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Review Article

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Microbial Bioremediation Strategies for Sustainable Wastewater Treatment

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Abstract
Wastewater treatment uses various techniques to remove contaminants such as heavy metals, hydrocarbons, and organic substances—by-products of agriculture, industry, and human activity. Microbes play a crucial role in eliminating hazardous substances through a process known as bioremediation. Bioremediation is a novel and promising technology that offers several advantages over conventional techniques for waste removal. It is flexible, cost-effective, and eco-friendly, and thus holds great potential for wastewater treatment. A diversity of microbial organisms, like algae, fungi, yeast, and bacteria, perform methylation and have the ability to modify and detoxify pollutants. This review outlines microbial approaches to wastewater treatment and contextualizes them within physical, chemical, biological, and membrane-based treatment frameworks. Key microbial technologies include biodegradation and activated sludge systems. Despite these advancements, challenges remain. These limitations include inconsistent efficiency across varying environmental conditions, difficulties in scaling up from lab to field applications, and challenges in maintaining active microbial populations. The current article outlines various strategies employed for biodegradation, highlighting their efficacy, recent advancements, and the challenges associated with their implementation and commercialization.

Keywords:
wastewater treatment; pollution; microorganisms; bioremediation; sustainability

1. Introduction

Water pollution and its treatment have become major global concerns. Contaminants are mainly released through industrial activities—such as fertilizer production, mining, and pesticide manufacturing—or as domestic effluents. The release of hazardous waste affects human health and disturbs the aquatic ecosystems.

UN World Water Development Report of 2024 states that an estimated 80% of the wastewater that is released into the environment has been adequately treated, more so, in countries under the low- and middle-income group [1]. The World Health Organization (WHO) reported in 2024 that over 2 billion people drink water contaminated with feces, causing nearly 485,000 deaths from diarrhea each year [2]. The UNEP Global Environment Outlook (2024) also reported that more than 60% of freshwater bodies across the world are either moderately or severely polluted, comprising a wide range of contaminants, from nutrient overloads (eutrophication) to emerging pollutants, namely pharmaceuticals, microplastics, and personal care

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products [3]. These alarming statistics necessitate urgent efforts to develop sustainable and efficient wastewater treatment (WWT) solutions. Recent developments in biological WWT encourage researchers to improve microbial bioremediation technologies to ensure the availability of purified water [4,5].

Microbial bioremediation offers an eco-friendly, cost-effective alternative that aligns with global initiatives, namely Sustainable Development Goal (SDG) 6.3, aiming to halve the concentration of untreated wastewater as well as significantly enhance its recycling and safe reuse via nature-based solutions (NbS) [6]. In the bioremediation process, contaminated soil and water are treated using microbial species such as bacteria, fungi, and yeast. Bioremediation is described as the application of biological procedures for removing, attenuating, or transforming pollutants. Aquatic ecosystems are the earliest and most severely impacted ecosystems in every nation, whether due to pollution from a single point source or multiple sources. Major primary sources are released directly into the stream. Common sources of environmental contamination include effluents from industrial and municipal activities, run-off and leachate from solid waste disposal sites, industrial drainage, and discharges from vessels. Urban run-off from undeveloped regions, agricultural run-off from fields and orchards, are other secondary sources of water pollution. Water contamination has severe consequences not only on aquatic life but also on birds and terrestrial animals. Polluted water kills aquatic life and hinders their ability to reproduce. Consequently, water becomes unsuitable for household or human use, and in extreme circumstances, it even poses a risk to human health. Application of bioremediation can lower the financial and environmental costs associated with waste disposal [7]. Many treatments typically involve seeding polluted water with competent microflora that can degrade hazardous materials to hasten the bioremediation process [8].

2. Principle of Bioremediation

M. Robinson introduced the concept of using microorganisms for bioremediation [9]. The principle of biological remediation relies on biodegradation [10]. The process is commercially feasible and environmentally friendly, but its effectiveness varies with the region [11]. The microorganisms employed in bioremediation have the physiological ability to decompose and detoxify water contaminants [9,12]. It is an on-site, cost-effective strategy [13]. These microbial consortia can be generated by supplying nutrients, introducing electron acceptors, and modifying humidity and temperature in various ways [14].

During bioremediation, microorganisms utilize these contaminants as sources of nutrients or energy. In some cases, native microorganisms present at the site actively participate in the treatment process, while in other situations, specific microbial strains are introduced to the site through bioreactors [9]. Effective bioremediation depends on the proliferation and activity of microorganisms and environmental conditions affecting microbial development and degradation [12].

Therefore, in broad terms, bioremediation relies on selecting appropriate microorganisms at suitable sites for the efficient degradation of toxicants under optimal environmental conditions. By converting waste into carbon dioxide, biomass, water, or other non-toxic materials, bioremediation mineralizes waste and minimizes the requirement for further treatment [12]. Figure 1 illustrates the various stages of wastewater treatment, which are categorized into preliminary, primary, secondary, and tertiary processes.

Besides processing urban debris and wastewater, microorganisms can also decompose pesticides, chemical waste generated from agriculture, fuel remnants, and imperishable compounds such as chlorofluorocarbons, chlorinated solvents, and several organic materials. During the process, microorganisms may be introduced into the polluted site from their place of origin, or they may be isolated and endemic in the polluted area. Microbial population transforms contaminants through reactions involved in their metabolism. Behavior of several microbial species is also a major factor involved in the biodegradation of a contaminant [12,15].

3. Sources of Water Pollution

Water contaminants comprise domestic and industrial waste, and can be broadly classified as chemical pollutants, pharmaceutical contaminants, and irrigation discharges. They constitute infectious agents, microbial toxins, and spores in water bodies that affect the day-to-day water requirements [16]. Certain microbial pathogens that contribute to water pollution are responsible for causing waterborne diseases. These organisms include fungi, bacteria, protozoa, viruses, roundworms, and flatworms [5]. *Enterococcus faecalis, Enterobacter cloacae, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris*, or *Pseudomonas aeruginosa* account for opportunistic pathogens, which affect immunocompromised patients and cause systemic infection. Moreover, *Shigella* and *Salmonella* sp. or strains of *Escherichia coli* are leading causes of waterborne diseases [17,18].

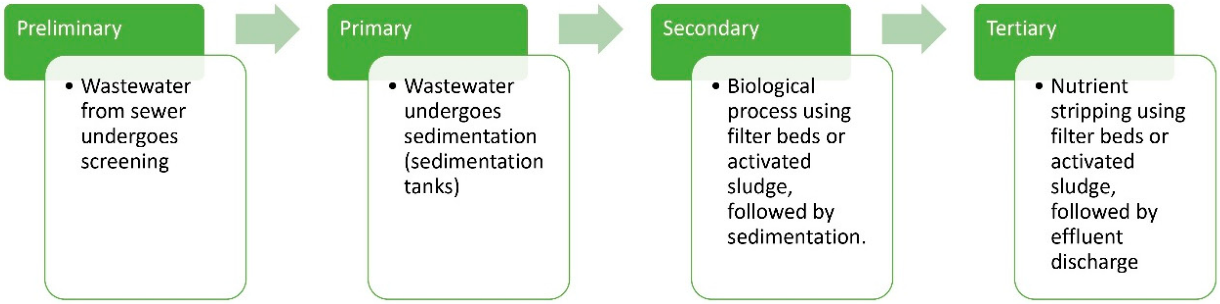

Figure 1: Stages of Wastewater Treatment.

