



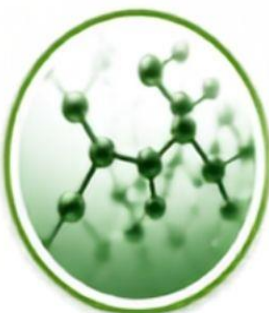
BIOREMEDIATION STRATEGIES

FOR EMERGING POLLUTANTS:

RECENT DEVELOPMENTS AND
SUSTAINABLE PERSPECTIVES



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Bioremediation Strategies for Emerging Pollutants: Recent Developments and Sustainable Perspectives

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ABSTRACT

Recently, environmental pollution caused by xenobiotics and other recalcitrant contaminants is now recognised as a serious hazard to people and the natural environment. The presence of many pollutants heavy metals, polychlorinated biphenyls (PCBs), plastics, and various agrochemicals in the environment is due to their toxicity and persistence (non-biodegradability) in the environment. Bioremediation is becoming a popular clean-up method for toxic waste, from polluted environments. In polluted sites bioremediation employs many microorganisms (aerobes, anaerobes) to treat contaminated sites. In bioremediation, microorganisms are the primary agent in the degradation, detoxification and immobilisation of hazardous wastes and contaminants. The primary objective of bioremediation is to degrade or convert pollutants into less toxic or non-toxic forms. Bioremediation can be accomplished through two approaches: ex situ and in situ. The selection of a proper approach for bioremediation depends on several factors, including costs, types of pollutants, and concentrations. Therefore, a proper bioremediation technique was selected. The review focuses on current advances in bioremediation techniques, including how microorganisms degrade various pollutants, and the potential future direction of bioremediation as a means to reduce the total amount of pollution worldwide.

Keywords: Bioremediation, PollutantsMicrobes, Environment, Sustainable technologies

1.Introduction

The world has seen significant changes in its natural resources due to pollution by industry. Because of this, the quality of fresh water has decreased as a direct result of human activities like mining and the disposal of toxic metal waste from steel manufacturers, battery manufacturers, and power producers, all of which have created serious environmental issues. The various types of waste products such as oil, plastic and heavy metal, all cause damage to the environment. Heavy metals are a form of pollution that naturally occur in large quantities within the lithosphere (Earth's crust) and due to being nonnon-biodegradable, are extremely challenging to decompose. Heavy metals are typically extracted from ores in rock formations, but high-level exposure to heavy metals can also enter the ecosystem. Heavy metals that have entered the ecosystem typically remain toxic for a longer length of time[1]. Many of these pollutants not only affect the environment but also have mutagenic effects on human health. Heavy metals that are absorbed by humans will build up in the brain, liver, and kidneys. In addition to affecting human health, heavy metals have a variety of effects on animals, including increased risk for developing cancer, nervous system damage, slow growth, and even death [2]. Heavy metals can also decrease food quality and quantity by preventing nutrients from being absorbed into the plant, stunting plant growth and impacting other physiological metabolic processes. Contaminated soils are currently being treated with chemical, biological and physical remediation methods; however, physicochemical methods create large amounts of pollution and waste so they are not considered to be good methods to remediate soils [3]. Environmental pollution is being removed using an economically feasible (or viable) and readily applicable (or usable) method; that method is bioremediation, which removes pollutants from the environment [4]. Soil microorganisms play an important role in the following activities: promoting plant development, assisting insect control, conserving soil, recycling nutrients, and controlling pollutants [5]. Bioremediation has improved over time with respect to efficiency, cost and public acceptance [6]. Bioremediation studies are typically centred around bacteria, but archaea also have significant roles to play in bioremediation and can assist with many of the same kinds of processes that are completed with the use of bacteria. Bioremediation will be used to improve

a lot of previously degraded places that have been impacted by an unpleasant environment of an industrial site. Bioremediation can also be used to remove pollutants from hyperthionic, acid, hyperthionic or basic industrial wastes [7,8]. Research shows that using multiple living organisms can increase the effectiveness and outcomes of bioremediation and promote increased diversity of microorganisms available to assist in bioremediation efforts. [8,9]. Researchers worked with bioremediation systems to remove organic compounds as well as inorganic pollution from the environment [10,11,12]. *Aspergillus sydowii* treated samples containing organophosphates like chlorpyrifos, methyl parathion, or profenofos by bioremediation technology while endophytic fungi treated samples containing chloramphenicol [13,14]. Another example involves *Cymbella* sp. detoxifying naproxen- contaminated groundwater, achieving 97.1% efficiency [15]. Bioremediation as an approach was an attempt to put the specific microbial enzymes to work to destroy hydrocarbons by breaking them into less-violent compounds. Various approaches, such as using genetically-modified microorganisms that might eliminate petroleum natural, naphthalene, toluene, benzene, and some xenobiotic compounds, have maintained the latter course of study on and off [16]. Ambient temperature, aerobic or anaerobic conditions, and ecosystem nutrient availability are competing factors influencing bioremediation for effective outcome. Emerging environmental sectors such as persistent organic contamination, whereby heavy metals, toxins, and air pollutants of both synthetic or natural origin are introduced into ecosystems mainly through anthropogenic activities, present significant threats to plants, animals, and humans [17]. Bioremediation is one of the most cost-effective and environmentally friendly biotechnological developments. In waste management, bioremediation is the predominant method. Bioremediation has the ability to break down persistent organic pollutants that are difficult to degrade, and these pollutants are considered to be alien biological materials. This review discusses the current methods being used in bioremediation, as well as the new advances in bioremediation techniques to achieve environmentally friendly detoxification and the ultimate breakdown of many different organic and inorganic pollutants used to control environmental contamination.

