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Review

Sustainable and Advanced Strategies for Bioremediation of Highly Contaminated Wastewater

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Abstract

The risk of contamination of the vital resource of water continues to increase and represents an urgent problem for modern society. Globalisation, industrialisation and technological progress have led to the need to treat more and more wastewater streams before they can be released into the environment. A high chemical and biochemical oxygen demand as well as the sum of dissolved and suspended organic and inorganic components are the main characteristics of highly contaminated wastewater. Research into environmentally friendly and sustainable technologies is becoming increasingly important in wastewater treatment. Bioremediation utilises the ability to restore the biogenic elements of the environment and is an environmentally friendly method for removing contaminants from the surrounding ecosystem. Forming microbial consortia that exhibit both excellent biosorption properties and a high resistance to toxic conditions is crucial for the biodegradation of complicated systems, such as highly contaminated wastewater. The development of systematic biological molecular tools can further improve the bioremediation process. By integrating innovative technologies with the already existing natural microbial capacity, it is possible to further improve the sustainability of biological treatments of wastewater streams while preserving the natural environment.

Keywords: highly contaminated wastewater; bioremediation; natural and synthetic potential for biodegradation

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1. Introduction

In light of increasing ecological challenges, the issue of water contamination is moving into the focus of science and society. The growing world population combined with intensive industrial development leads to the generation of highly contaminated wastewater, including from the distillery, brewing, paper, and pharmaceutical industries, as well as landfill leachate [1,2]. This wastewater is contaminated with various substances such as nutrients containing nitrogen and phosphorus, heavy metals, pharmaceuticals, and pathogenic microorganisms. Ecosystems and human health are seriously jeopardised by such contaminants. The high concentrations mentioned above, combined with their long-term stability and resistance to natural degradation processes, are a prerequisite for the development of efficient wastewater treatment processes that comply with increasingly stringent environmental regulations [3]. Addressing this challenge effectively requires the use of advanced technologies and methods as well as a comprehensive assessment of their environmental and economic sustainability in the long term [4,5].

Among the various treatment approaches, bioremediation is characterised by its ecological compatibility, effectiveness, and cost efficiency [6]. The innovative technology described below serves as an environmentally friendly alternative to conventional physico-chemical treatment approaches, which are often associated with the use of hazardous chemicals and high energy consumption [7]. In addition, bioremediation offers a financially viable solution for wastewater treatment as it eliminates the need for expensive equipment and infrastructure [8]. The value of bioremediation lies in its ability to adapt to different ecological conditions and contaminants, making it incredibly versatile in tackling pollution problems. This approach not only helps to clean up highly contaminated wastewater but also promotes the regeneration of ecosystems and the restoration of natural water purity and biodiversity [9].

Microorganisms such as bacteria, fungi, or algae use their specific enzymatic potential to metabolise the contaminant and convert it into non-toxic products and by-products. Certain microorganisms are particularly good at metabolising heavy metals, including *Pseudomonas aeruginosa*, *Geobacter* spp., *Cunninghamella elegans*, and *Rhodopseudomonas palustris*. Others, such as *Bacillus subtilis*, *Pseudomonas putida*, *Gleophyllum striatum*, and *Penicillium chrysogenum*, mainly metabolise organic compounds such as pesticides or petroleum derivatives [10]. Understanding the interactions between microorganisms and contaminants serves as a basis for the effective implementation of the biodegradation process [11]. In addition, monitoring systems of interest enables the rapid detection and resolution of potential problems resulting from changes in the composition and contamination of wastewater [12].

Although the primary mechanism of bioremediation is a biological process, scientific and technological developments are essential for its optimisation and effective management. Interdisciplinary collaboration between microbiologists, chemists, technical engineers, and IT experts facilitates the development of comprehensive solutions that can effectively address the complex wastewater problem. The use of biotechnological innovations makes it possible to analyse the genetic material of microorganisms in detail, which leads to a better understanding of their biodegradation ability [13]. Genome editing technologies and synthetic biology offer new opportunities to identify and enhance the bioremediation potential of microorganisms, which can improve the biodegradation of contaminants under controlled ex situ conditions [14].

The main objective of this study is a thorough review of wastewater management, focussing on highly contaminated wastewater in different forms. It examines how natural and technologically improved methods can help to increase the efficiency of their treatment. From a bioremediation perspective, it will examine how specific microorganisms, whether naturally occurring or genetically modified, can be the key to success in the specific treatment of wastewater. While there are several reviews on bioremediation, rapid environmental challenges and recent technological advances call for an updated and focused review that specifically addresses the complexity of highly contaminated wastewater characterised by high contaminant loads and resistance to natural degradation. The aim is to provide a comprehensive overview of existing and potential treatment approaches and to emphasise the importance of an integrated approach that bridges natural and advanced biotechnological methods. Understanding and improving bioremediation will not only have a positive impact on the environment but will also enable more sustainable management of water resources in the future, while identifying knowledge gaps and future research directions to support practical applications.

2. Highly Contaminated Wastewater

To ensure that emissions do not exceed the specified limits, wastewater must be treated thoroughly and in compliance with legal requirements before being discharged

into the natural environment. Knowledge of the composition of wastewater makes it possible to treat certain types of wastewater in a targeted manner [4,5]. Wastewater can be categorised according to its origin into sanitary, industrial, rainwater, and leachate, while according to its degree of contamination into low, medium, and highly contaminated wastewater. This categorisation facilitates the understanding and procedure for wastewater treatment. The chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH value, temperature, colour, odour, concentration of suspended solids (TSS), and total nitrogen (TN) and phosphorus (TP) are used as indicators for the condition of the water. Analysing these indicators provides information about the water properties and changes in composition [3,15].

Various methods are used for biological treatment: biofilters, anaerobic bioreactors, membrane bioreactors, sequencing batch reactors, lagoons, etc. [16]. In wastewater treatment, various aerobic and anaerobic processes are used to remove dispersed and dissolved organic substances, depending on the origin and composition of the wastewater. Membrane bioreactors and activated sludge processes are amongst the two commonly used aerobic processes for wastewater treatment. Anaerobic processes are more effective in treating highly contaminated wastewater (COD > 3000 mg O_2/L), produce less sludge due to slower microbial growth, and recover energy from methane produced as a by-product of the anaerobic degradation of organic matter. However, anaerobic treatment processes are less efficient than aerobic processes, nitrogen compounds cannot be oxidised, and the process is very sensitive to slight changes in process parameters [3,17].

2.1. Leachate

Leachate is wastewater produced by leaching and various biochemical processes from waste materials over a certain period of time. This wastewater may contain large amounts of easily and poorly biodegradable organic matter, with humic substances being an important category. It may also include nutrients such as nitrogen and phosphorus compounds, as well as contaminants such as heavy metals and plasticisers [18]. Microorganisms utilise the easily accessible dissolved biodegradable organic compounds through enzymatic reactions. In addition, enzymes help break down solid organic material, releasing highly bioavailable compounds. The hydrolysis of biowaste and the dissolution of organic and inorganic molecules can contribute to the dispersion of substances into the liquid phase [19]. Microbial cells, including bacteria and fungi such as *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas* sp., *Staphylococcus aureus*, *Staphylococcus xylosus*, *Staphylococcus hominis*, *Staphylococcus warnerii*, *Streptococcus* sp., *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., and *Fusarium* sp. have been isolated and found in landfill leachate according to the literature reports [20,21].