4. Water Pollutants

4.1. Inorganic Chemicals

Various pollutants exist under this category, including heavy metals, hydrocarbons, inorganic anions, pesticides, radioactive substances, cosmetics, and medication. Their presence can lower water suitability for use by biological organisms residing in large concentrations. Industrial waste with Hg, Cd, and Cr, agricultural and domestic waste containing nitrogen, along with naturally occurring F, As, and B, can be considered as sources. Human activities like substandard sanitation, hazardous farming methods, and industrial wastes lead to the addition of heavy metals to water [19].

Inorganic contaminants are not easily decomposed; they gradually settle into the aquatic environment and become hazardous to aquatic life. The category of inorganic water pollutants is composed of heavy metal halides, trace elements, radioactive compounds, inorganic salts, cyanides, sulfates, cations, and oxyanions [19–21].

Massive amounts of hazardous heavy metals and other contaminants, like As, Cd, Cr, Cu, Co, Hg, Ni, Pb, Sn, and Zn, are found in industrial effluent. Toxic heavy metals may originate from various sources, including mine waste, electroplating, hospital waste, sewage, smelters, battery factories, dye and alloy companies, and electronic factories. Natural or man-made sources of water might contain these heavy metals. Examples of natural causes include volcanic eruption, soil erosion, and rock disintegration, while human activities leading to water contamination include burning fossil fuels, mining, landfilling, urban water runoff, irrigation, processing of metals, manufacturing of printed circuit boards, colour dye production, and several other activities. Consequently, water is not accessible for use by common people [19,22,23].

4.2. Organic Compounds

Numerous chemical contaminants found in wastewater include pesticides, herbicides, fertilizers, phenols, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), heterocyclic aliphatic compounds, agricultural runoffs, bacteria, sewage, and effluents from the food processing industry. Wastewater from industrial and agricultural processes has organic components. It includes wastewater from farms that contain high levels of herbicides or pesticides, coke plant wastewater carrying different types of PAHs, chemical industry wastewater that contains different toxic compounds, including PCBs and polybrominated diphenyl ethers (PBDEs), food industry wastewater, and municipal wastewater. These organic contaminants in water pose a hazard to human health and the environment [19,24].

5. Effects of Water Pollution

Anthropogenic and many industrial activities generate heavy metals, which contaminate water and cause severe harm to marine habitats. They are not biodegradable and harm animals and plants, which means they pose an appreciable risk to both life and the surroundings [4]. Pollutants can exert different effects depending on their sources and types.

Certain types of waste, including dyes, heavy metals, and various organic contaminants, are known to be carcinogenic. Chemicals that damage the endocrine system and affect human and non-human animal reproduction and growth include some hormones, medicines, cosmetics, and waste generated from products of personal care [25].

The following are some detrimental effects of contaminated water on human health and the environment as a whole [26]:

(a) **Health Impact**

- ❖ One of the main causes of waterborne illnesses, such as cholera, typhoid, hepatitis A, and dysentery, is contaminated water.
- ❖ Cancer, neurological abnormalities, and disorders of reproduction are just a few of the severe health consequences that can result from exposure to harmful substances in contaminated water.

(b) **Environmental Effects**

- ❖ Water pollution may affect aquatic habitats, interfere with fish reproduction, and cause the death of fish.
- ❖ The loss of biodiversity is the result of all of these.Eutrophication, which results in algal blooms that lower water oxygen levels, can be brought on by an excess of nutrients from agricultural runoff

(c) **Economic Impacts**

- ❖ Reduced agricultural output, higher medical expenditures, and lost tourism revenue are just a few of the substantial financial consequences that water contamination can have.
- ❖ Fish populations are impacted by water pollution, which lowers harvests and causes financial losses for the fishing sector.
- ❖ The cost of eliminating contaminants from waterways and regenerating harmed ecosystems can be substantial.

(d) **Other Effects**

- ❖ There is a shortage of clean water available for drinking, irrigation, and industrial usage when freshwater sources are rendered unsuitable due to water pollution.
- ❖ This may make challenges with water scarcity worse.

6. Wastewater Treatment Methodologies

Major methodologies involved in treating the wastewater are physiological and biological processes. Conventional physicochemical methods employed are precipitation, evaporation. Osmosis, electrochemical treatment, ion exchange, and sorption (Figure 2). They are neither cost-effective nor environmentally friendly [27–29]. Biological methods are preferred as they are efficient in removing minute concentrations of metal ions and other waste materials.

7. Characteristic Features of Biological Wastewater Treatment

Biological WWT is eco-compatible and cost-effective. It has a high metal binding potential of microbial consortium, which can remove heavy metals from a contaminated site effectively. It is highly effective even at low concentrations and has no adverse effects on aquatic ecosystems. Biological treatment is highly effective, as the microbial population easily adapts to the environment [28,30–32].

8. Need for Microbial-Dependent Remediation of Polluted Water

To safeguard both human health and the environment, microbial bioremediation rapidly and affordably immobilizes or eliminates pollutants [33–35]. Exogenous, specialized microorganisms or genetically modified microbes are being studied in various ways to improve the process [36]. Microbial remediation is capable of efficient and cost-effective contaminant removal, depending on various spatial and temporal factors, including the pollutant, the hydrogeologic environment, microbial ecology, and others. By adding nutrients (mainly nitrogen and phosphorus), oxygen as an electron acceptor, and substrates like toluene, phenol, and methane, or by presenting microbes with preferred catalytic properties, the bioremediation action through microbes is increased [37,38].

Therefore, in general, the bioremediation technique relies on locating the desired microorganisms at an appropriate location for efficient degradation under requisite environmental conditions. Biological treatment procedures turn trash into water, carbon dioxide, plant matter, or other benign compounds, thereby causing waste to mineralize and eliminating the need for additional treatment procedures. The term "bioremediation" refers to the handling of a wide variety of substances [12].

In addition to processing of urban trash and wastewater, microbial populations can also be employed for decomposition of pesticides, chemicals of agricultural waste, derivatives of fuel oil, and non-perishable compounds like chlorinated solvents, chlorofluorocarbons, and several more organic compounds. The metabolic activities of many organisms can also lead to the breakdown of chemicals [12].

9. Microbial Activity in the Treatment of Wastewater

Apart from the existing WWT techniques, microbial population plays a significant role in the degradation of wa-

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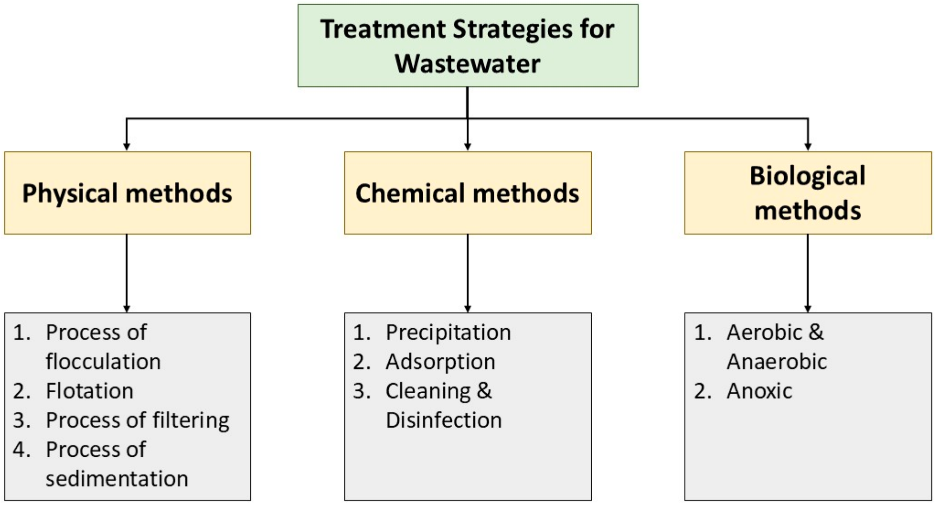

Figure 2: Strategies of Wastewater Treatment.

ter pollutants. Factors enhancing the technology involve the community of microorganisms, their structures, adaptability to the environmental conditions, and optimization of the biological systems. The potential of microbial WWT has become more stringent with the development of cultivation-independent techniques and a suite of molecular methods.