2. Microbial approaches to Bioremediation

Microorganisms play an important role in keeping biological equilibrium by being a part of nutrient cycles. The nature of bioremediation means to utilize certain groups of organisms including bacteria, algae, fungi and yeast to clean the environment of pollutants [18]. Microorganisms have been found to grow in extremes from -196 degrees Fahrenheit (-127 Celsius) up to 1200 degrees Fahrenheit (600 degrees Celsius) on the presence of hazardous materials and waste streams to be remediated. They are ideal candidates for remediation because they have a vast ability to adapt to various conditions as well as forms of life [19]. Carbon is an essential nutrient for all types of microorganisms. In performing bioremediation, many different types of microbes can be used from a variety of habitats; these include *Achromobacter*, *Alcaligenes*, *Xanthobacter*, *Arthrobacter*, *Pseudomonas*, *Bacillus*, *Mycobacterium*, *Corynebacterium*, *Flavobacterium* and *Nitrosomonas* [9].

2.1. Aerobic

Many different kinds of environmental contaminants can be bioremediated by various types of microorganisms in an aerobic environment. For example: *Bacillus*, *Pseudomonas*, *Sphingomonas*, *Flavobacterium*, *Nocardia*, *Rhodococcus* and *Mycobacterium* are examples of aerobic (oxygen-loving) bacteria that can break down many different complex organic materials [20]. Microorganisms have been shown to degrade pesticides, alkane hydrocarbons and polycyclic aromatic hydrocarbons. Many microorganisms use the contaminants they degrade as their carbon and energy source [21]. In the aerobic bioremediation process, oxygen acts as the limiting factor for the microorganisms' growth.

2.2. Anaerobic

Bioremediation involves using microorganisms to clean up environmental pollution from chemicals such as polychlorinated biphenyls, chlorinated solvents, etc., through natural metabolic processes. As more organizations search for environmentally friendly ways to remove pollutants from industrial waste, using amphiphilic bacteria that biodegrade and convert pollutants into non-toxic compounds is becoming more common in bioremediation, including both anaerobic and aerobic processes. Examples of such microorganisms include *Pseudomonas*, *Aeromonas*, sulfate-reducing bacteria, etc., which can function effectively under anaerobic conditions [22]. Garg and Tripathi conclusively reported that azo dye degradation occurs through anaerobic reductive biochemical processes based on the reducing potential of these bacteria by producing electrons through the oxidation of organic substrate(s) [23]. In anaerobic conditions and through controlled conditions, the effective color removal of azo dyes should also depend on the electrochemical properties of the microorganisms performing decolorization [24]. In an industrial landscape, azo dye will continue to be decolorized anaerobically in moving forward, and

through controlled decolorization events, organizations will progressively accumulate time-variant decolorized metabolite(s) (DMs). However, the external accumulation of time-variant DMs created through controlled processes will allow for improved research [23].

3.Environmental and Biological Factors Affecting Bioremediation

The use of microorganisms (e.g. bacteria, algae, fungi) and plants is known as bioremediation. This employs natural organisms to break-down, change, remove, isolate or immobilize toxic chemicals from contaminated/protected environments. Microorganisms use chemical processes known as enzymes to speed-up the breaking-down of contaminants through chemical reactions [25,26]. For microorganisms to be able to break-down contaminants, they must make contact with certain substrates that will provide the energy and nutrients required for their growth. Various factors can affect the process of bioremediation, which include: physical, chemical, biological, soil type (e.g. sandy vs clay), the nature of the X source (carbon/nitrogen), and the type of microorganisms (e.g. single vs consortium) [27]. Microbial consortia exhibit multifunctional capabilities along with resilience qualities through different species working together to optimally utilize all available substrates; thereby producing more efficient bioremediation than a single microbial organism [28]. According to a study on bioremediation, carbon is among the key nutrients to facilitate in situ bioremediation through stimulating metabolic processes within natural microbial communities and assisting in accelerating the rate of bioremediation for the breakdown of currently present contaminants. On top of being amongst the main nutrients, bioremediation will use more than any other type of additive, organic carbon. Organic carbon can ferment and produce hydrogen gas through anaerobic microorganisms [29]. During another study on bioremediation, soil type was identified as having a major impact on bioremediation and removal efficiencies for contaminants were different with waste products being removed better from sandy soil than from clayey soil [30]. For bioremediation to succeed, there needs to be proper access to the microbial population that was responsible for removing contaminants as well as the physical and chemical properties of the environment, as displayed in (Table 1) which outlines what type of contaminants exist and what are the soils they are located in and/or what is the depth of the contaminant and how accessible it will be.