2.2. Distillery Wastewater

Wastewater from distillation plants is characterised by a complex combination of organic and inorganic contaminants that remain in the environment over the long term. These contaminants include phosphates, sulphates, phenols, phthalates, and heavy metals such as iron (Fe), chromium (Cr), lead (Pb), and nickel (Ni). They are known to have mutagenic, genotoxic, and endocrine-disrupting effects. Due to the factors mentioned above, including the high concentration of dissolved solids (over 23,000 mg O_2/L) and the increased chemical and biochemical oxygen demand, treatment of this wastewater before discharge into the environment is mandatory. To facilitate the biodegradation of such complex wastewater systems, it is important to cultivate a microbial consortium that exhibits both heavy metal tolerance and biosorption capabilities, promoted by the ratio of cell wall surface area to active chemisorption sites. These prerequisites are possessed

by some strains of the genera *Micrococcus* sp., *Flavobacterium*, *Pseudomonas* sp., *Bacillus* sp., and *Enterobacter* sp. [22,23]. Tripathi et al. [24] investigated the effects of indigenous *Klebsiella pneumoniae* from distillery effluents and bioaugmented *Enterobacter cloacae*. The results showed the exceptional metabolic affinity of these species towards fatty acids, carbohydrates, and other hydrocarbon compounds present in distillery effluents.

2.3. Pharmaceutical Wastewater

The pharmaceutical industry produces highly contaminated wastewater characterised by residues of unreacted chemical precursors, antibiotics, drugs, endocrine disruptors, antidepressants, beta-lactams, fat regulators, cosmetics, detergents, and more [3,9]. These contaminants have extremely dangerous and toxic effects on the environment, especially on the aquatic ecosystem, and consequently on human health. Once absorbed into the body, the drug is metabolised, and the resulting secondary metabolites are excreted into municipal wastewater. The persistence of pharmaceuticals in the environment is relatively low, but their widespread distribution in terms of quantity and discharge rate does not facilitate their transformation or degradation. Physico-chemical characteristics of pharmaceutical effluents include intense colouration and unpleasant odour, increased chemical and biochemical oxygen demand, significant organic carbon content, dissolved and dispersed particulates, and a wide pH range from 3 to 11. Treatment of such effluents can be extremely difficult and requires a specific strategy, often using a combination of different physical, chemical and biological treatment technologies [25,26].

2.4. Brewery Wastewater

The waste streams generated during beer production are easily biodegradable, making biological processes particularly suitable for treatment. These streams are enriched with high-quality nutrients from sugar and starch and are free from impurities such as pharmaceuticals. The inoculated microbiological cultures involved in the fermentation process create optimal conditions for the growth and multiplication of biomass and facilitate the metabolism of suspended and dissolved substances in the wastewater [27]. While the use of bacterial cultures is a conventional treatment method, the use of fungi offers additional advantages in terms of both the quality and quantity of activated sludge produced. The proliferating mycelium effectively converts organic compounds into biomass consisting of yeasts, moulds, and higher fungi, which can subsequently be utilised as a component of animal feed. According to the available literature [28], the following fungal species are commonly used for the bioremediation of brewery wastewater: *Agaricus bisporus* M7215, *Lentinula edodes* M3782, *Pleurotus ostreatus* M2140, *Trichoderma harzianum* CBS 226.95, and *Trametes versicolor* M9912.

2.5. Paper Industry Wastewater

The pulp and paper industry consumes natural, inorganic, and organic resources as well as considerable amounts of water at all stages of production, resulting in the generation of wastewater that is highly coloured and toxic due to the presence of modified and chlorinated high-molecular-weight lignins [29]. The resulting effluents are very difficult to handle as they contain polymers, biocides, surfactants, and extremely hazardous chemicals such as dibenzo-p-dioxins and dibenzofurans. In the study conducted by Ayed et al. [30], the bacterium *Pseudomonas putida* showed exceptional efficiency in reducing COD by 92% in a 10-day period, emphasising its importance for biodegradation and potential commercial applications. In addition, Gugel et al. [31] investigated the biodegradation of synthetic dyes and lignin by putrefactive fungi, including: *Bjerkandera adusta*, *Phanerochaete chrysosporium*, and *Trametes versicolor*. *Bjerkandera adusta* showed a remarkable ability to biodegrade without altering the pH, which offers promising prospects for bioremediation. These

results emphasise the importance of an integrated approach to wastewater treatment that enables effective contamination reduction and water recycling, thus contributing to the sustainability of the industry.

2.6. Municipal Wastewater

Wastewater from the public sewerage system, i.e., sanitary, stormwater, or industrial wastewater, is a mixture of complex inorganic and organic compounds. These contamination indicators include elevated levels of COD, BOD, total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP), and chlorine (Cl), as well as detergents, heavy metals, metabolised drugs, and the presence of coliform bacteria [32]. Legal regulations prescribe three treatment stages for the treatment of such wastewater, including physical, chemical, and biological processes [33]. Using 16s rRNA technology, a diverse microbial consortium was identified in the membrane bioreactor (MBR) that can effectively degrade contaminants. The species that make it up include: β-Proteobacteria (27%), Bacteroides (25%), α-Proteobacteria (14%), as well as other phylogenetic groups such as δ-Proteobacteria, Acidobacteria, Actinobacteria, and Firmicutes (34%) [34].

Literature reviews show different methods and their respective efficiency in the treatment of highly contaminated wastewater in different industries [22,23,26,33,35,36]. Key indicators such as COD, BOD, pH, TN, TSS, conductivity, and colour reflect the extent of contamination. The efficiency of different treatment technologies such as two-stage bioreactors, sequencing batch reactors (SBR), membrane bioreactors (MBR), and the use of specific microorganisms such as *Pseudomonas putida*, *Aeromonas* sp. and *Pleurotus ostreatus* show a significant reduction in COD and BOD values (Table 1). These examples illustrate the progress and potential of biological and integrated approaches to effectively treat and mitigate the environmental impact of highly contaminated wastewater.

2.7. Field-Level Applications of Bioremediation

The Kalina pond bioremediation project in Poland represents the comprehensive implementation of an integrated field-scale remediation strategy combining mechanical, physical, and biological methods. The key to success was the application of a unique autochthonous extremophilic bacterial consortium, which led to a significant reduction in persistent organic contaminants. Within four months, the COD value fell by 72% and reached a reduction of 86% by February 2023. Polycyclic aromatic hydrocarbons (PAHs) decreased by over 97%, while the total amount of phenols and monoaromatic hydrocarbons (BTEX) decreased below the detection limit. The water quality improved significantly: The dissolved oxygen increased from 0.0 to 2.5 mg/L, the redox potential changed from −200 mV to +140 mV, and the TOC decreased from 240 to 64 mg/L. The treatment also led to a visible biological recovery, including the reappearance of algae, zooplankton, amphibians, and fish [37]. A complementary field-level application of microbial remediation was carried out at Lake Jelonek in the Gniezno Lake District, where a large-scale sedimentation and biofiltration system with specialised microbial consortia was used to reduce the in-flow of biogenic nutrients and other contaminants. Although there is no direct data on the concentration of contaminants in the lake, the system was designed to significantly limit the nutrient load entering the aquatic ecosystem from outside, thus acting as a preventive and sustainable bioremediation solution. Taken together, these cases emphasise the practical relevance, scalability and ecological value of bioremediation technologies in restoring degraded aquatic habitats under real environmental conditions. They also demonstrate how bioaugmentation strategies can be tailored to site-specific challenges and provide flexible and effective tools for ecosystem restoration [38].