Using the culture-independent techniques (DGGE: Denaturing Gradient Gel Electrophoresis) [39], molecular methods (T-RFLP, Cloning, FISH) [5,40,41] describe microbial community structure present in polluted water bodies. These techniques were entirely conducted using these molecular methods and metagenomic studies. Briefly, the research outcomes explained that removal of contaminants from activated sludge is promoted by phylum Proteobacteria, along with other groups like Chloroflexi, Bacteroidetes, Actinobacteria, Firmicutes, Planctomycetes, and many more in varied concentrations [42–44]. The basis of pollutant degradation involves carbohydrates, proteins, and amino acid derivatives or the metabolic products formed from aromatic compounds [45]. Key genera of microbes effective in wastewater treatment are summarized in Table 1(a–c).

The major microbial species associated with efficient wastewater treatment (Figure 3) include lactic acid bacteria and photosynthetic bacteria [46]. The microbial consortium involved in bioremediation of wastewater includes several bacterial species like *Arthrobacter, Achromobacter, Alcaligenes, Pseudomonas veronii, Acinetobacter, Corynebacterium, Flavobacterium, Micrococcus, Sphingomonas, Rhodococcus, Nocardia, Mycobacterium, Bacillus cereus, Kocuria flava, Sporosarcina ginsengisoli, Vibrio, Lactobacillus plantarum, Lactobacillus casei, Streptococcus lactis (lactic acid bacteria), Rhodopseudomonas palustris*, and *Rhodobacter sphaeroides* (Photosynthetic bacteria). Fungal species like *Penicillium canescens, Aspergillus fumigatus*, and *Aspergillus versicolor* are also involved in the process of bioremediation. Yeasts like *Saccharomyces cerevisiae* and *Candida utilis* form part of the consortium. Algae like *Cladophora fascicularis*, *Spirogyra* sp., *Cladophora* sp., and *Spirulina* sp. are also involved in bioremediation [46–49].

10. Bacteria-Dependent Bioremediation

Bacteria involved in the degradation of pollutants in wastewater treatment are predominantly aerobic, as their activity requires a significant amount of oxygen. Facultative and obligate anaerobes may also be present temporarily during treatment processes. Additionally, a few anaerobes, such as 18 species of *Longilinea, Desulforhabdus, Georgenia, Thauera, Desulfuromonas*, and *Arcobacter* genera, actively participate in the treatment processes and are released into the water bodies through sewage systems [50,51]. Among the anaerobes, *Methanosarcina, Methanosaeta,* and *Clostridium* are responsible for methane fermentation, though other species aid in the breakdown of complex organic macromolecules into simple compounds [52–55].

Bacteria are widely used in wastewater treatment, owing to a wide enzymatic activity and their prevalence in sewage water [56]. Bacterial cells typically range in size from 0.5 to 5 μm, depending on various shapes, like spherical, spiral, straight, and curved rods. Depending on their shape, the bacterial cells are observed singly, in pairs, or

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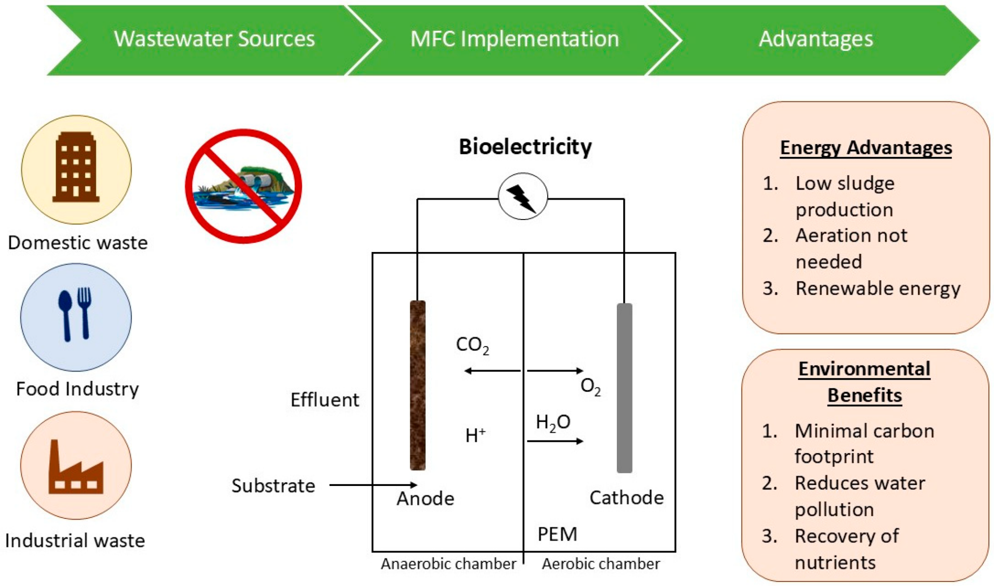

Figure 3: Workflow of Wastewater Treatment.

even in chains [57]. There are two major categories of bacteria: heterotrophic and autotrophic. Autotrophic bacteria utilize inorganic compounds as sources of carbon and energy, while heterotrophic bacteria such as *Pseudomonas, Flavobacterium, Achromobacter*, and *Alcaligenes* use organic materials. Heterotrophic bacteria are further classified based on their need for oxygen into:

(i) aerobic bacteria, which require free oxygen for the breakdown of organic matter,
(ii) anaerobic bacteria, which grow in the absence of oxygen to break down organic matter,
(iii) facultative bacteria, which disintegrate organic materials under both aerobic and anaerobic conditions.

11. Bioremediation by Aerobic Bacteria

Aerobic bacteria are most frequently employed for biological wastewater treatment, including trickling filters and activated sludge processes. The following equation describes the process:

$$\text{Organic waste} + \text{Aerobes} \rightarrow CO_2 + H_2O + \text{Energy} \quad (1)$$

They facilitate the breakdown of organic matter. Such bacteria operate as autocatalysts and decompose organic matter under aerobic conditions. Based on factors such as pH, temperature, and the type of biological reactions involved, different concentrations of aerobic bacteria are used. Among these, the activated sludge process utilizes the highest concentration of bacteria. For converting a significant volume of feedstock in aerobic WWT, the activated sludge procedure is a straightforward and economically viable practice. Anaerobic bacteria have a substantially slower metabolic rate than aerobic bacteria. However, a major limitation of the process in aerobic conditions is the production of excessive biomass, often known as clarification sludge. In addition, it is quite cumbersome, to manage and dispose of this enormous amount of sludge, which has major environmental consequences, such as direct and indirect greenhouse gas emissions. Further, excessive concentration of heavy metals and other hazards decreases the use of sludge as fertilizer for agriculture, necessitating its processing and treatment before final placement on land [58]. Moreover, dumping sludge in landfills can lead to the leaching of hazardous metals and organic pollutants into nearby soil and groundwater sources, thereby causing secondary pollution [59]. Several AGT (Advanced Green Technology) techniques are currently being applied either alone or in conjunction with conventional WWT techniques.

