately 10 million tonnes per year.[23] The next most hazardous substance is lubricant oil, a common essentiality that is normally used to run several engines and machines. A subsequent accumulation of these oils from the auto-mechanic workshops may result in serious environmental problems because of its hazardous nature.[24] Oil spills, either it could be crude oil or refined oil, is also significantly intimidating the health of the environment and living creatures including humans.

Oil spills are mainly due to the release of crude oil from tankers, offshore platforms, drilling rigs and wells, as well as spills of refined petroleum products and their by-products, heavier fuels used by large ships such as bunker fuel etc.[25] Spilled oil can also contaminate drinking water supplies. For example, in 2013 two different oil spills contaminated water supplies for three hundred thousand people in Miri, Malaysia, eighty thousand people in Coca, Ecuador.[26] In 2000, springs were contaminated by an oil spill in Clark County, Kentucky.[26] Though the oil spill incidents are relatively showing a downward trend from 28.1 numbers of spills in 1990 to 5.2 number of spills in 2010, but it has not been completely stopped. In reference to this, the two large recorded oil spills that occurred in the year 2015 in Singapore and Turkey can be accounted. Removal of these compounds from the environment always remains a challenging task for the people globally. In this context, there is a significant need for remediation measures to protect the environment as well as to secure the drinking water resources.[27] The presence of these substances in the environment not only pose health hazards to the people globally but also raises a threat to the environmental food chain due to their toxicity, mutagenic and carcinogenic properties.[7]

Limitations of present disposal techniques

There are several methods that have been designed for the removal or elimination of these hazardous toxic pollutants from the soils. Some of the methods used for the disposal of the hazardous pollutants from the environment include-incineration, recycling and landfills.[28] Disposal of the toxic waste by the process of incineration is not a good approach as incineration of the waste materials results in a large amount of toxic emissions such as carbon monoxide and methane. The release of these gases to the atmosphere ultimately brings severe changes in climate.[29] At the same time, disposal of the used materials like polyethylene bags, rubber tyres, etc into the landfills will pose serious threats not only to the soil microflora but also proves hazardous to the mankind. Existing literature tried focussing on the biodegradation of polythene because of the demerits of the existing disposal strategies for Polythenes like incineration, dumping into landfills, recycling that is not only a hazard towards environmental health but also highly expensive, time-consuming and labour intensive.[30]

Some other techniques that are under development includes extraction of pollutants with organic solvents, oxidation of organic pollutants under subcritical or supercritical conditions, vitrification, electro-reclamation, dehalogenation of chlorinated organic compounds, chemical reduction or oxidation of contaminants, steam stripping, plasma torch techniques etc.[31,32] Out of these techniques mentioned above, the biological method for the removal of soil contaminants was considered as the most potent and environmentally friendly approach.[33] This approach is covered under an umbrella term called bioremediation. By definition, bioremediation is described as the use of microorganisms to destroy or detoxify waste materials.[34,35] Biological approach for elimination of soil contaminants involves a wide array of microorganisms. The reason behind this is their biodiversity and vast catabolic potential.[36,37] For effective removal of the recalcitrant compounds from the soil, the best biological approach is considered to be the "*in-situ* bioremediation". This process of detoxification targets the harmful chemicals by mineralisation, transformation, or alteration.[38,39] Bioremediation at its natural pace is incapable of eliminating the toxic and hazardous waste from the environment considerably. In order to enhance the degradation rate of the waste through bioremediation, bioremediation is been coupled with two other approaches named as bioaugmentation and biostimulation.

Bioaugmentation

Bioaugmentation is the method of application of autochthonous or allochthonous wild type or genetically modified microorganisms to polluted hazardous waste sites in order to accelerate the removal of undesired compounds.[3] Figure 1 outlines the process of bioaugmentation. Bioaugmentation is mainly undertaken in oil contaminated environments as an alternate strategy for bioremediation.