Table 1. Crucial Parameters for Effective Microbial Bioremediation.

Factors	Remarks	References
Biological Parameters	Microorganisms in soil compete with each other for sources of carbon and they also predate on protozoa and on bacteriophages, therefore they can influence the rate of degradation of organics. The rate of derivatization will be affected by the amount of contaminants and the levels of catalysts. The rate of contaminant biodegradation will also be increased or decreased by the presence of expressed enzymes in the soil. Additionally, expressed enzymes that are involved with the metabolism of the contaminant must have an affinity for the contaminant and be in sufficient availability for the enzymes to increase or decrease the oxidation of the contaminants. The biological factors that are of primary concern are: interaction (competition, predation, and succession)/population size/population composition.	[31,32]
Nutrients	Microbial growth and development as well as the rate and efficiency at which microorganisms biodegrade organic matter are influenced by the availability of nutrients. The bacterial C:N:P ratio must be optimized for improved performance with regard to the biodegradation of organic matter, particularly when essential nutrients (e.g., N and P) are present. The microorganisms need nutrients such as carbon, phosphorous, and nitrogen for growth and metabolism; the ability to degrade hydrocarbons in a nutrient-limited environment will also be affected by the amount of hydrocarbon present. When nutrients are added to cold environments, the metabolic activity of the microorganisms will increase, thereby increasing the biodegradation rate. The availability of nutrients in aquatic environments is a limiting factor on the rate of biodegradation.	[33,34]
Oxygen	Organisms that decompose without the use of oxygen help increase biodegradation rates. Since most biological organisms need oxygen for living, anaerobic decomposition happens where most of their waste will occur. Adding air to most will speed up hydrocarbon metabolism.	[35]
Moisture level	To meet their growth targets, microorganisms need adequate moisture. Excess moisture will diminish biodegradation processes.	[36]

Temperature	Temperature is the primary environmental factor affecting the ability of microorganisms to grow and survive, as well as the characteristics of hydrocarbons. In colder areas of the world — such as the Arctic — the rate of natural oil degradation is slow, meaning that microbes have a greater 'burden' to clean up an oil spill than in warmer areas. Also, when temperatures drop below freezing, the subzero water causes the microbial transport pathways to be frozen; therefore, it is almost impossible for these organisms to perform their normal metabolic functions. The degradation of various compounds occurs at various temperatures because the temperature regulates the metabolic turnover of the enzymes responsible for the degradation process. In addition, temperature affects microbial physiological characteristics, and generally increases the rate of bioremediation. The rate of microbial activity typically increases with increased temperature, then declines sharply when the temperature increases or decreases to a point that thermodynamically limits the capacity for microbes to continue functioning.	[37,38]
PH	The pH of a given compound affects the metabolic activity of microbes and will in turn affect the ability to remove that compound from the environment. The pH of soil, and thus the acidity/alkalinity of the soil, may be used as an indicator to predict microbial growth. pH changes of even a small level may significantly affect microbial metabolic processes.	[39]
Metallic ions	Metals are important for both bacteria and fungi; however, excess amounts of metal can inhibit metabolic processes within cells. Both direct and indirect effects of metals in the environment influence how quickly compounds degrade.	[40]
Microbial Organism	The presence of high levels of some toxic substances may be detrimental to microorganisms, thereby inhibiting their ability to facilitate the decontamination of an area. The degree of toxicity is influenced by the type of toxic substance, its concentration and which microorganisms are present.	[41]
Site Evaluation and Selection	A thorough investigation is required prior to suggesting any bioremediation remedy; this investigation will determine the extent of contamination at the site. The actual procedures used to select a site include locating both the horizontal and vertical extents of contamination, determining what parameters will be measured and where sampling will occur, and describing how samples will be collected and analyzed.	[42]

4. Fundamental Principles of Bioremediation

Bioremediation is when organic waste materials are broken down biologically in a controlled manner. Using bioremediation allows for the biological degradation of harmful materials and/or detoxification through the provision of nutrients and other chemical substances to allow organisms to optimally function. Each metabolic stage has a number of enzymes that play important roles in their respective stages [24,43]. Enzymes belong to the oxidoreductases, lyases, transferases, and hydrolases groups. Many enzymes have non-specific and specific substrate affinities, allowing them to be able to degrade a wide variety of substrates. In order for bioremediation to be a successful entity there needs to be an enzymatic influence to degrade the pollutant. Environmental factors need to be adjusted to enhance microbial growth and degradation, during the bioremediation process, as this process only takes place if the environment is conducive to the growth and movement of microbes [38,43]. Bioremediation occurs naturally and supports the use of biodegradable products and fertilizers to enhance the process. A major aspect of bioremediation technology is through the biodegradation of harmful organic pollutants, such as carbon dioxide and water to non-toxic or naturally occurring inorganic compounds that are safe for use by humans, plants, animals, and aquatic life [44].

5. Classification of Bioremediation Techniques

Bioremediation can be applied in different ways and some of the most common methods used are presented here (Figure 1).