12. Bioreactors

(i) **Fixed Bed Bioreactor-** The multichambered tanks that collectively make up this bioreactor contain closely packed chambers of permeable porous plastic, ceramic, and foam. In this setup, wastewater flows over an immobilized media bed, which is composed with sufficient surface area for the development of a tough and resilient biofilm. This reduces the costs associated with sludge formation and removal [60,61].

(ii) **Moving-Bed Bioreactor-** These reactors have aeration tanks with small, polyethylene movable biofilm carriers that comprise an internally tethered vessel by sieves for media retention. These types of bioreactors can treat wastewater with an elevated Biochemical Oxygen Demand (BOD) within a constrained space, eliminating the requirement for plugging. They are followed by a secondary clarifier, where extra sludge settles down, passes through a filter, and is then removed as solid waste [62].

(iii) **Membrane Bioreactors**- They employ an advanced technique for wastewater treatment (WWT), using membrane filtration to separate suspended solids more effectively than traditional methods such as sedimentation or settling. The concept of filtration enables effective operation with long solid residence times, enhanced mixed liquid suspended solids (MLSS), to produce significantly superior outcomes than the traditional activated sludge procedure [63].

(iv) **Biological Trickling Filters**- They work by pumping air or water through a medium of ceramics, foam, gravel, sand, and other materials. The media are designed to build up a surface biofilm. To accelerate the disintegration of organic compounds in air or water, biofilms can contain both aerobic and anaerobic microbes. This technique is frequently used to remove H_2S from municipal wastewater [64]. Figure 4 gives a detailed representation of a wastewater treatment plant.

13. Bioremediation by Anaerobic Bacteria

Due to strict environmental regulations and policies, anaerobic treatment has significantly increased in popularity despite the drawbacks of aerobic treatment, like high energy cost and sludge (Figure 5) [65]. Anaerobic bacteria decompose organic pollutants present in wastewater and derive energy from nitrates and sulphates [66] as depicted in Equations (2) and (3):

$$\text{Organic waste} + \text{Nitrate} \rightarrow CO_2 + N_2 + \text{energy (bound to oxygen in nitrate)} \quad (2)$$

$$\text{Organic waste} + \text{sulphate} \rightarrow CO_2 + H_2S + \text{energy (bound to oxygen in sulphate)} \quad (3)$$

These anaerobic reactions have a slower metabolic rate, necessitate a large population of bacteria, and take a very long time to reduce organic compounds [67]. However, the technique has many advantages [68,69]. Due to the absence of oxygen, aerosol formation is also prevented, making this method energy-efficient. More than 95% of the organic material is converted into combustible gases; hence, it provides a practical illustration of waste disposal. To optimize the advantages of both aerobic and anaerobic treatment methods, increasing attention is given to their strategic and precise integration. To obtain the desired result, several modifications have been explored to improve process efficiency [70]. A notable example is the combined approach of the two processes, in which one portion of wastewater is treated by aerobic processes and the other by anaerobic processes. This integrated methodology lowers P levels in the effluent along with the odour and sludge formation. Distillery treatment of wastewater is a prime example of a mixed process, which first performs anaerobic treatment to produce biogas before moving on to an aerobic process to meet wastewater regulations [65].

Additionally, one of the most significant and notable uses of microbes for WWT is the ”Microbial Fuel Cell” (MFC) method of producing bioelectricity. It exemplifies cutting-edge technology for microbial metabolism-based generation of power [71]. This method makes use of microbes, particularly bacteria, to convert chemical energy created during the oxidation of organic and inorganic materials found in effluent into electrical energy. To effectively generate electricity from wastewater released by paper, agro-based, and dye industries, several bacteria, including *Klebsiella pneumonia, Shewanella oneidensis, Nocardiopsis* sp., *Escherichia coli*, *Pseudomonas* sp., and *Streptomyces enissocaesilis*, are employed [72,73]. In an MFC, the cathode and anode compartments are typically separated by a proton exchange membrane, similar to other fuel cells [74]. Protons and electrons are released as a consequence of the oxidation of organic-containing wastewater in the anodic portion. By traversing the membrane and outer circuit, the electrons and protons migrate from anode to cathode, generating an electric current in the process. As a result, MFC is reliable for generating electricity as it is affordable (uses polluted water as a medium), clean, renewable, and produces no harmful byproducts [71,72].

14. Fungal Dependent Bioremediation

”Fungi possess unique metabolic capabilities that enable them to degrade and eliminate a wide range of contaminants; therefore, fungal bioremediation presents a viable and sustainable approach to addressing environmental contamination. Laccases, peroxidases, and hydrolases are

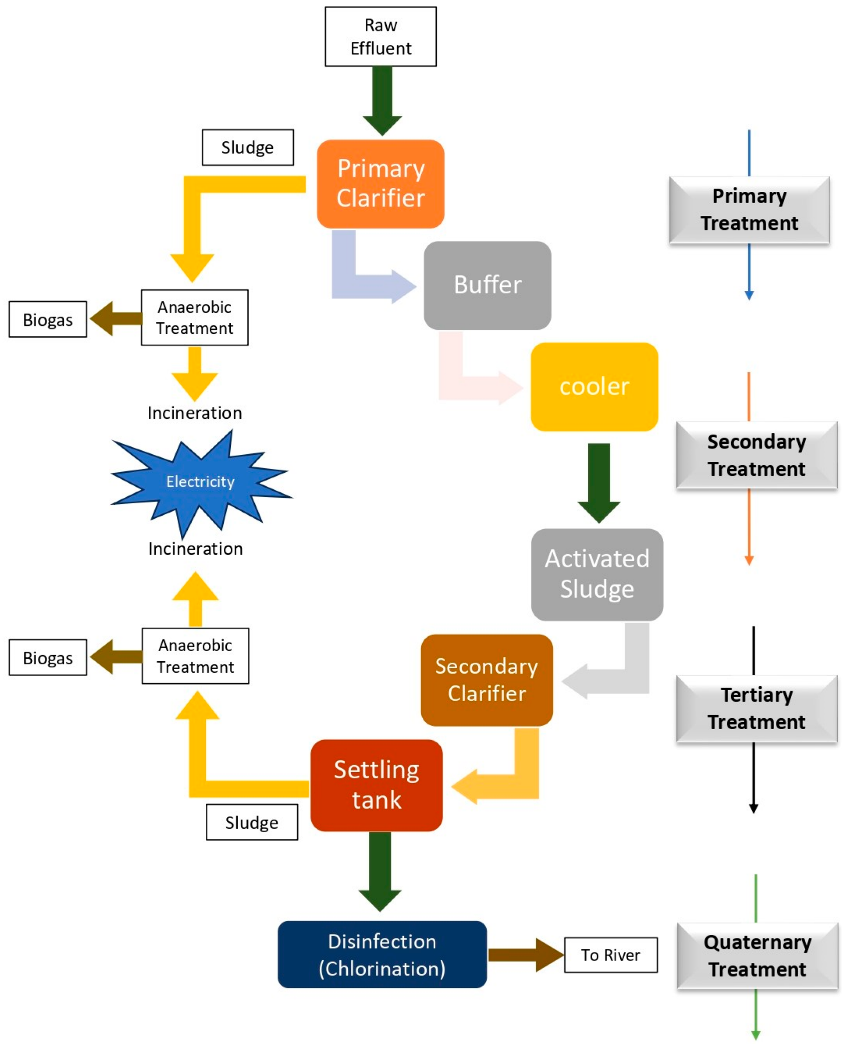

Figure 4: Schematic representation of a wastewater treatment plant.