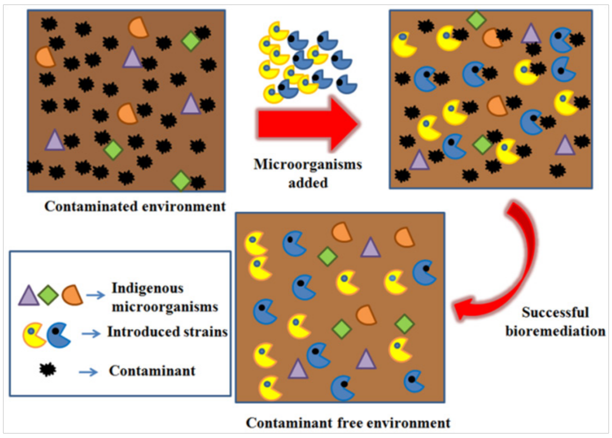

Figure 1 The pictorial diagram of bioaugmentation.

Principle of bioaugmentation

The rationale of this approach is to enhance the degree or rate of degradation of the complex pollutants by the addition of pollutant-degrading microorganisms.[40,41] Enhancing the microbiota of a contaminated site will not only enhance the elimination of the pollutants from the particular site but also at the same time increases the genetic capacity of the desired site. Therefore, bioaugmentation corresponds to an increase in the gene pool and, thus, the genetic diversity of the site. In principle, this genetic diversity could be increased by augmenting the microbial diversity.[42,43]

Factors influencing bioaugmentation

The success of bioaugmentation process depends mainly on the adaptation of the microbial consortia to the site that needs to be decontaminated. The success of the process also relies on the ability of the newly introduced microbial consortia to compete with the indigenous microorganisms, predators and various abiotic factors.[25] Previous studies have shown that it improves the biodegradation capabilities of the contaminated soil by improving the efficiency of the remediation process.[3,44] Bioaugmentation has been mostly undertaken in soils with a lower number of pollutant-degrading microorganisms

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and with compounds that require multi-process remediation.[3,45] Apart from these, there are several other parameters that control the rate of bioaugmentation occurring in soil. Some of the noted parameters or factors that influence the process of bioaugmentation are pH, temperature, moisture, organic matter content, aeration, nutrient content and soil type.[3,46] Lack of any of these soil parameters under natural condition makes the remediation process inefficient.[46–48] Existing literature stated that *Burkholderia* sp. FDS-1 can optimally degrade nitrophenolic pesticides at a temperature of about 30ºC and at a slightly alkaline pH. As mentioned, the diverse catabolic activities of the microbial cells are mostly due to the presence of catabolic genes and enzymes.[49–52]

Moreover, the existence of a variety of adaptation strategies in microorganisms such as the ability to modify the cellular membrane to maintain the necessary biological functions,[53,54] the production of surface-active compounds such as biosurfactants[55] and of the use of efflux pumps to decrease the concentration of toxic compounds inside the cells[53,56] makes them a significant weapon for the remediation of contaminated soils. One of the most important soil parameters influencing the effectiveness of bioaugmentation is organic matter content. It plays a crucial role in bioavailability of pollutants and impairs the survival of inoculated strains and their ability to degrade contaminants. In this context, the studies carried out by Mrozik and Piotrowska-Seget 2010 and Greer and Shelton 1992 was quite convincing.[3,57] They reported that degradation of 2, 4-dichlorophenoxyacetic acid was found to be optimum at high organic matter soil in comparison with soil containing lower organic matter. In addition to this, the moisture availability in the soil also influences the rate of bioaugmentation. In this context, the studies carried out by Ronen et al.,[58] can be taken into account.

The article studied the effect of water content on the survival of *Achromobacter piechaundii* TBPZ and the degradation rate for tribromophenol. They observed that in the presence of 25% and 50% water content in the soil, the degradation rate was found to be rapid whereas with the decrease in the water content the degradation rate decreases. Existing literature revealed that low soil water content decreased the activity of bacteria mainly due to diffusion limitation of substrate supply and adverse physiological effects associated with cell dehydration.[3,59] Similarly, the studies carried out by Kim et al.,[60] revealed that *Pseudomonas spadix* BD-a59 grow very fast in slurry systems amended with sterile soil in comparison with that of the soil that was combusted earlier for the elimination of organic matter. This particular study made by Kim et al.,[60] gave an idea of the essentiality of the insoluble organic compounds for the BTEX degrading microorganisms.