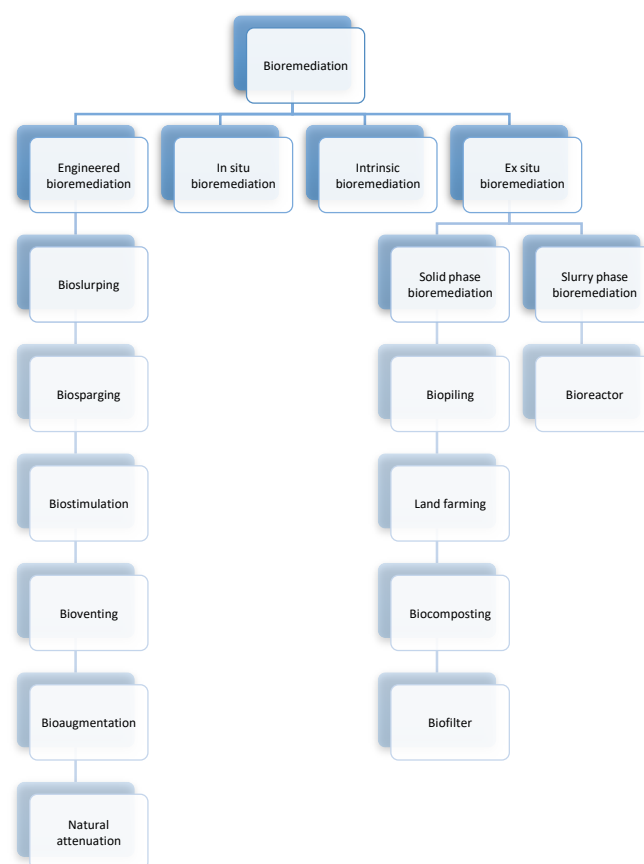


Figure 1. Various Bioremediation Techniques.

Bioremediation is an environmentally safe and modern technique used to clean up and remove contaminants from the environment. It provides a very cost-effective, reliable, and environmentally friendly means of cleaning up our planet. Many new methods have recently been developed and now are included as part of the bioremediation process. Bioremediation can be performed using either an in-situ or an ex-situ method; however, ex-situ bioremediation is much more costly, requires more equipment, and takes longer to complete than put in an appropriate form. This is why the in-situ method is usually the preferred bioremediation method. There are several limitations that can be associated with bioremediation methods, as indicated in Table 2.

In-situ bioremediation refers to "in-place" methods for treating contaminants directly at the source of the contamination without necessarily excavating or removing the contaminated matrix (i.e., soil or water). In the case of mass pollution, this is typically favored over other methods because it is a more economical option and creates less impact to surrounding areas [45].

Ex-situ bioremediation refers to removing the contaminated material (i.e., excavating the contaminated soil or pumping out contaminated groundwater) and treating that material at a different location from where it was originally found [46]. Ex-situ bioremediation methods are generally less efficient and more disruptive than in-situ methods, but they allow for greater control over environmental parameters and can often speed up remediation time[45].

Table 2. Challenges and Limitations of Bioremediation technique.

Methods	Limitations	References
Biopile	Weathering can change the chemical form through the creation of more hydrophobic materials, thereby limiting biopiling's potential for biodegradation.	[47]

Windrows	The main issue of studying lenticular forms in-situ, however, may be that they can be very difficult to forecast. There has been some success with forecasting certain sub-meso-scale convergences; however, litter windrows have a far greater issue due to their dependency on litter loading as this creates an additional source of uncertainty.	[48]
Bioventing	Although this technique is effective, it can only be applied to treat very contaminated soil ecosystems by utilizing the lowest layer of it.	[49]
Bioreactor	A factor limiting membrane bioreactors' (MBRs) use at high mixed liquid suspended solids (MLSS) levels appears to be the very low to nonexistent amount of oxygen being transferred to the MBR when using standard diffused aeration systems (such as fine or coarse bubble diffusers). Therefore further study is needed to determine the limitations placed on traditional bubble diffusers (measured in terms of alpha factor) under the specific combination of operational parameters (high MLSS).	[50]
Landfarming	One disadvantage of this technique is that the results of the objectives set forth within the constraints have to be strictly complied with in order to not be seen as impossible solutions. Organic waste can cause difficulties by causing anoxic conditions at times that are detrimental to the growth of plants. To keep the pre-existing soils in good condition, it is usually recommended not to add more organic matter to the soil over time.	[51]
Phytoremediation	The phytoremediation approach took the form of phytoextraction and rhizodegradation to restore surface-polluted soils in surface soils. This method can take a significant amount of time and will not remove all contaminated soils.	[52]
Intrinsic in-situ bioremediation	Another major limitation on in situ bioremediation is that the subtype of remediation technique requires soil with good permeability for both sub-surface and in-situ bioremediation.	[53]

6. Bioremediation Strategies for Various Contaminants

6.1. Bioremediation Strategies for Organic Pollutants

As a result of their current prevalence and levels in the environment, organic chemicals (OCs) such as flame retardants and biocides pose a serious danger to virtually all living organisms on Earth. Typically, nearly all OCs are subject to microbiological degradation; for example, polychlorinated biphenyls (PCBs), polybrominated biphenyl ethers (PBEs), and polycyclic aromatic hydrocarbons (PAHs) would not be considered "permanent contaminants" because they can all be degraded by microorganisms into either lesser-toxicity or total innocuous products through the biological process known as biodegradation [54]. In order to gain organic carbon and energy, microorganisms consume the organic content. Usually, an isolated individual microbial species is unable to degrade an organic substrate [55], yet has a high level of success as part of a microbial community. Microbial communities also confer resistance, ability to degrade chemicals, and tolerance through the transfer of genetic materials between species within the community. Due to the lack of internationally endorsed standards and methodologies for the identification of microorganisms, many OC-degrading microorganisms are misidentified [56].