among the few of many enzymes that fungi possess and facilitate the degradation of heavy metals, complex organic compounds, and xenobiotics into less toxic forms. Due to their adaptability, fungi can be used in a variety of environmental settings, such as soil, water, and air remediation (Table 1b). Recently, the fungal phyla include: *Ascomycota, Basidiobolomycota, Basidiomycota, Calcarisporiellomycota, Chytridiomycota, Entomophthoromycota, Entorrhizomycota, Glomeromycota, Kickxellomycota, Monoblepharomycota, Mucoromycota, Neocallimastigomycota, Olpidiomycota*, and *Zoopagomycota* [75–77].

15. Algae-Dependent Bioremediation

Various microalgal species have demonstrated remarkable abilities for the bioremediation of nutrients, heavy metals, emerging contaminants, and pathogens from wastewater, including *Chlorella, Phormidium, Limnospira* (previously *Arthrospira, Spirulina*), and *Chlamydomonas* [78].

Photosynthetic microorganisms such as microalgae, eukaryotic algae, and cyanobacteria exhibit immense potential in the biodegradation of contaminated water [79]. This is an ecologically safe and sustainable technique for removing heavy metal contaminants, nutrients, and several organic pollutants from wastewater derived from municipal and industrial sources [80]. The technique involving algal species for biodegradation is called “phycoremediation” [81,82]. *Chlorella vulgaris, Chlorella* sp., *Tetraselmis* sp., *Scenedesmus* sp., *Picochlorum* sp., etc. are the algal species that are most frequently utilised for phycoremediation [83]. *Anabaena* species, *Dolichospermum* species, *Hapalosiphon* species, *Scytonema* species, *Leptolyngbya* species, *Chroococcus* species, *Pseudospon-*

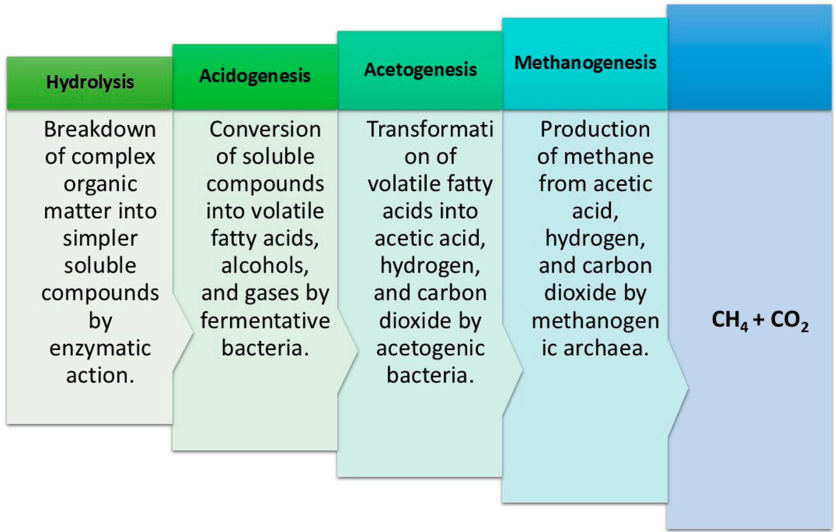

Figure 5: Showcasing stepwise anaerobic digestion.

giococcus species, *Gloeocapsa* species, *Lyngbya* species, *Oscillatoria* species, and *Synechocystis* species are among the several cyanobacterial strains.

16. Archaea

Integrating resource recovery and energy production into the clean water generation process highlights the crucial role of archaea-based technologies in wastewater treatment. Archaea play a vital role in transforming contaminants into sustainable resources. Archaea remain poorly understood, especially compared to bacteria, which have been extensively studied in wastewater treatment systems. Insufficient literature is available that explains the metabolisms of a few significant archaea and the ecological patterns of archaea in a complex wastewater microbiome. Infrastructure Aging: Many WWTPs still use obsolete equipment, which results in inefficiencies and higher maintenance expenses. It will cost a lot of financial resources to upgrade these facilities [84].

17. Microbial Removal of Inorganic and Organic Constituents from Wastewater

17.1. Removal of Inorganic Constituents

17.1.1. Nitrogen Removal

In WWT, nitrification (conversion of ammonia to nitrite and then to nitrate (nitrification)) and denitrification (conversion of nitrite or nitrate into gases like N_2O and N_2) are major mechanisms for the removal of nitrogen waste. The existence of ammonia and nitrite contribute to eutrophication and is harmful for aquatic bodies. Therefore, oxidation of ammonia is facilitated by aerobic and anaerobic oxidizers. The microbes involved in these processes are proteobacteria and anammox (ammonia oxidation) bacteria [85].

17.1.2. Phosphorus Removal

The concentration of phosphorus in water bodies gives rise to eutrophication and affects environmental conditions. The biological processes involved in the removal of phosphorus-containing waste involve enhanced biological phosphorus removal (EBPR), and putative polyphosphate-accumulating organisms (PAOs). Microbes accumulate phosphorus intracellularly as polyphosphate and are then eliminated by wasting phosphorus-rich sludge. This process is facilitated by glycogen-accumulating organisms (GAOs) as they compete with PAOs [86].

17.2. Organic Matter Removal

Degradation of organic waste from contaminated water is enhanced in the presence of filamentous bacteria. These bacteria are specifically added to biological wastewater plants and bioreactors for waste removal. The removal is facilitated by the formation of bioflocs, a technology that improves the efficiency of fish feed utilization and maximize aquaculture productivity [87], particularly in activated sludge systems. They perform well in adverse conditions of reduced chemical oxygen demand or under substrate-limited circumstances. The active role of filamentous bacteria in the removal of organic matter became more stringent with the development of molecular techniques like FISH and high-throughput sequencing techniques. Excessive growth of filamentous bacteria causes operational problems in wastewater plants. The condition of this overgrowth is defined as bulking, which gives rise to deterioration in the settleability of bioflocs. As a result, the efficacy of the process is reduced; therefore, leads to poor pollutant separation in the final effluent [5,88].

17.3. Carbon Mineralization

Anaerobic digestion breaks down complex carbon compounds. The predominant genera involved in this method are archaea, which are often introduced into wastewater systems. They are prokaryotes, and the classified phylum involved are *Euryarchaeota*, which are currently grouped in the form of six established orders (*Methanomicrobiales, Methanobacteriales, Methanopyrales, Methanococcales, Methanosarcinales, Methanocellales*) and also as a proposed order (*Methanomassiliicoccales*). During the process, Archaea utilize restricted substrates like H_2, CO_2, methylated compounds, and acetate. It generates methane like a value-added by-product, which exclusively involves methanogenic archaea [5].