Spectrum of microbes used in bioaugmentation

Existing pieces of the literature showed a varied role of bio-augmenting microorganisms in the augmentation of the contaminated sites. The studies carried out by Wang et al.,[61] and Xu et al.,[62] reported the biodegradation efficiency of *Burkholderia pickettii* against Quinoline. It was observed that though the indigenous organisms of the contaminated site were incapable of degrading Quinoline but these organisms in cooperation with the newly introduced Quinoline degrader *Burkholderia pickettii,* can degrade Quinoline (concentration of 1mg/g of soil), within 6 and 8 hours. The experiments carried out by Tchelet et al.,[63] showed the efficiency and capability of β-Proteobacterium *Pseudomonas* sp. strain P51 to degrade chlorinated benzenes. Their results demonstrated the possibility of successful bioaugmentation by applying preselected strains to degrade poorly degradable substance like TCB in the contaminated site. A wide variety of microbial strains or microbial consortia can be employed for the process of soil augmentation. Contaminants in the soil not only affect the soil quality but also the soil microbial communities are known for rendering several important functions in the soil.

Selection of appropriate microorganism: Choosing the best tool

Successful soil augmentation requires appropriate selection of microbial strains or microbial consortia. The selection of a suitable strain or a suitable microbial consortium requires the consideration of few of the features of microorganisms like fast growth, easily culturable, ability to withstand high concentrations of contaminants and to survive in a wide range of environmental conditions. There are several approaches that can be considered for selection of a suitable bacterial strain. One of the approaches involves isolation of bacterial strain from a contaminated soil followed by its culturing under laboratory conditions for its pre adaptation and finally augmented back into the same contaminated soil. This approach is called as re inoculation of soil with indigenous microorganisms. Table 1 shows the success of bioaugmentation in polluted environments. Tribedi et al.,[64] had isolated an organism *Pseudomonas* sp. AKS2 from landfill soil and characterised the polyethylene succinate (PES) degradation activity in laboratory condition. They also showed that the isolated organism AKS2 exhibited PES degradation in soil contaminated with PES without damaging soil ecological balance, proving an efficient bioaugmentation tool against PES remediation.[44] Similarly, another approach for augmentation of contaminated soil can be through selection of appropriate microorganisms from different polluted sites with similar contaminants and finally augmenting them into the desired contaminated site.[41,65,66]

Table 1 Microorganisms used in bioaugmentation studies

Microorganism used	Pollutants degraded
Pseudomonas putida PaW340/pDH5	4 chlorobenzoic acid[93]
Cupriavidus necator RW112	Chlorobenzoates Arochlor 1221 and 1232[94]
Burkholderia xenovorans LB400 (ohb)	Arochlor 1242[95]
Pseudomonas fluorescens RE	2,4-dinitrotoluene[96]
Pseudomonas fluorescens MP	2,4-dinitrotoluene[96]
Pseudomonas putida KT2442	Napthalene[87]

Table continued...

Microorganism used	Pollutants degraded
Pseudomonas fuorescens F113rifpcbrmBP1::gfpmut3	Biphenyl, polychlorinated biphenyl[97]
Rhodococcus sp. StrainRHA1	4-chlorobenzoate[98]
Eschirichia coli AtzA	Atrazine[99]
Pseudomonas sp.Pseudomonasputida B13ST1(pPOB)	3 phenoxybenzoic acid[100]
Pseudomonas fluorescens F113rifPCB	Biphenyl, polychlorinated[101]
Pseudomonas fluorescens CS2	Biphenyl Ethylbenzene[102]
Pseudomonas putida BCRc14349	Phenol, trichloroethane[103]
Rhodococcus sp.F92	Various petroleum products[104]
Arthrobacter, Burkholderia, Pseudomonas, Rhodococcus etc.	Petroleum hydrocarbons[105]

Biostimulation: an efficient strategy of bioremediation

Biostimulation is a remediation technique that is highly efficient, cost effective and eco-friendly in nature. Biostimulation refers to the addition of rate limiting nutrients like phosphorus, nitrogen, oxygen, electron donors to severely polluted sites to stimulate the existing bacteria to degrade the hazardous and toxic contaminants.[4,67–69] Figure 2 outlines the process of biostimulation.