For each substrate, the identification of the appropriate species/strains of microbes is essential for the successful bioengineering of the microbe. Biodegradation by naturally occurring organisms, when provided with an abundant supply of available organic carbon and energy in the environment, is an excellent alternative to the use of recombinant techniques to degrade resistant organic materials. Microbial growth and proliferation are enhanced by the fluctuations in the chemical gradients of their environment, allowing them to maximise their growth in an optimal environment [57].

6.2. Bioremediation Strategies for Inorganic Pollutants

Mining, power generation, metallurgical industries and chemical manufacture all give rise to common inorganic contaminants. Examples of contaminants resulting from those emissions are toxic chemical elements and/or their compounds [58]. Due to the fact that it is unacceptable to deposit toxic elements into surface soils or waters, the concern of toxic element disposal and its associated health consequences, is one of the key issues for those who are concerned with the environment [59]. Microorganisms cannot degrade metals that are in ionic form; however, it is important to recognize that the microorganisms typically transform a metal's oxidation state in order to achieve stabilization of it as an ionic element. The metabolism and detoxification of all biologically available elements by microorganisms is the same as the effects of any other nutrient within a microbial cell [60]. Microbes' ability to adapt to the presence of heavy metals (e.g., iron, zinc, chromium, magnesium, mercury, and barium) in textile waste was illustrated by the multidrug-resistant strain of *Pseudomonas aeruginosa* T-3 isolated from tannery effluent [61,62]. These findings support the idea that microorganisms adapt to fluctuating conditions in their environment. Evidence supporting this includes the use of a plasmid-encoded gene for copper and cadmium metal resistance found in the bacterium *Pseudomonas putida* PhCN [63]. Using both plasmid-based and biochemical techniques derived from the metallothionein gene (*Neurospora-oderiacrasa*) for Cd uptake enabled the creation of recombinant *Escherichia coli* allowing cadmium uptake; the result was very rapid cadmium accumulation compared to the donor organism [64]. Using plasmids, researchers created a gene that encodes for a peptide containing multiple histidine residues (a so-called polyhistidine peptide). This peptide was incorporated into both *Staphylococcus carnosus* and *Staphylococcus xylosus* genes allowing these bacteria to fix nickel ions from solution [65].

7. Recent Developments and Challenges in Bioremediation

7.1. Bioinformatics based Strategies for Bioremediation

Bioremediation is a method of waste management that focuses on the cleanup of contaminated areas or sites using biological agents. It is specifically related to using an organism to either eat or neutralize pollutants [20]. Bioremediation is an attempt to use different biological databases (such as chemical structural and compositional databases, RNA/protein expression databases (Bioinformatics), organic compound databases, databases of catalytic enzymes, microbial degradation pathway databases, and comparative genomic databases) to determine the mechanism of degradation that will occur to a pollutant when an organism that will perform bioremediation performs the bioremediation process on the pollutant [66]. To study bioremediation and create better technologies for cleaning up the environment, a variety of bioinformatics tools are used to analyze the data from each of these different sources. This is important because over time there has been a lack of information regarding the influencing factors of the growth and metabolism of microorganisms that can perform bioremediation, therefore there have been a number of applications in this area that have been unsuccessful. Microorganisms that perform bioremediation have been characterized, and thus the mineralization pathways and mechanisms have been mapped out through the use of bioinformatics [67]. This research crosses over both computer science and biology, in particular computers are used to store (computer databases of DNA), manipulate (certain sequences of DNA, RNA, and proteins), and access (the completed genome (i.e., DNA, RNA, Amino Acids) of an organism) from databases of DNA and RNA for bioremediation research [65,68].

7.1.1. Omics Based Tools for Bioremediation

Bioremediation research may benefit from using genomics, transcriptomics, metabolomics, and proteomics because these technologies show how well DNA sequence correlates with levels of metabolites, proteins, and mRNA. These tools can be used to evaluate the effectiveness of an in situ bioremediation process [69,70] (Figure 2).