17.4. Other Complex Molecules

Moreover, a few bacteria have the potential to remove complex pollutants by generating electricity. There are some bacteria recognized for their ability to transfer electrons towards a working electrode and are categorized as microbial fuel cells (MFC). The operating principle is based on electrical performance and the mechanisms of electron and ion transport. Diversity in microbial populations within wastewater offers a broader range of MFC communities for the process. The efficiency of biodegradation through MFCs was supported by studies based on fingerprinting methods [89–92].

Table 1: Depicts the microbial consortium involved in treating wastewater.

(a): Bacterial Consortium Treating Wastewater		
Water Pollutants	**Microbial Diversity**	**Mechanism of Action**
Nitrogenous waste removal	Monophyletic classes- - Betaproteobacteria ammonia decomposers (like *Nitrosomonas* and *Nitrosospira*) - Gammaproteobacteria Nitrosococcus (except *Nitrosococcus mobilis*, which is a beta-proteobacterium)	Ammonia oxidation
	Aerobic nitrite bacteria (NOB) - - Alphaproteobacteria (*like Nitrobacter* 2014), - Gammaproteobacteria (like *Nitrosococcus*) - Nitrospirae (like *Nitrospira*)	Nitrification
	- *Alcaligenes* - *Pseudomonas* - *Methylobacterium* - *Bacillus* - *Paracoccus* - *Hyphomicrobium*	Denitrification
Phosphorous waste removal	*Acinetobacter*	Putative PAO
	Rhodocyclus related organisms	Enriched in EBPR reactors for phosphorous degradation.
	Accumulibacter	Concerning phosphorus and carbon utilization by the microorganism.

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Table 1: *Cont.*

(a): Bacterial Consortium Treating Wastewater		
Water Pollutants	**Microbial Diversity**	**Mechanism of Action**
Organic Matter Removal	Filamentous Bacteria - Alphaproteobacteria (similar to '*Nostocoida*'), Gammaproteobacteria (e.g. *Thiothrix* and similar microbes) - Chloroflexi - Actinobacteria (*Candidatus* 'Microthrix', Mycolata) - *Nostocoida limicola'* I and II, *Mycobacterium fortuitum*	
Complex molecules	Electrogenic Bacteria - *Geobacter* sp., - *Shewanella* sp., - Phototrophic bacteria (like *Rhodopseudomonas* sp.)	Based on electrochemical activities of microbial communities.
Carbon Mineralization	Euryarchaeota - *Methanobacteriales*, - *Methanococcales*, - *Methanomicrobiales*, - *Methanosarcinales*, - *Methanopyrales*, - *Methanocellales* - *Methanomassiliicoccales*	Generates a value-added by-product, methane.
(b): Significant Fungi in Treating Wastewater [77,93]		
Water Pollutants	**Fungi Species**	**Mechanism of Action**
Heavy metals (e.g.Pb, Cd, Cr, Hg)	- *Aspergillus niger* - -*Trichoderma harzianum* - -*Penicillium simplicissimum*	Release organic acids that chelate metals and facilitate the removal of heavy metals.
Hydrocarbons	- *Phanerochaete chrysosporium* - *Pleurotus ostreatus*	These fungi are present in contaminated soil and possess ligninolytic enzymes. Laccase and peroxidase break down complex hydrocarbons into simpler compounds.
Dyes (e.g., azo, anthraquinone dyes)	- *Trametes versicolor* - *Pleurotus ostreatus*	Laccase and manganese peroxidase enzymes degrade the dyes.
Pesticides	- *Phanerochaete chrysosporium* - *Trametes versicolor* - *Bjerkandera adjusta* - *Pleurotus* sp.	Hydrolysis and oxidation through enzymatic pathways.
Pharmaceuticals (e.g., antibiotics)	- *Pleurotus ostreatus* - *Aspergillus fumigatus*	Enzyme-based oxidation and hydroxylation; cytochrome P450-mediated metabolism.
Phenolic compounds	- *Phanerochaete chrysosporium* - *Trichoderma harzianum*	Oxidative breakdown mediated by peroxidases and laccases.
Nitrogenous compounds (e.g., ammonia, nitrates)	- *Aspergillus oryzae* - *Rhizopus* spp.	Assimilatory and dissimilatory nitrate reduction; ammonium assimilation.

Table 1: *Cont.*

(b): Significant Fungi in Treating Wastewater [77,93]		
Water Pollutants	**Fungi Species**	**Mechanism of Action**
Endocrine-disrupting compounds (EDCs)	- *Trametes hirsuta* - *Lentinula edodes*	Oxidation and polymerization using laccase.
Chlorinated compounds	- *Ganoderma lucidum* - *Cladosporium resinae*	Reductive dechlorination and enzymatic oxidation.
Microplastics & synthetic polymers	- *Aspergillus tubingensis* - *Pestalotiopsis microspora*	Depolymerization via hydrolases and esterases.
(c): Significant Algal species in Treating Wastewater [94]		
Water Pollutants	**Algal Species**	**Mechanism of Action**
Heavy metals (e.g., Pb, Cd, Cu, Zn)	- *Chlorella vulgaris* - *Scenedesmus obliquus*	Biosorption and bioaccumulation through cell wall binding and intracellular uptake.
Nutrients (Nitrate, Phosphate)	- *Chlorella pyrenoidosa* - *Spirulina platensis*	Uptake via active transport and assimilation into biomass.
Dyes (e.g., methylene blue, Congo red)	- *Oscillatoria* sp. - *Nostoc* sp.	Adsorption on mucilaginous sheath and enzymatic breakdown.
Pharmaceuticals & Personal Care Products (PPCPs)	- *Chlamydomonas reinhardtii*	Enzymatic degradation, sorption, and photodegradation.
Phenols & Aromatic Compounds	- *Anabaena cylindrica* - *Chlorella minutissima*	Biodegradation is facilitated by oxidative enzymes that incorporate substrates into metabolic pathways.
Pesticides (e.g., atrazine, lindane)	- *Scenedesmus dimorphus*, - *Ankistrodesmus* sp.	Biotransformation using detoxification enzymes.
Organic load (BOD, COD)	- *Spirulina maxima* - *Chlorella ellipsoidea*	Reduction through oxygenation and microbial symbiosis enhances organic matter breakdown.
Oil and Hydrocarbons	- *Dunaliella salina* - *Botryococcus braunii*	Bioemulsification, adsorption, and partial degradation.
Endocrine Disruptors (e.g., Bisphenol A)	- *Chlorella sorokiniana*	Laccase-like activity and photolytic transformation.

18. Mechanism of Action

Bioremediation involves eukaryotes as well as prokaryotes in the elimination of toxic elements from water bodies. The methods employed and promoted in the biological transformation include bioleaching, bio-extraction, biosorption, bioencapsulation, and bioremediation [95, 96].

Furthermore, bioremediation is classified as biosorption and bioaccumulation. These are based on the physicochemical interactions of microbes and pollutants. Factors affecting biosorption include pH, biomass concentration, temperature, and particle size [4]. Both dead and living biomass can be used in biosorption, which does not rely on cellular metabolism. Whereas, bioaccumulation involves intracellular and extracellular processes, in which passive uptake has a restricted and non-specific role [31]. Hence, living biomass is involved in bioaccumulation. This process (biosorption and bioaccumulation) is promoted by microbes (Figure 6), as they possess different macromolecules, like polysaccharides and proteins. They have many charged groups like thioether, carboxyl, sulfydryl, phenol, imidazole, carbonyl, amino, amide, ester sulfate, and hydroxyl [97,98]. The cell wall composition of microorganisms encourages the adsorption of the contaminants [31]. Therefore, algae act as biosorbents and produce less or negligible toxic substances [1]. The potential of microorganisms involved in biodegradation is mentioned in Table 2.