Table 1. Examples of wastewater treatment with different technologies and microorganisms.

Wastewater Type	COD, mg O_2/L	BOD_5, mg O_2/L	Other Contamination Indicators	Treatment Example	Reference
Industrial–Distillery	80,000–100,000	40,000–60,000	pH 3.9–4.5 TN 680–900 mg/L EC 6.3–6.7 ms/cm Dark brown colour	COD reduced following treatment in a two-stage bioreactor: *Pseudomonas putida* 44% and *Aeromonas* sp. 66%, within 24 h.	[22,23]
Landfill Leachate	100–79,000	3–25,000	pH 5.8–8.5 TSS 13–5000 mg/L TN 5–13,000 mg/L	COD removal efficiency through SBR treatment ranges from 62 to 75%, data significantly vary depending on landfill age and climatic conditions.	[35]
Industrial–Pharmaceutical	250–60,000	1–21,000	pH 6.6–9.4 TSS 60–360 mg/L EC 518–2840 ms/cm	MBR technology for pharmaceutical industry wastewater removed over 95% of COD and 99% of BOD.	[26]
Industrial–Breweries	80,000–90,000	8000–65,000	pH 5.1 TN 730–778 mg/L TP 367–385 mg/L	Highly loaded brewery wastewaters treated with *P. ostreatus*, COD removal rate was 77%.	[28]
Industrial–Pulp and Paper	6300–6500	860–950	pH 5.5 TSS 1309 mg/L Intense colouration, dissolved lignin, high concentration of chlorinated compounds	Highly loaded wastewaters from the paper industry treated with *Bacillus subtilis* and *Micrococcus luteus*, COD removal percentage 97%, BOD 87% over 9 days.	[36]
Municipal	500–1200	200–600	pH 7.5 TSS 258–282 mg/L TN 50–55 mg/L TP 7.4–7.5 mg/L Turbidity 221–244 NTU	Treatment in an aerobic MBR removed 89% of COD value.	[33]

COD: Chemical Oxygen Demand, BOD_5: Biochemical Oxygen Demand over 5 days, TN: Total Nitrogen, TP: Total Phosphorus, EC: Electrical conductivity, TSS: Total Suspended Solids, SBR: Sequencing Batch Reactor, and MBR: Membrane Bioreactor.

3. Fundamental Principles of Bioremediation Technology

Microbiologists began exploring the advantages of bioremediation in the 1940s, but it was not until the early 1990s that it became a modern technology in the truest sense of the word in the United States. The U.S. Environmental Protection Agency (EPA) conducted a series of laboratory and field studies in response to a major oil spill. During this time, bioremediation technology was highlighted in trade and scientific journals as the first choice for revitalising biogenic systems [39]. By utilising biological processes and microbial enzymatic activity, bioremediation represents a novel, effective, and affordable approach to preserving contaminated environmental elements such as soil, sediment, and water. A wide range of organisms, including bacteria, fungi, algae, microalgae, and plants, are capable of converting a chemical component from a complex organic form into a simpler, more environmentally friendly form [1,40]. Contaminants are usually degraded and detoxified by various physical and chemical processes such as coagulation, filtration, adsorption, chemical precipitation, electrolysis, and ozonation. The costs associated with these processes, the problems with disposing of secondary waste, and the formation of hazardous by-products, which can be more dangerous than the original substance, make them particularly unreliable. In contrast, bioremediation offers clear advantages over physical techniques (e.g., excavation, thermal treatment) and chemical processes (e.g., catalysis, redox reactions, and adsorption). Bioremediation is characterised by its high effectiveness, cost efficiency, and environmental compatibility and can be improved by biostimulation and bioaugmentation [7,41]. The most important factors influencing the biodegradation of contaminants are listed in Table 2. Depending on the site of application, bioremediation strategies can be categorised as in situ or ex situ methods (Figure 1) [1].

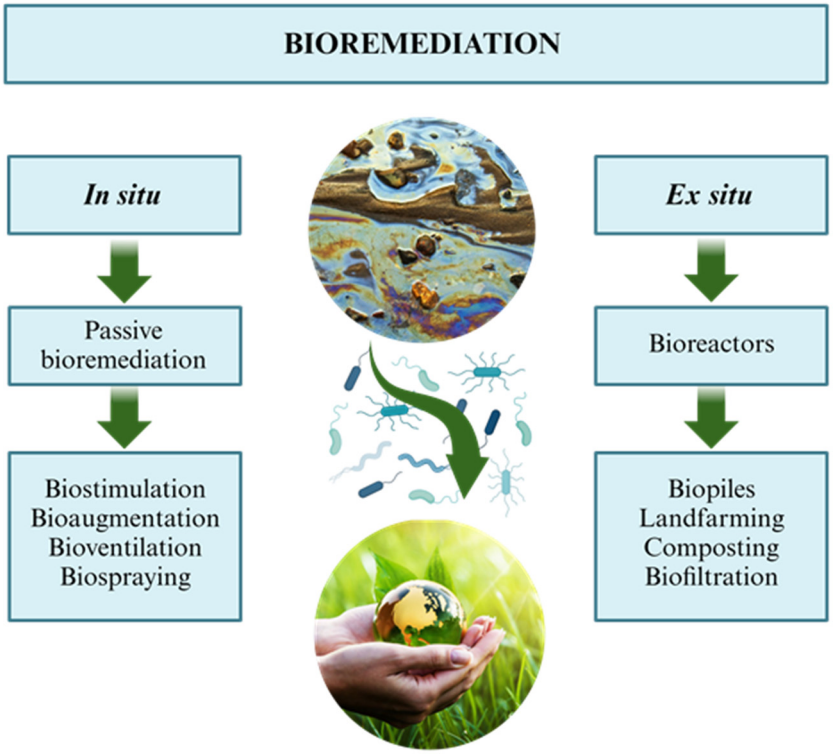


Figure 1. Types of bioremediation according to the location of implementation.

Table 2. Factors affecting the ability of contaminants' biodegradation [1,42].