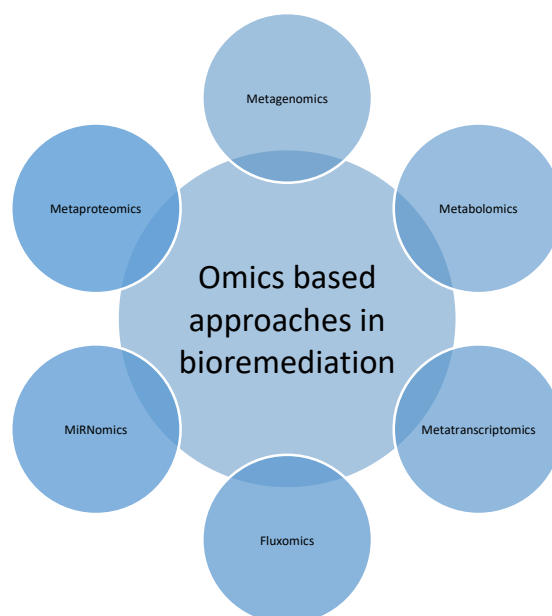


Figure 2.Omics based approaches for the Bioremediation of Environmental Contaminants.

7.1.2. Genomics

The microbial bioremediation field is a new area of genomics where we can analyze microorganisms' ability to utilize all their genetic information to inform us about their behavior. Bioremediation relies upon a number of different types of microorganisms [71]. Different genomic tools can assist us in understanding the processes of biodegradation. These genomic tools include; PCR, isotope distribution analysis, DNA hybridization, molecular connectivity, metabolic footprinting, and metabolic engineering. To perform genotypic fingerprinting, there are many PCR-based fingerprint methods such as amplified fragment length polymorphisms (AFLP), amplified ribosomal DNA restriction analysis (ARDRA), automated ribosomal intergenic spacer analysis (ARISA), terminal-restriction fragment length polymorphism (T-RFLP), randomly amplified polymorphic DNA analysis (RAPD), single strand conformation polymorphism (SSCP), and length heterogeneity [72]. RAPD can be applied in studying the soil microbial community by detecting bacterially related microorganisms, developing functional structural models, and creating genetic fingerprints [73]. LH-PCR can be utilized to measure the natural length variation among/within SSU rRNA genes in microbial communities. Different microbial taxonomic groups can be profiled simultaneously using T-RFLP [74]. There are many different molecular techniques that researchers commonly use to assess the relationship between soil microorganisms and their environment. Examples of molecular techniques include: quantitative reverse-transcription polymerase chain reaction (qRT-PCR), stable isotope probing (SIP), fluorescence in situ hybridisation (FISH), microradiography (MR), and genetic fingerprinting (GF).

7.1.3. Transcriptomics and Metatranscriptomics

The transcriptome provides information on the cellular phenotype, interactome, genome, proteome and therefore can be viewed as the critical link between them. It supplies the number of genes that were being actively transcribed for specific time and environmental conditions, and therefore, helps us understand how the control of gene expression is crucial in adapting to changes in the environment for survival. Transcriptomics allows us to view the entire human genome at the same time, and we can do so by using DNA microarrays which are quite powerful in determining mRNA expression levels [75]. In order to conduct a transcriptome analysis, one would first isolate and enrich all the mRNA, then synthesize cDNA from the mRNA and then sequence the cDNA transcriptome. Utilising DNA microarrays provides one with the ability to analyse and study almost every gene in an organism's mRNA expression profile [76]. Transcription mRNA profiles, or transcriptomics / metatranscriptomics are important as they provide functional insights into the activities of environmental microbial communities [77]. By using metagenomics, genome binning and metatranscriptomics throughout the

entire biodegradation process, to observe and study the relationships developed through syntrophism of various microbes as well as those developed through complementary metabolic pathways [78]. Metatranscriptomics is one method that can be used to observe gene expression by scientists conducting environmental microbial community research [79].

7.1.4. Proteomics and Metabolomics

In contrast to metabolomics, which looks at all metabolites made by an organism in a specific time frame or environment, proteomics is concerned with all proteins expressed in a cell at a given time and location [80]. Proteomics has been used to study protein abundance, looking at how composition varies over time, and identify the key microbes of those proteins [81]. In addition to genomics, functional analysis of microbial communities will prove to be more effective and predictive. There are two ways to use metabolomics to assess biological systems. The first method does not require any prior knowledge of the biological system's metabolic pathways; as long as there are enough metabolites in the sample collected, they can all be recovered and analysed to give a lot of data allowing us to make connection between some of the samples through metabolic pathways. The second approach is to do targeted studies to determine specific metabolic pathways or specific biomarkers based on previous research [82]. Numerous techniques such as metabolite profiling, foot printing and target analysis are used to identify and quantify the many types of metabolites produced by microorganisms as part of microbial metabolomics [83]. Both the proteome and metabolome will be used to assess potential for bioremediation without the use of cells.

7.2. Nanotechnology Based Approaches for Bioremediation

Nanotechnology uses a unit of measure called a nanometer, which is the tiniest measurement unit. Nanotechnology's capabilities enable the removal of many toxic materials due to its special features when dealing with other hard-to-remove contaminants. Another area where this view toward the use of nanotechnology is found is in the way we treat water. Nanofiltration has arisen as an environmental-friendly approach for water treatment, thanks to advances in nanotechnology [83].

7.2.1. Microorganism and Nanotechnology

Using effective organisms (EM) for water treatment will allow farmers to use effluent from EM water treatment for irrigation purposes [84]. Nanotechnology and EM have both been used to treat drinking water as well. Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds that have multiple fused benzene rings and are found in the environment everywhere due to the presence of most toxic recalcitrant organic pollutants in the global environment, i.e., PHA's [85]. In Ramos et al. [86], researchers successfully used whole fungal cells (i.e., *Trichoderma* spp.) to produce and extract silver nanoparticles for various applications.