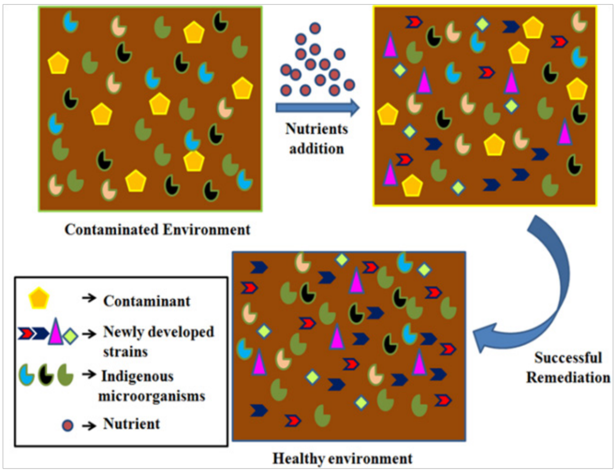

Figure 2 The pictorial diagram of biostimulation.

The mechanism of stimulation: Role of rate limiting nutrients

The addition of rate limiting nutrients improves the degradation potential of the inhabitant microorganisms efficiently as it significantly accelerates the decontamination rate.[41,70] Among all of the bioremediation techniques, biostimulation is considered to be the most efficient method for remediation of hydrocarbons.[41] Existing works of literature have established biostimulation as an important remediation tool for the degradation of hydrocarbons especially petroleum products and its derivatives.[71] To be specific, petroleum contaminated sites having less efficient and metabolically poor microbial population can be remediated significantly by the addition of some of the rate limiting nutrients or through the process of biostimulation.[4,72] This is mostly due to the easy availability of carbon (C) source which is one of the rate-limiting nutrients required by the indigenous microorganisms for their metabolic activities from the petroleum contaminants. So it can be said that just meagre addition of few of the other rate limiting nutrients except carbon onto the soil boosts up the petroleum degradation rate significantly. In addition to the aforementioned rate limiting nutrients, implementation of certain other nutrient rich organic matter can also trigger the remediation process extensively. In this context, the study carried out by Sarkar et al.,[73] is worth mentioning. They have observed that addition of bio solids (nutrient rich organic matter) obtained from the treatment of domestic sewage and inorganic fertilisers, rich in nitrogen and phosphorus gears up the degradation rate of petroleum hydrocarbons up to 96%.

Factors influencing the biostimulation

Bioremediation of a polluted site through biostimulation is influenced by few of the environmental parameters like pH, moisture content, temperature, etc.[24] In addition to these factors, the prevailing environmental physiology also influences biostimulation rate. In this context, the example of bioremediation process in the marine environment can be taken into account. In the marine ecosystem, the bioremediation rate is significantly low as it becomes fairly difficult for the microorganisms to target the polymer for degradation as there are high chances that the polymer will get diluted or washed out by the wave action. It is not always necessary that addition of nutritious matter onto the soil will enhance remediation process but there are examples that reflect the adverse effects of excessive addition of nutrients onto the soil. The experimental work carried out by Nikolopoulou & Kalogerakis[70] showed that higher concentrations of N and P sources can cause eutrophication, which enhance the algal growth and ultimately reduce the dissolved oxygen concentration in the water resulting in the death of several aquatic lives.[70] Thus, we can say that dependency of biostimulation on environmental factors can limit the progress or efficiency of the method being adopted.[74] To be specific, biostimulation works efficiently towards the elimination of complex contaminants from the ecosystem by maintaining a proper balance between the desirable and undesirable addition of rate limiting nutrients onto the soil.

Application of biostimulation

The major contaminants that can be successfully remediated through biostimulation are petroleum hydrocarbons, sulphate and

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polyester polyurethanes. Sulphate contamination of groundwater is a threat not only to the environmental ecology but also towards human health. This can be remediated biologically through the process of biostimulation. It requires amendment of electron-donor that will enhance sulphate reduction and thus remediate the same.[75] In the case of polyester polyurethanes, biostimulation plays a significant role in enhancing its degradation rate in soil. The polyester polyurethanes (PU) are a diverse group of synthetic polymers with many industrial and commercial applications, including insulating and packaging foams, fibers, fabrics, and synthetic leather goods.[76,77] These polymers contain intra-molecular bonds analogous to those found in biological macromolecules (such as ester and urethane linkages). It is due to the presence of these intramolecular bonds that enhances microbial degradation as these bonds act as a site for microbial attack.[77–79] Pre-existing literature regarding petroleum contaminated sites indicated that biostimulation stands out to be the best approach in those cases where microbial population gets acclimatised due to exposure to hydrocarbons at contaminated sites.[40,71,80] Thus, in the due course of remediation process, the adapted populations showed higher remediation rates in comparison to those with no contamination exposure history. This is an indicative study reflecting the efficiency of biostimulation of polluted sites. Table 2 has been introduced in the manuscript to show the success of biostimulation in polluted environments.