Environmental Components	Soil, Water, Sediment
Inoculum Concentration	Optimal amount of added autochthonous/exogenous microbiological cultures for a successful biodegradation process
Oxygen Concentration	Depends on the specific needs of individual microorganisms; obligate aerobes, obligate anaerobes, facultative anaerobes, aerotolerant anaerobes, microaerophiles
Metal Concentration	Essential metal concentration is necessary, heavy metal concentration is inhibitory
Moisture	Depends on the environmental component; the optimal ratio is 60%
Salinity	Halotolerant (0–5% NaCl), mildly to extremely halophilic microorganisms (2–30% NaCl)
Nutrients (C:N:P)	100:10:1
Temperature	20–30 °C; enzyme activity range and highest efficiency of biodegradation
pH	6–8

Figure 2 presents a process flow diagram of wastewater treatment based on the activated sludge system. The dynamic and functionally diverse mixed microbial community responsible for the formation of activated sludge is capable of degrading complex organic contaminants and converting heavy metals into less hazardous by-products. Structural stability, metabolic synergies between different microbial strains, and adaptability to process conditions are required for its effectiveness. Activated sludge serves as a primary biomass source for biodegradation, but also as a biosorbent that absorbs suspended pollutants on its surface. In this way, the overall efficiency of wastewater treatment is improved. The microbial community of activated sludge, which is mainly composed of bacteria, yeasts, algae, and protozoa, produces enzymes that enable the degradation of a wide range of pollutants. Key functions of microbial enzymatic biodegradation and biotransformation include aerobic and anaerobic degradation of complex organic compounds, processes of bioaccumulation, the biosorption and bioprecipitation of heavy metal compounds, the oxidation of ammonia and nitrite, and the removal of nitrogen and phosphorus-rich nutrients. During the biodegradation processes, microorganisms produce extracellular polymeric substances (EPS) which facilitate the flocculation of activated sludge flocs. Such sludge can sediment and separate from the wastewater by forming stable aggregates. EPS plays an important role in biofilm production, which is characterised by a high density of microbial communities. In addition to extracellular polymers, some microbes release soluble microbial products (SMP) that act as buffers against pH fluctuations in the environment. Proteobacteria, which carry out nitrification and denitrification processes and are also involved in the removal of organic pollutants, make up the majority of bacterial cultures (up to 60%). Bacteroidetes, which usually degrade complex chemical compounds, are the second most abundant group (10–20%), followed by Actinobacteria and Chloroflexi. Archaea are rare in aerobic environments; they are mainly involved in anaerobic methanogenesis processes [43–45].

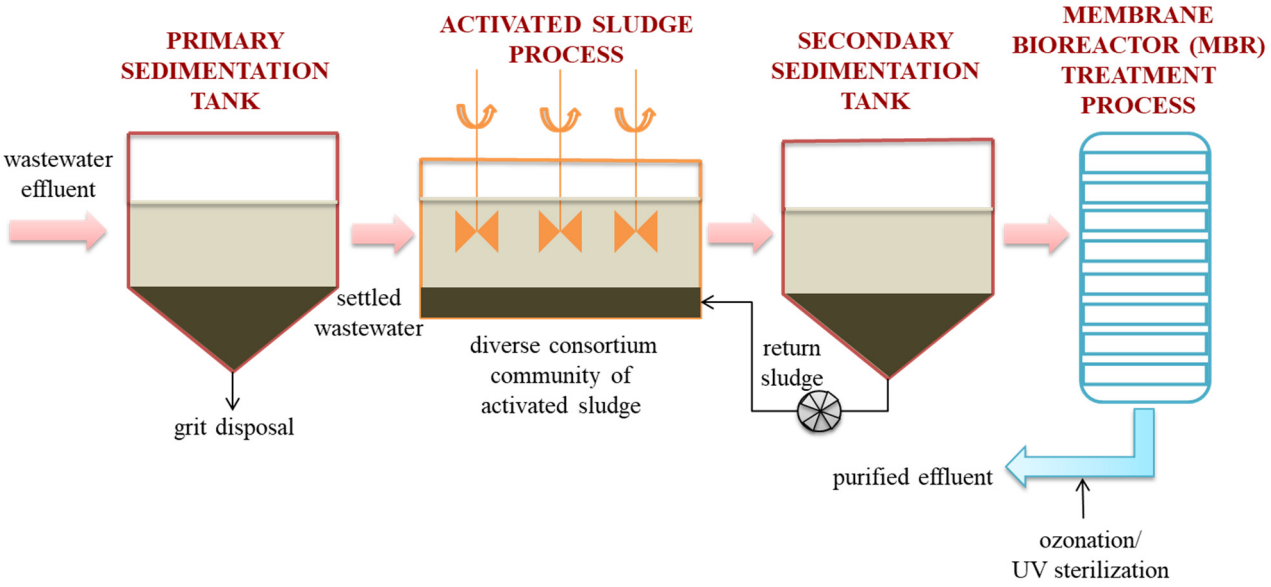


Figure 2. Process flow diagram of wastewater treatment using activated sludge system.

In practise, bioremediation technology still faces various socio-economic, political, and legal obstacles, although it is a highly developed, economical, and environmentally friendly technique for treating highly contaminated wastewater and other growing environmental issues. The regional development background and the particular state institutions of each country are directly related to these challenges. The prospective and long-term benefits of green remediation technologies tend to be less recognised by government authorities and decision-makers, leading to socio-economic barriers. Acceptance of bioremediation could be further limited by low community participation and public awareness, leaving conventional physico-chemical treatment processes as the first choice, as they are often proven and familiar in the operational context. In addition, investors may be discouraged from exploring biological alternatives due to the high initial investment required for pilot plants and research infrastructure for the development of bioremediation. In a comprehensive study by Ibáñez et al. (2024) [46], the authors applied an Impact Pathway Approach (IPA) together with Environmental Life Cycle Costing (eLCC) to evaluate a case study on in situ metal (loid) precipitation. The country's political and economic support mechanisms, such as government subsidies and favourable credit conditions, as well as technological optimisations at the process level, such as increasing the volume of water treated to 90 m^3/year and reducing the discount rate to 2%, were found to be crucial for the sustainability and overall profitability of bioremediation technologies. The IPA focussed on reducing the medical costs associated with human and animal arsenic exposure, but neglected long-term environmental benefits such as biodiversity restoration, which could increase the overall effectiveness of bioremediation. Despite the promising results of such assessments, these types of assessments are still rarely integrated into standard policy-making and remediation planning procedures [46,47]. As with other cutting-edge biotechnologies, the practical application of bioremediation technologies often faces obstacles due to the need for strict authorisation procedures and delicate administrative approvals, although the regulatory and legal framework for their implementation is generally positive. In the European Union, important legislation such as the Industrial Emissions Directive (IED) and the Water Framework Directive (2000/60/EC) must be observed when deploying new technologies. The Nature Restoration Law, the EU's first legally binding law on nature restoration, was adopted in 2024 and contains specific guidelines for the restoration and rehabilitation of degraded natural sites. Due to the ecological hazards

and potential for competing interactions with native microbial communities found in the vicinity of contaminated sites, these legal instruments also impose strict restrictions on the use of genetically modified organisms (GMOs) in bioremediation [48,49]. In developing countries in Asia and Africa, regulatory standards remain inconsistent, and there is no harmonised legislation for the application of bioremediation technologies. In addition, the effectiveness of environmental standards is hampered by inadequate enforcement of pollution monitoring in industrial and manufacturing facilities. Although the regional Asian Environmental Compliance and Enforcement Network (AECEN) prescribes methods for the management and monitoring of environmental zones, its regulations are not binding [50]. The successful use of bioremediation techniques in practise, with strong political support from government agencies, requires concerted efforts by a number of national organisations, including the ministries of environment, science, and health. Developing and emphasising long-term remediation strategies using environmentally sustainable practises is as important as choosing short-term, recognisably quick fixes often associated with conventional physico-chemical treatments. Despite geographical differences, countries need to develop appropriate funding models with the support of global regulators and dedicated environmental funds to ensure the long-term sustainability of bioremediation initiatives. Building comprehensive environmental management systems and achieving long-term maintenance results require such integrated and proactive approaches [51,52].