7.2.2. Bioremediation of Hydrophobic Pollutants Using Engineered Polymeric Nanomaterials

The interaction between organic pollutants, including petroleum hydrocarbons & polycyclic aromatic hydrocarbons (PAHs), with the soil surface decreases their available & mobile forms, decreasing the overall impact of these pollutants on the environment. Polymeric nano-network particles (PNPs) increase both the solubility of phenanthrene (C₁₄H₁₀) as well as the lability of phenanthrene to be removed from contaminated aquifer media [87]. Polymeric nanoparticles (PNPs) are manufactured by using precursor chains from polymeric (ethylene) glycol modified based urethane acrylate (PMUA). PMUA nanoparticles are designed to preserve their physical characteristics in the presence of a wide range of species found in a bacterial population [88].

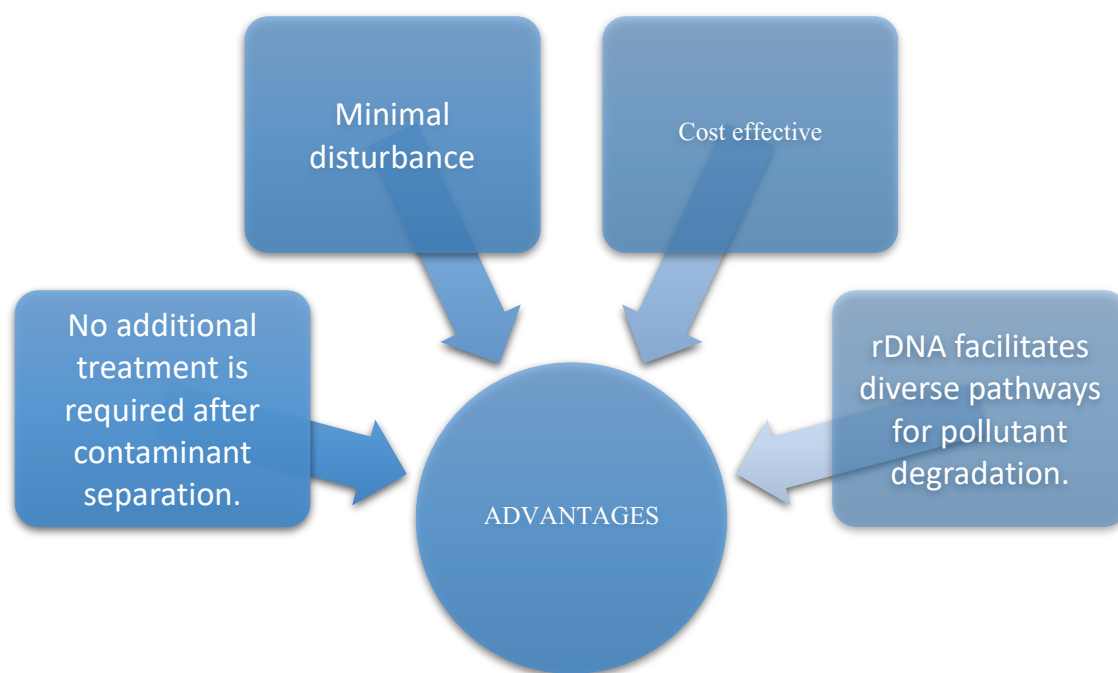
7.3. Genetic and Metabolic Pathway Engineering

Gene Editing" refers to scientific technical developments that enable the rational development of genetically-created fragments at the level of the entire genome that incorporate a specific addition, deletion or substitution of DNA molecules. Transcriptional activators, such as TALENs, ZFNs and CRISPRs, each have transcriptional activators used in multiple kinds of gene editing methods (researchers have reported that CRISPR-Cas represents the most effective and simple method to edit gene expression) [89]. The DNA-binding domain of TALEN consists of the host's specific complementary sequence (to the DNA-binding element of TALEN). Once the TALEN binds to DNA, it creates double-strand DNA breaks (or DSBs) by exposing sticky ends in order to stabilize corner stub structure of DSBs. In contrast to TALEN, ZFNs also have a DNA-binding domain that consists of a 30-

amino acid sequence. The Fok1 cleavage domain of ZFNs is responsible for the generation of DSBs at the target sequence of the host. A fresh molecular perspective on the use of the composite endonuclease of TALEN and ZFN nucleases toward solving the molecular problems associated with DSBs was warranted[90,91].The CRISPR-Cas system is characterized by two major attributes: 1) the presence of complementary sequence similarity and 2) the ability to carry out multiple gene editing processes at one time[92,93].So-called "Streptococcus pyogenes" (the cause of strep throat) contributes its own form of "resistance" against viruses through a CRISPR-Cas system, which uses a guide RNA to link crisper-derived RNA (bCRRNA) with the trans-acting anti-sense RNA (bdrRRA) that helps target the CRISPR-Cas9 enzyme to the desired site for genetic modification ; therefore, creating double-stranded breaks (DSBs) in the target DNA sequence at the specific site where bGRNA identifies. The work being conducted on the knock-in and knockout effects of these tools is currently being examined for potential applications in bioremediation studies (see below, sections 15.1-3, p. 11-55)[93]. Researchers have accepted CRISPR-Cas systems as viable tools for genetic manipulation in model organisms such as Escherichia coli and Pseudomonas aeruginosa. Bioremediation studies also use the CRISPR-Cas system to explore new ways to create gRNAs to produce specific target genes in non-model species such as Rhodococcus ruber TH, Achromobacter sp. HZ01, and Comamonas testosteroni [94].