The process of bioremediation is facilitated by complexation reactions, sorption, variation in pH, bioaccumulation, precipitation, and encapsulation.

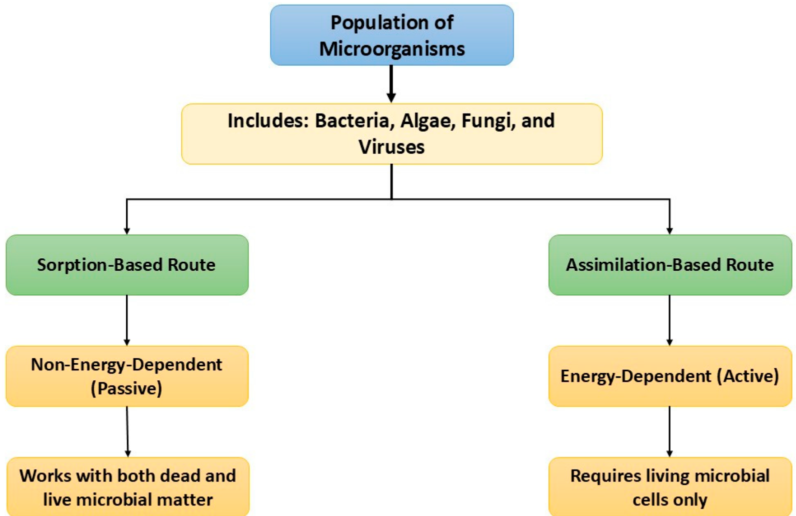

Figure 6: Schematic representation of the degradation of wastewater by microbes.

19. Molecular and Omics Approach

Adoption of bioinformatics by using information from multiple biological databases, including databases of chemical structure and composition, RNA/protein expression, organic compounds, catalytic enzymes, microbial degradation pathways, and comparative genomics, could lead to the objectives of bioremediation [99]. All of these sources are interpreted using a range of bioinformatics methods to investigate bioremediation and develop more efficient environmental cleaning technologies. Only a small number of bioremediation applications have been made because of the lack of information on the variables influencing the growth and metabolism of microorganisms with bioremediation potential [100]. Bioinformatics has been used to map out the mineralization pathways and processes of these bioremediation-capable bacteria and to profile them [101]. Proteomics, metabolomics, transcriptomics, and genomics can all be used to enhance bioremediation investigations. These methods facilitate the assessment of the in-situ bioremediation process since it may correlate DNA sequences with the number of metabolites, proteins, and mRNA, leading to biomarker exploration also [102–104].

20. Genetics

The study of bioremediation bacteria has given rise to a new area of genetics. This method is predicated on microorganisms' capacity to thoroughly analyze their genetic material within cells. Numerous bacteria are used in bioremediation [105]. Genomic technologies like PCR, isotope distribution analysis, DNA hybridization, molecular connectivity, metabolic footprinting, and metabolic engineering are utilized to gain a better understanding of the biodegradation process. 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 are among the PCR-based methods available for genotypic fingerprinting. RAPD can be applied to the study of soil microbial communities to generate genetic fingerprints, build functional structural models, and evaluate naturally related bacterial species [106]. A combination of molecular techniques, including genetic fingerprinting, microradiography, FISH, stable isotope probing, and quantitative PCR, can also be used to study the interactions between pollutant bacteria and natural variables. The quantity and appearance of taxonomic and operational gene markers in the soil can be ascertained by quantitatively analyzing the soil microbial communities using PCR.

Using cluster-assisted analysis, which analyzes fingerprints from several samples, it may be possible to gain a deeper understanding of the relationships between varied microbial populations [104].

21. Transcriptomics

The transcriptome is a vital connection between cellular phenotype, interactome, genome, and proteome that describes the association of genes under specific parameters.

The ability to regulate gene expression is essential for environmental adaptation and, consequently, for survival. Transcriptomics provides a comprehensive understanding of this process across microbial genomes involved in bioremediation. DNA microarray analysis is a potent technique in transcriptomics for determining the amounts of mRNA expression [107].

22. Proteomics and Metabolomics

Proteomics pertains to the total proteins expressed in a cell at a specific location and time, as opposed to metabolomics, which is involved with the total metabolites generated by an organism in a specific time or environment [108]. Proteomics has been used to identify important proteins linked to microbes, analyze protein abundance and compositional changes, and more [109]. Therefore, functional analysis of microbial communities involved in bioremediation becomes more practical and has greater potential than genomics. On the other hand, metabolomics studies are used to analyze biological systems. Implementing these approaches, the identification and recovery of a large number of metabolites in the sample produces an immense quantity of data that can be further utilized to demonstrate variations in the metabolic activity, physiological state, and adaptive responses of microorganisms under different environmental conditions [110].

Table 2: Bacteria are used in the treatment of wastewater.

S.No.	Bacteria/ Species/ Genus	Bacterial Characteristics	Factors Temperature/ pH/Time/ Inoculum	Type of Pollutant	Degradation %	References
1.	*Bacillus amyloliquefaciens* NSB4	*Bacillus amyloliquefaciens* a Gram-positive, aerobic bacterium in soil	25 °C/ voltage below 300 mV/15 Days/40 mL inoculum	Organic pollutant	The obtained result showed a 90.46% reduction in COD of wastewater.	[111]
2.	*Bacillus aryabhattai DDN*	*Bacillus aryabhattai* is a *rhizobacterium* that promotes plant growth and colonizes plant roots.	pH 8–8.7	Sewage Water Pollutants	The obtained result showed a reduction in BOD (65.81%) and COD (58.02%) after 21 days	[112]
3.	*Pseudomonas aeruginosa*	Gram-negative, aerobic, rod-shaped bacterium	-	Dairy wastewater	The obtained result showed a 60% reduction in COD and BOD levels	[113]
4.	*Pseudomonas zhanjiangensis* 25A3E	-	10 °C/96 h		The obtained result showed 72.9% success in removing chemical oxygen demand (COD), 70.6% success in removing ammoniacal nitrogen (NH4+-N), and 69.1% success in removing total nitrogen (TN)	[114]
5.	*B. subtilis*	*Bacillus subtilis* is a Gram-positive, rod-shaped bacterium/optimal growth temperature 30–35 °C	7.12 pH/72 h	Organic pollutant	The obtained result showed a reduction in BOD from 352.18 to 32.56 mg/L COD from 125.12 to 74.28 mg/L of wastewater.	[115]