Bioaugmentation and Biostimulation

The addition of indigenous or exogenous microorganisms that are capable of biologically degrading contaminants is known as bioaugmentation [53]. A distinction is made between non-specific bioaugmentation, which does not require the prior assessment of microbiological properties (e.g., activated sludge), and highly specific bioaugmentation, in which pure strains of microorganisms or precisely defined microbial communities with known biodegradation potential are added [5]. Bioaugmentation methods include the addition of a pre-adapted pure bacterial strain, pre-adapted consortia, genetically modified microorganisms, or the incorporation of relevant biodegradation genes packaged in vectors and conjugated into indigenous microorganisms. The effectiveness of contaminant transformation by individual microorganism species generally decreases when complex contaminants are involved, which can create stress conditions and disrupt metabolism. To overcome this challenge, microbial consortia or communities are often used. The metabolic diversity and resilience required for practical applications in the field are ensured by using a microbial consortium instead of a pure culture [54]. The microbial biomass used as inoculum in bioaugmentation processes is usually produced in bioreactors before being introduced into the contaminated natural environment. Under real conditions, the inoculated biomass is exposed to various biotic and abiotic stress factors, which mainly affect its abundance and biodegradation potential. Knowledge of the properties of the contaminated medium and the operating conditions of the system is critical to avoid negative results, as a lack of knowledge reduces the likelihood of successful integration of the inoculum [55–57]. Activated sludge processes are among the most widely used processes, varying in configuration and performance, but generally consisting of aerobic, anoxic, or anaerobic biomass cells that are biodegradable under continuous flow conditions. These processes can be operated in mixed or batch systems or plug-flow systems [2]. The use of carrier materials can physically support the biomass and improve access to nutrients, moisture retention, and aeration, thus increasing the survival rate. Encapsulation or immobilisation of microbial cells further improves the survival rate by protecting the cells from harsh environmental conditions. Encapsulation prevents exposure to toxic chemicals, reduces damage to cell membranes, controls nutrient flux, reduces concentrations

of harmful compounds in the microenvironment, and provides protection from competition and predators. Several materials have been extensively studied and evaluated for the encapsulation or immobilisation of cells, including agar, agarose, alginate, gelatine, gellan gum, kappa carrageenan, acrylic copolymers, polyurethane, and polyvinyl alcohol gel. Compared to an identical quantity of free cells, encapsulated cells have a higher contaminant degradation rate [58]. Bioaugmentation can be broadly divided into two types: cellular bioaugmentation, which involves the direct introduction of specialised microbial strains capable of degrading certain contaminants, and genetic bioaugmentation, a more indirect strategy that focuses on improving the genetic potential of indigenous microbial communities. In the latter, catabolic genes, usually located on mobile genetic elements (MGEs) such as plasmids or transposons, are introduced into the native microbiome. This approach is based on horizontal gene transfer (HGT), a natural process in which genetic material is exchanged between microorganisms independently of reproduction. In genetic bioaugmentation, a donor bacterium carrying catabolic plasmids is introduced into the contaminated environment. These plasmids are then transferred to indigenous microbial populations by HGT mechanisms such as conjugation, transformation, or transduction. This provides the resident bacteria with new degradation processes, increasing both the diversity and efficiency of the microbial community in degrading pollutants. Compared to cellular bioaugmentation, genetic bioaugmentation offers several advantages. As the functional genes are spread within the existing microbial community, the adapted indigenous microbiota retains its ecological fitness and is better suited to survive under site-specific environmental conditions. Over time, this results in an evolutionarily stable and functionally enhanced microbiome, leading to improved degradation rates and more sustainable bioremediation outcomes [59]. As an element of bioremediation, biostimulation involves the promotion of microbial activity to support the biodegradation, biotransformation, and biomineralization of environmental contaminants. To promote the growth and activity of microorganisms that are naturally involved in the degradation of contaminants, nutrients such as carbon, nitrogen, or phosphorus are added in this method. In addition to the addition of nutrients, it is important to optimise other process variables such as temperature, pH value, moisture content, and the concentration of potentially hazardous and suppressive contaminants in the substrate. The addition of oxygen or alternative electron acceptors can improve the aerobic biodegradation and oxidation of organic matter in the wastewater. Care must be taken to maintain a balance between providing sufficient nutrients and avoiding excessive (inhibitory) concentrations, particularly of nitrogen and phosphorus, which can lead to eutrophication and a subsequent reduction in dissolved oxygen levels in the system [60]. In addition, the addition of organic material such as molasses or algal extract to contaminated systems provides additional nutrients for microorganisms, which accelerate growth and reproduction and thus promote the biodegradation of contaminants. In certain scenarios, sulphates or nitrites can be used as electron acceptors to stimulate anaerobic microorganisms to biodegrade under low-oxygen conditions [61].

The synergistic application of these two approaches leads to a significant reduction in the chemical and biochemical oxygen demand in highly contaminated wastewater, achieves a harmonised nitrogen concentration, and influences microbial diversity [62,63]. Table 3 provides a comprehensive overview of various biological wastewater treatment methods, with a specific focus on key performance criteria including process efficiency, field applicability, cost-effectiveness, environmental footprint, scalability, and implementation time. The table systematically compares these methods to highlight their relative advantages and limitations in real-world bioremediation scenarios. This comparative approach enables the identification of the most suitable technologies for specific wastewater profiles, especially in contexts that demand sustainable, adaptable, and economically viable treatment solutions.

Table 3. Evaluation of biological treatment technologies for wastewater: technical and environmental criteria.

Type of Method	Removal Efficiency	Applicability	Cost-Effectiveness	System Scalability	Environmental Footprint	Process Implementation Time	References
Biostimulation	Enhanced biodegradation increases with increased microbial activity, with an average improvement of 80% to over 90. Some by-products remain as partially degraded compounds.	In situ application for the treatment of contaminated soil layers and surface waters by adding nutrients, oxygen, or electron donors/acceptors. Ex situ application in reactor systems to optimise the biodegradation process. Possible complications include excessive stimulation of the process (overgrowth of biomass and lack of oxygen in the soil pores).	A cost-efficient process. The operating costs include the procurement of nutrients (substrate, nutrients, and oxygen) and transport vehicles to bring them to the intended location, without the need for expensive equipment.	High scalability for the specified process. Easy expansion of scalability from pilot plants to large wastewater treatment plants and contaminated areas.	It is an innovative green treatment method, as it only minimally disturbs the natural balance of ecosystems. No additional energy consumption is required.	The achievable contamination reduction is 50–70% over several months, depending on the initial contaminant load and the chosen treatment approach (in situ field application vs. ex situ reactor systems).	[53,64,65]
Bioaugmentation	It significantly accelerates the degradation rate of persistent organic pollutants. Studies show varying effectiveness and the possibility of partial degradation of xenobiotics. Both indigenous and exogenous microbial communities require association with biostimulation techniques.	Broadly applicable for ex situ (biofilters, reactor systems) and in situ applications (field sites, surface water, and groundwater). Process control (C/N/P, pH, and dissolved oxygen) is required to maintain biomass viability and metabolic activity in the field.	Less costly than conventional physico-chemical treatment processes, as only reactors for the cultivation of biomass are required as process equipment. The process costs increase with the energy requirement if larger quantities of biomass have to be produced (aeration, mixing).	Favourable scale-up potential from laboratory to pilot plant and field applications but requires strain adaptation. Ex situ implementation is often limited by competing interactions between introduced strains and the indigenous microbiota.	Favourable ecological effects, as natural microorganisms are used as inoculum, thus eliminating the need for costly and aggressive chemicals. Particular caution is required when genetically modified strains are used, as they could be competitive with indigenous strains in the ecosystem.	When using tested microbial consortia and under controlled conditions, optimal degradation kinetics generally require weeks to months to fully remediate a site while maintaining environmental safety and reducing by-product production.	[56,66,67]