Table 2 Reports on biostimulation based bioremediation

Nutrients used	Target pollutants
Animal manure and sewage sludge	Atrazine and alachlor[106]
Activated sludge	Atrazine and simazine[107]
Plant residues, ground seed, or commercial meal	Alchlor, metolachlor, atrazine and trifluralin[108]
Cellulose, straw and compost	Atrazine[109]
Cornmeal, ryegrass and poultry litter	Cyanazine and fluometuron[110]
Dairy manure	Atrazine[111]
Maize straw	Methabenzthiazuron[112]
Ammonium nitrate, potassium nitrate and ammonium phosphate	Atrazine[113]
Phosphorus	2,6 Di chloro benzonitrile and atrazine[114]
Nitrate	(R)-mecoprop[115]
Nitrate and phosphorus	Isoproturon[116]
Tryptic soy broth	Dichlofop[117]
Mannitol	Atrazine[118]
Minimal nutrient medium, casamino acid, glucose and phosphate	2,4-Di chloro phenoxy acetic acid, methylchlorophenoxypropionic acid[115]

Advantages and pitfall of biostimulation

The primary advantage of biostimulation is that it is done by native microorganisms that are well suited to the environment and are already well distributed spatially.[41] Secondly, biostimulation is an eco-friendly and cost effective technique which can be performed anywhere[81] and lastly, biostimulation helps in the degradation of contaminants internally which prevents any kind of disturbances to the environment. In spite of its high efficiency in the remediation process, biostimulation has certain well defined disadvantages. The major disadvantage of biostimulation is its dependency on environmental factors that controls its potentiality. Secondly, when the contaminants are firmly engrossed to the soil particles or the contaminant is non biodegradable, then biostimulation cannot be executed. Thirdly, biostimulation is extremely site specific and requires immense scientific observation.[81]

Roles of environmental genomics on bioremediation

Environmental functional genomics is highly significant for understanding the gene arrangement and metabolic properties of microorganisms residing in a particular environment. For an in-depth study of few of the non-cultured, significantly potential microorganisms playing pivotal role in ecological balance, environmental genomics is of great importance.[82] Environmental genomics is essential for those habitats that harbours a wide array of microorganisms involved in the transformation of organic nitrogen, carbon and phosphorus.[83–85] This study enables in understanding and revealing the gene pool of the microbiota associated with the particular habitat.[86,87] In the present times, people have adopted bioremediation and biostimulation as efficient disposal and remediation strategies without having the knowledge about the degradation pathways going on in the process of remediating and eliminating polymers from the environment. This is mostly due to our inability in isolating majority of earth's microorganisms and cultivating them in appropriate media though there is huge advancement in the microbial techniques.[88] Inspite of the molecular techniques there is very less known about the protein coding genes and thereby the microbial diversity existing in the environment.[89] Metagenomics or environmental genomics takes up a significant place in analysing the existing microbial communities.[90] Few of the advantages of environmental genomics or Metagenomics are-helps in the search for new catabolic genes for the degradation of a wide range of xenobiotic, aromatic compounds, etc. It also enables in screening clones capable of expressing certain desired traits on certain appropriate media.[91,92]

Conclusion

The earth is at a severe risk due to rapid industrialisation and urbanisation resulting in serious environmental issues that include global warming, acid rain, eutrophication, loss of microbial functional diversity of soil, depletion in soil quality and much more. Moreover, dumping of toxic waste into the soil like rubber, polyethylene, PCB, etc raised a severe threat against the soil microbial community responsible for several important ecological processes. The most potential disposal strategy among all was believed to be bioremediation. This is because of its eco-friendly and cost effective nature. The current study extensively focuses on the benefits of applying bioremediation using bioaugmentation and biostimulation as a proposed disposal strategy on the environment to compensate the loss incurred as a consequence of the implementation of other disposal strategies.

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None.

Conflicts of interest

The authors declare that there is no conflicts of interest.

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