Table 2: *Cont.*

S.No.	Bacteria/ Species/ Genus	Bacterial Characteristics	Factors Temperature/ pH/Time/ Inoculum	Type of Pollutant	Degradation %	References
6.	*Bacillus spizizenii* DN	Gram-positive, rod-shaped/obligate anaerobe	-	Textile waste water	The obtained result showed 97.78% decolorization, whereas on adding *Bacillus spizizenii* DN metabolites 82.92% decolorization was seen, post incubation of 48 h in microaerophilic conditions.	[116]
7.	*Bacillus aryabhattai* B8W22	-	pH 8.0/30 °C	Phenol in wastewater	The obtained result showed 99.96% degradation of phenolic water.	[117]
8.	*Bacillus velezensis*	*Bacillus velezensis* is a gram-positive, aerobic bacterium		Brewery wastewater-	The resulting bioflocculant exhibited effective wastewater treatment with removal success of 72.0% turbidity, 62.0% COD, and 53.6% BOD.	[118]
9.	*Bacillus subtilis*	-	-	Pharmaceutical wastewater	The Result obtained showed COD reduction 150 mg/L from 395 mg/L initial raw wastewater value, and with a removal efficiency of 62.03% after 14 days. BOD was reduced to 45 mg/L after 14 days with a reduction efficiency of 75.5%	[119]
10.	*Pseudomonas aeruginosa*	-	pH 5 and aluminium resistant up to 250 mg/L	Aluminium removal and recovery from wastewater	The obtained result showed 46.08 ± 1.95% of 50 mg/L aluminium removal by *P. aeruginosa* isolated from wastewater	[120]
11.	*Bacillus* sp. K5	-		Municipal wastewater treatment	The obtained result showed high efficiency in removing nutrients e.g., for COD (90 ± 100%) and NH4+-N (85 ± 100%) removal was observed.	[121]

Table 2: *Cont.*

S.No.	Bacteria/ Species/ Genus	Bacterial Characteristics	Factors Temperature/ pH/Time/ Inoculum	Type of Pollutant	Degradation %	References
12.	*Serratia marcescens Abhi 001*	A Gram-negative, rod-shaped bacterium, which produces a red pigment at room temperature	18 h	Phenolic compound (P cresol) in wastewater	The obtained result showed 85% degradation of phenols in wastewater.	[122]
13.	*Bacillus sterother-mophilus* ABO11	*Bacillus stearother-mophilus*, also known as *Geobacillus stearother-mophilus*/Prefer 30–75 °C temperature/Gram positive/rod shaped/spore forming	Maximum growth was observed at 40 °C, pH 8 and using NH4Cl as a nitrogen source	Removal of phenol from wastewater	The result obtained showed 100% of degradation after 10 days.	[123]

23. Comparative Analysis of Microbial-Dependent Remediation

The bioremediation approach has its advantages and disadvantages. A few of these are summarized below [104]:

23.1. Advantages of Bioremediation

i. Naturally, waste treatment strategy for polluted materials like soil is time-consuming. The number of microorganisms that can break down the pollutant decreases. Though the byproducts, such as carbon dioxide, water, and cell biomass, are typically harmless to life forms or the environment.

ii. It requires minimal work and is frequently performed on-site regularly, without interfering with the regular microbial activity. This eliminates potential hazards to the environment and human health, as well as the quantity of waste that is transported off-site.

iii. In contrast to other traditional techniques that are frequently employed for the cleanup of toxic hazardous waste for the treatment of oil-contaminated regions, it operates cost-effectively. Additionally, it facilitates the full breakdown of pollutants; a large number of dangerous hazardous substances can be converted into less damaging products, and contaminated material can be disposed of.

iv. In the natural process, no hazardous chemicals are used. Fertilizers, in particular, are added to nutrients to promote rapid and vigorous microbial growth. The toxic compounds are completely eliminated due to bioremediation, which converts them into innocuous gases and water.

v. Due to their inherent role in the environment, they are easy to use, less labour-intensive, and inexpensive.

23.2. Disadvantages of Bioremediation

i. It is limited to biodegradable substances. Not all substances undergo a rapid and thorough breakdown process.

ii. Certain novel biodegradation products might be more hazardous than the original substances and persist in the environment.

iii. The bioremediation process is microbial consortium specific, which requires suitable environmental and optimal growth conditions for degradation.

iv. Promoting the process from bench and pilot-scale to large-scale field operations is a challenging task. There may be solids, liquids, or gases that are contaminants. It frequently takes longer than alternative treatment options like incineration or soil excavation and removal.

23.3. Limitations of Microbial-Dependent Remediation

Only biodegradable compounds can undergo bioremediation, and not all break down quickly or completely. In the environment, biodegradation products could be more hazardous or persistent than the parent molecule [124].

i. **Specificity-** Biological processes depend on the availability of metabolically competent microbial populations, proper environmental growth conditions, and the right amounts of nutrients and pollutants are all crucial site elements that are necessary for success.
ii. **Bulk Production-** Scaling up the bioremediation process from pilot and batch scale investigations to large-scale field operations is challenging.
iii. **Technological Enhancements-** To develop novel engineered bioremediation methods that work at sites with composite combinations of toxins that are not evenly distributed in the environment, more study will be required. It could exist in the form of solids, liquids, or gases.
iv. **Time Consuming-** Compared to alternative treatment options, such as excavating and removing soil from a contaminated site, bioremediation is more time-intensive.

24. Future Perspectives and Conclusions

The commercial application of microbial WWT depends on factors such as ecology, microbial diversity, implementation, mechanism of action, sensitivity, and specificity. Microbial treatment of wastewater is involved in both existing and conventional techniques, but the outcome is boosted by a better understanding of the microbial diversity, their metabolic and biological processes. Therefore, prospects can be improved through omics-based research. Genetic engineering, the development of novel microbial species using recombinant DNA technology, is a promising tool in bioremediation. These provide new insights into a host of complex and diverse microbial consortia. The emergence of biotechnological studies has improved knowledge of gene function, regulation, and metabolic potential.

Efforts are currently underway to achieve the Sustainable Development Goals (SDGs) in a reliable and cost-effective manner. To overcome the existing gaps execution of Artificial Intelligence (AI) is anticipated to enhance the process of bioremediation [125]. Novel techniques such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) can be utilized to integrate genetic data more effectively into computational modeling and system-level simulations. Hence, research in this field could lead to a better understanding of bioremediation processes.

List of Abbreviations

AGT	Advanced Green Technology
AI	Artificial Intelligence
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
EBPR	Enhanced Biological Phosphorus Removal
EDCs	Endocrine Disrupting Compounds
FISH	Fluorescence In Situ Hybridization
GAOs	Glycogen-accumulating Organisms
MFC	Microbial Fuel Cell
MLSS	Mixed Liquid Suspended Solids
PAHs	Polycyclic Aromatic Hydrocarbons
PAO	Polyphosphate-Accumulating Organisms
PBDE	Polybrominated Diphenyl Ethers
PCBs	Polychlorinated Biphenyls
T-RFLP	Terminal Restriction Fragment Length Polymorphism
WHO	World Health Organization
WWT	Wastewater Treatment

Author Contributions

T.C.: conceptualization, writing original draft; T.S.: conceptualization, writing original draft; N.K.: analysis, review, and editing; T.B.: conceptualization, review & editing; D.T.: conceptualization, review & editing; R.T.: review & editing; T.B and D.T.: designing and visualization of the figures. All authors have read and agreed to the published version of the manuscript. It is declared that all the figures used in the manuscript are original and self-drawn, though idea was taken from the published articles and references for the same has been mentioned.

Availability of Data and Materials

Not applicable. This is a review article, and all data analyzed or discussed in this manuscript are derived from previously published studies, which are appropriately cited in the references.

Consent for Publication

No consent for publication is required, as the manuscript does not involve any individual personal data, images, videos, or other materials that would necessitate consent.

Conflicts of Interest

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