Table 3. *Cont.*

Type of Method	Removal Efficiency	Applicability	Cost-Effectiveness	System Scalability	Environmental Footprint	Process Implementation Time	References
In situ bioremediation	A number of variables influence efficacy, including chemical composition, bioavailability of contaminants to microorganisms, environment, and appropriate process control.	Application of a biodegradation technique in situ at the site of contamination without relocation. This includes techniques such as biosparging, bioventing, biostimulation, bioaugmentation, and phytoremediation, which require a minimum of physical intervention.	This approach is less costly than ex situ treatment as it does not require excavation, transport, or processing in specialised facilities. Infrastructure investment costs are limited to minor construction projects, process control equipment, biomass spraying systems and water recirculation devices. The overall implementation of the project takes longer.	High adaptability—the process can be adapted to the size of the contamination site. For smaller areas, there are technical solutions such as injection wells and special drainage systems, while for larger sites, methods for irrigation with nutrients or the application of biomass are used. Maintaining uniform process conditions proves difficult in extensive, heterogeneous areas.	The most environmentally sustainable remediation approach that only minimally disturbs the balance of ecosystems. As no material transport is required, the risk of secondary emissions is eliminated and environmental damage caused by excavation work is avoided. Energy consumption is limited to the operation of pumps and compressors.	The biodegradation process requires a longer duration, as natural degradation processes are slow by nature and often take years. This makes long-term monitoring necessary. For urgent remediation scenarios involving highly toxic contaminants, treatment in an ex situ reactor is a more suitable alternative.	[68–70]
Ex situ bioremediation	Exceptionally high efficiency under controlled conditions enables complete degradation with more than 99% contaminant removal. Strict process control (pH, temperature, and agitation) combined with biofilters removes virtually all known organic contaminants.	Applicable when physical access to contaminated zones is possible, e.g., industrial wastewater streams. High implementation complexity with excavation, transport, multi-stage treatment, and subsequent by-product/sludge management. Requires constant monitoring by operators, full process automation, and comprehensive maintenance support. Technically more demanding than in situ processes.	Significant initial costs due to excavation, transport, reactor system, plant design, and waste management. Treatment is faster and more efficient, reducing long-term monitoring costs. Economic compensation can be achieved through anaerobic reactor treatment with biogas production.	The scalability is high—it ranges from small reactor systems for limited quantities of wastewater to larger technical units for the treatment of large quantities, which require higher investment costs. Spatial and temporal components become significant challenges for scaling.	Moderate sustainability and environmental footprint compared to passive methods; higher resource requirements for excavation/transport fuel and process energy requirements. Better resource utilisation is achieved through biogas production during anaerobic treatment or recycling of wastewater. Long-term benefits for the ecosystem result from the rapid removal of contaminants.	Rapid contaminant removal. In specially designed reactors with contaminant-specific inoculum, biodegradation takes place within days to weeks. Complete remediation of the site is achieved within months, whereas years are required for passive treatment methods.	[70–72]

Table 3. *Cont.*

Type of Method	Removal Efficiency	Applicability	Cost-Effectiveness	System Scalability	Environmental Footprint	Process Implementation Time	References
SBR (Sequencing Batch Reactor)	A well designed and operated SBR system achieves 90–98% removal of COD, BOD, and suspended solids. The system has proven itself with an 85% COD reduction in wastewater from the textile industry, 97% reduction in initial BOD_5 levels in landfill leachate, and 95% ammonia removal. Batch operation improves microbial resistance to toxic conditions.	Excellent for wastewater treatment through different process cycles: inoculation, aeration, settling, and discharge of wastewater. Suitable for various wastewater streams, including landfill leachate, wastewater from the food/pharmaceutical industry, and wastewater from the agricultural industry.	A cost-effective and efficient process that does not require separate tanks, clarifiers, or sedimentation tanks—all treatment takes place in a single reactor tank, which significantly reduces investment costs. Although the operating costs include the energy required to implement the process, these are offset by the rapid completion of treatment and the high quality of the wastewater, resulting in long-term savings.	Excellent scalability: from household/commercial SBR units to large municipal SBR plants. Small systems use a single reactor, while larger plants require parallel SBR units (modular operation). Effective automatic cycle monitoring by the operators is essential.	Low environmental footprint. Space-efficient design eliminates the need for separate basins. Intermittent operating mode: no continuous oxygen supply required—aeration is phased (pumps are deactivated during settling and draining phases). Extended endogenous respiration reduces the production of active sludge as secondary waste.	A fast cyclical process with daily operating cycles of 5–8 h ensures a constant, visible daily treatment performance. Although the settling time is extended to 60 min, this duration is justified as it eliminates the need for additional clarifiers.	[73–75]
MBR (Membrane Bioreactor)	A high-efficiency system—100% removal of suspended solids and up to 99% reduction in COD and BOD values. The wastewater often fulfils the legal discharge standards without the need for additional treatment. It is not effective at removing nutrients (N/P) unless designed with anoxic phases or chemical precipitation. It has the best removal efficiency compared to other biological treatment methods.	In practise, it is suitable for treating a wide range of municipal and industrial wastewater that place high demands on wastewater quality. The system can be used as a larger stationary plant or as a smaller mobile unit in containers. It is a complex treatment system that combines activated sludge and membrane processes. Automation is essential. It is the most technologically sophisticated of all biological treatment systems.	High costs in terms of investment and maintenance. In addition to the activated sludge module, additional filtration equipment such as cleaning systems, pumps, and similar components is required. Considerable energy expenditure for operation: sludge aeration and pressure for membrane processes. Additional filtration or disinfection is not required. The costs are justified by the high efficiency of the process.	Scaling is feasible in practice—from small modular field units to large municipal wastewater treatment plants. Most systems consist of several membrane modules. Larger scaling means higher costs, but also higher process efficiency (larger systems are more energy efficient per wastewater flow unit).	The high energy consumption means that more space is required, but this is offset by returning the wastewater to the cycle at a high quality. The production of sewage sludge is reduced by up to 30% compared to conventional biological methods, as the biomass remains in the system for longer. The plant requires less space, which minimises the need for construction. The process remains ecologically sustainable due to its high purification performance.	The targeted degradation of contaminants is achieved by a high inoculum mass, which enables treatment within a few hours. Membranes continuously separate the treated wastewater stream, eliminating the sedimentation time. This results in a fast, highly efficient treatment process with continuous water inflow and outflow.	[76–78]

4. Naturally Occurring Potential for Biodegradation

While organic contaminants are utilised by microorganisms as a source of carbon and energy, heavy metals cannot be biodegraded. Instead, they pass from one organic complex or oxidative state to another, changing their degree of water solubility and reducing their toxicity [79]. The main advantage of microorganisms lies in their ability to convert heavy metals into less toxic, water-soluble forms through mechanisms such as leaching, chelation, redox conversion, and methylation [11]. Microorganisms degrade pesticides to simpler organic molecules or mineralise them completely to carbon dioxide, water, and other inorganic forms [80]. The biodegradation of plastics is promoted by hydrolytic enzymes and takes place in four phases: attachment to the surface, fragmentation, assimilation, and mineralisation. These and other contaminants are often found in soils and groundwater. The main challenge in removing these contaminants from the subsurface is that indigenous microorganisms are more effective than laboratory-grown microorganisms. The implementation of strategies tailored to the specific environmental conditions of the contaminated area is usually required for a bioremediation programme to be truly effective [81].