8.Advantages and Disadvantages

Bioremediation offers a range of advantages and disadvantages and limitations including;



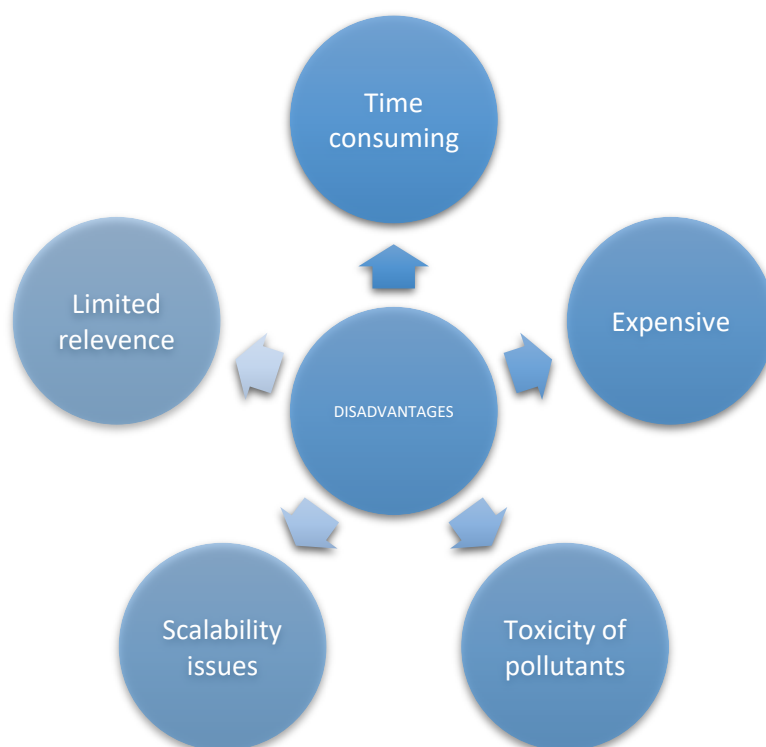


Figure 3. Advantages and Disadvantages of Bioremediation.

9. Future perspectives and Conclusion

As a growing field, omics will play a prominent role in many industries including microbial remediations of pulp and paper, textiles, food, dairy, wood, fisheries, water and soil treatment, waste remediation, remediation of heavy metals and hydrocarbons. There must be an effort to mine bioremediation data to understand the different pathways of degradation as well as the use of new algorithms to easily assimilate bioremediation data into simulation and numerical models will require standardized protocols that encompass the collection, re-positioning, exploration, and transmission of bioremediation. New biomarkers would assist researchers in better understanding bioremediation processes. The study of using all the omics data combined with genetic engineering techniques on the microbial remediation process would create a much clearer understanding of this process. The study of phytoremediation's contribution to reducing environmental pollution would also benefit this field of study. Phytoremediation offers several benefits in comparison to other types of remediation processes including lower costs, greater public support, and higher capacity to degrade the pollutants [95]. Groundwater and air pollution, as well as toxic waste by-products generated during the manufacturing of semiconductors, are major environmental concerns; examples include: glycol ethers, hydrochloric acid (HCl), xylene, hydrogen fluoride (HF), and methanol [96]. The pharmaceuticals that will be created to maintain their chemical structure and morphology for long periods of time or that will never break down make them a unique environmental threat. Pharmaceutical contaminant refers to contaminants that persist in the environment for long periods of time. Many urban and rural sources of groundwater are indeed contaminated with trace amounts of various medicines, including; contraception, anti-convulsant drugs, analgesics, and antidepressants [97]. Solar facilities emit fewer GHGs, even including air pollutants (such as carbon monoxide, volatile organic compounds, nitrogen oxides and carbon dioxide) than traditional fossil fuel facilities during operation [98]. Plants modified by biotechnology can bioremediate certain contaminants through molecular processes that are specific to the plant's metabolism; however, the location of the enzymes, genetic sequences or operons required to create the bioremediative process is currently unknown [99]. Even though genomics, metabolomics, and proteomics are used to find potential solutions for specific pollutants, next in bioremediation research is finding and comparing gene and protein sequences that remove pollutants. GMOs can clean up much waste water and polluted land [100]. Bioremediation can be an additional method along with

other physical and chemical methods to remove pollutants from the environment, and because it appears to be a long-term solution, there needs to be more exploration in this area.

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BIOREMEDIATION STRATEGIES FOR EMERGING POLLUTANTS:

Recent Developments and Sustainable Perspectives



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