In general, there are five important interactions between microorganisms and contaminants: biosorption, bioaccumulation, biotransformation, biodegradation, and biomineralization. The enzymes produced by microorganisms play a decisive role in bioremediation. The enzymes produced cause the reduction reaction of various ions such as nitrites, nitrates, tetrathionates, thiosulphates, etc. [7]. Enzymes serve as biocatalysts by reducing the required activation energy through the formation of transition states. Besides the numerous factors that influence the bioremediation process, such as the chemical composition of the contaminants, moisture content, and microbial diversity, an important aspect to accelerate the process, which is one of the greatest challenges, is the understanding of the genomics of the microorganisms. This understanding provides a deeper insight into the metabolic reactions that take place during biodegradation [82].

Communities of different microorganisms that are indigenous to contaminated sites and have significant metabolic potential form the basis for microbial bioremediation strategies. Such microorganisms can be further isolated and purified to learn specific details about microbial metabolites and degradation mechanisms. Currently, much of the research on bioremediation processes follows the principle of "treatability", where the rate of contaminant immobilisation or degradation is observed while samples from contaminated regions are incubated in a controlled environment. This approach can be used to assess the possible metabolic activity of microbial populations [13]. A major limitation arises from the difficulty of cultivating numerous microorganisms with remarkable biodegradation potential under laboratory conditions, which makes them inaccessible for extensive research and potential applications. Only a small percentage of microorganisms from different environmental samples can be successfully cultured in vitro [83]. Another environmental problem is the migration of contaminants, which can cross different states and easily reach organisms, accumulate, and permeate all trophic levels. Bioremediation has the potential to interrupt this sequence of events at a desired point, provided that the contaminants and the naturally occurring microorganisms, or those known to be effective in attenuating certain contaminants, are known [84]. Figure 3 illustrates the advantages and disadvantages associated with the use of bioremediation technology in the remediation of biogenic systems.

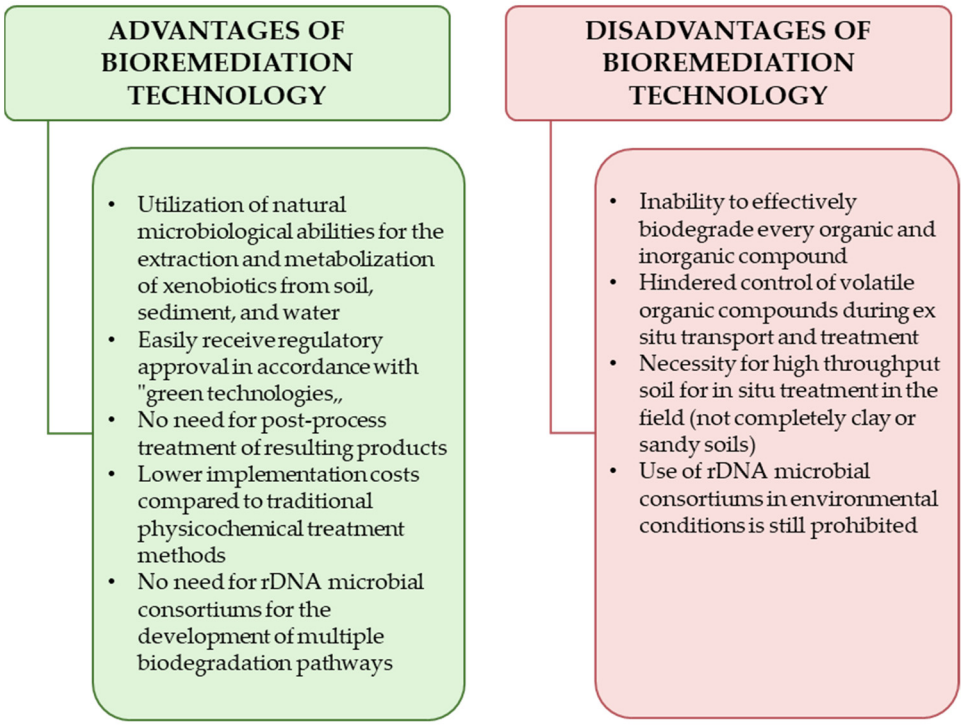


Figure 3. Advantages and disadvantages of using bioremediation in the remediation processes of biogenic systems.

5. Synthetically Engineered Potential for Biodegradation

Recent advances in molecular tools have revolutionised the assessment of previously unculturable microorganisms from the natural environment. The development of molecular, bioinformatics, and systems biology tools for bioremediation has enabled a deeper understanding of bioremediation mechanisms at the genetic level [14]. This has enabled the strategic exploitation of the synthetic potential of microorganisms, defined as their capacity to be genetically engineered or synthetically designed to express novel or optimised metabolic pathways for the efficient degradation of structurally complex and recalcitrant contaminants.

Further improvement of microbial remediation processes can be achieved through the successful application of genome editing and biochemical techniques, which can be used to modify existing strains to produce genetically modified organisms that can degrade multiple contaminants simultaneously. However, bioremediation processes for the elimination of xenobiotics are subject to certain limitations, in particular the requirement of optimal conditions for the metabolic activity of selected microorganisms, which are difficult to achieve in practise [85]. Various methods, such as horizontal gene transfer, molecular cloning, protoplast transformation, electroporation, as well as the conjugation and transformation of competent cells, are used for the successful development of genetically improved properties of microorganisms [86]. Research has shown that various naturally occurring microorganisms, including bacteria (e.g., *Pseudomonas*, *Alcaligenes*, *Cellulosimicrobium*, *Microbacterium*, *Methanospirillum*, *Bacillus*, *Sphingobium*, *Flavobacterium*, and *Rhodococcus*), moulds (e.g., *Aspergillus*, *Penicillium*, *Trichoderma*, and *Fusarium*), and yeasts (e.g., *Pichia*, *Rhodotorula*, *Aureobasidium*, and *Exophiala*) are effective in the biodegradation of xenobiotics from contaminated environments due to their exceptional bioremediation potential (Table 4) [40,82].

Table 4. Types of microorganisms with the potential to degrade a specific contaminant.

Contaminant Type	Chemical Formula	Natural Degradation Potential	Synthetic Degradation Potential	Reference
PAH (Polycyclic Aromatic Hydrocarbons)	C_nH_n	*Pseudomonas aeruginosa*, *Mycobacterium* sp., and *Rhodococcus* sp.	*Cycloclasticus* sp. P1	[87,88]
Phenol	C_6H_5OH	Microbial consortium: *Acinetobacter* sp., *Bacillus* sp., and *Pseudomonas* sp.	*Rhodococcus* sp. CS-1	[89,90]
Dioxins	$C_{12}H_{10-X}Cl_X$	*Bacillus megaterium*, *Norcardiopsis* sp., and *Geobacillus* sp.	*Sphingomonas wittichii* RW1	[91,92]
Diesel	$C_{12}H_{23}$	*Bacillus cereus*, *Bacillus sphaericus*, *Bacillus fusiformis*, and *Acinetobacter junii*	*Pseudomonas fluorescens* HK44, *Pseudomonas putida* KT2442	[93,94]
Heavy Metals (Cadmium)	Cd	*Eichhorina crassipes*, *Thlaspi caerulescens*	*Escherichia coli* JM109, *Pseudomonas putida* 06909, *Bacillus subtilis* BR151 (pTOO24)	[95]
Pesticides (DDT)	$C_{14}H_9Cl_5$	*Chryseobacterium* sp., *Trametes versicolor*	*Rhodococcus* sp. IITR0	[96,97]
Pharmaceuticals (Erythromycin)	$C_{37}H_{67}NO_{13}$	*Penicillium oxalicum* RJJ-2, *Penicillium restrictum*, and *Trametes versicolor*	*Delftia lacustris* RJJ-61	[98,99]
Plastic (Polyethylene, PE)	$(C_2H_4)_n$	*Rhodococcus ruber*, *Penicillium simplicissimum*, *Streptomyces* sp., and *Brevibacillus borstelensis*	*Pseudomonas* sp. AKS2	[100,101]

The concept of synthetic biology, illustrated in Figure 4, includes both top-down and bottom-up approaches, which are important for the development of specialised microorganisms for bioremediation. In the top-down approach, existing microorganisms are modified using advanced omics technologies such as genomics, transcriptomics, and proteomics. These tools help to identify the important metabolic genes responsible for the degradation of harmful substances, which can then be improved or reprogrammed using genome editing techniques such as CRISPR-Cas. The result is genetically modified bacteria with optimised metabolic pathways that are specifically designed for the degradation or conversion of certain contaminants in wastewater. The bottom-up approach, on the other hand, involves building bioremediation systems from scratch by assembling synthetic biological parts. This includes artificial enzymes, biology-inspired nanostructures, or synthetic vesicles that mimic cellular functions [85]. These components often work as cell-free systems that degrade contaminants as biocatalysts or bind toxic substances as biosorbents and remove them from the water. As shown in Figure 4, hybrid systems with improved bioremediation performance can be created by combining both strategies, i.e., the genetic modification of living microorganisms and the construction of synthetic biomaterials. For example, synthetic gene circuits developed in the laboratory can be introduced into microbial hosts, or synthetic enzymes can be used together with living microbes in advanced wastewater treatment reactors. This integrated approach is a promising way to increase the efficiency, specificity, and sustainability of bioremediation technologies, especially in the context of emerging contaminants and industrial contaminants that are resistant to conventional treatment methods [40,82,90].

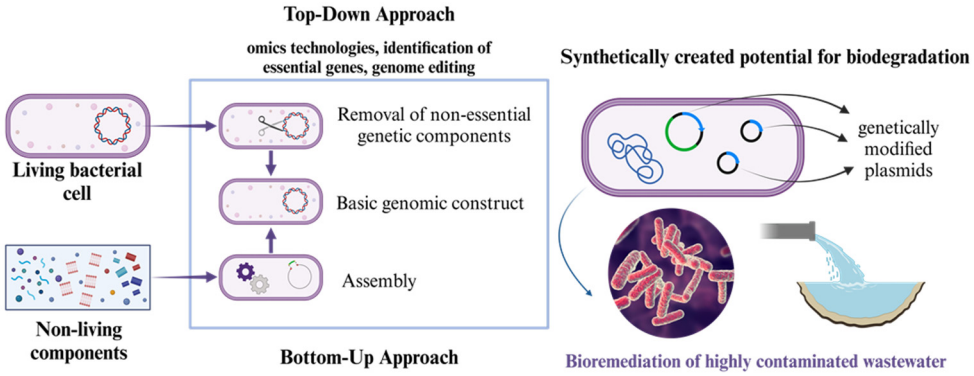


Figure 4. Synthetic biology and advanced genome editing technologies in bioremediation of highly contaminated wastewater.

6. Future Perspectives

Future developments in the field of bioremediation are likely to be characterised by the convergence of synthetic biology, computational modelling, and environmental monitoring technologies. One promising direction is the use of artificial intelligence (AI) and machine learning to model and predict the behaviour of microbial communities, enabling the development of tailored consortia with optimised degradation processes for specific contaminants and environmental conditions. Such predictive modelling could significantly reduce uncertainty in field applications and accelerate the transfer of laboratory results to real systems [102]. Another important area is the integration of biosensors that are either embedded in microbial systems or serve as external analytical tools for the real-time monitoring of contaminant concentrations, microbial activity, and system performance. These tools could support dynamic control strategies, improve safety and ensure compliance in complex treatment situations [103]. In parallel, the development of a sound regulatory framework for the safe and responsible use of genetically modified organisms in open environments is crucial. This includes establishing internationally harmonised guidelines

that take into account biosafety, ecological risks, and public concerns, while supporting innovation in synthetic biology-based remediation techniques [104]. Finally, more emphasis should be placed on interdisciplinary research that combines microbiology, environmental engineering, systems biology, and data science. Such collaboration is essential to overcome current limitations such as microbial competition, environmental unpredictability, and scalability and to achieve resilient, adaptable, and sustainable bioremediation strategies for the treatment of highly contaminated wastewater [105].

7. Conclusions

With advancing industrialisation and urbanisation, various anthropogenic activities significantly contribute to the introduction of toxic and hazardous contaminants into the environment. Highly contaminated wastewater is a common by-product of various technological production processes and contains elevated concentrations of contaminants. To ensure compliance with stringent legal standards for emissions from waste streams, treatment processes combine conventional physico-chemical methods with environmentally friendly biological techniques. Bioremediation is a highly complex process that necessitates a thorough understanding of the site, the microbial species present, the contaminants, and the process variables to control it effectively. By editing specific genomes and creating synthetic degradation potential, the biodegradation process can be accelerated and improved under controlled laboratory conditions. However, the application of such microorganisms in situ faces challenges such as their limited adaptability to environmental conditions, bioavailability of contaminants, and competing interactions with indigenous microbiological species. The potential for synergistically integrating existing natural and newly developed technologies plays a crucial role in enhancing the sustainability and effectiveness of treatment processes for highly contaminated wastewater. There is no doubt that a synergetic strategy that integrates many scientific disciplines—engineering solutions with aspects of digitalisation and biotechnology, supported by political and legal frameworks—is the way forward for biological wastewater treatment. Governments should provide incentives and subsidies to encourage the use of green technologies to remove persistent contaminants, especially those that are emerging. In addition, machine learning and predictive analytics driven by AI algorithms can be used to simulate and optimise the microbial inoculum, enabling the real-time optimisation of process parameters. Integrating such advanced systems with biosensors and smart sensors to continuously monitor key—and perhaps critical—process indicators can improve degradation efficiency. Hybrid systems that combine complementary physical and chemical processes, such as photocatalytic MBR systems or the combination of biostimulation/bioaugmentation with advanced oxidation processes (AOPs), should be used for the treatment of highly contaminated wastewater with difficult chemical compositions.

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