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Review Article

Microbial Bioremediation Strategies for Sustainable Wastewater Treatment

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Research Highlights

- Comprehensive overview of microbial strategies for sustainable wastewater treatment using microbial consortium.
- Explores advanced microbial biotechnologies, including biofilm reactors, microbial fuel cells, and membrane bioreactors for pollutant removal.
- Highlights the role of omics technologies (genomics, proteomics, metabolomics) in optimizing microbes for enhanced bioremediation.
- Compares microbial and conventional wastewater treatment methods in terms of cost, scalability, and environmental impact.
- Discusses current challenges, future directions, and the potential integration of AI and CRISPR-based approaches in microbial wastewater treatment.

Abstract

Wastewater treatment employs several techniques for removal of various contaminants, which are released as by-products of agricultural practices, industrial operations and human waste, such as heavy metals, hydrocarbons and organic substances. Microbes play a significant role in the elimination of these hazardous substances and the process involved is called bioremediation. Bioremediation is a novel and promising technology and has several advantages over conventional techniques for waste removal. It is flexible, cost-effective and ecofriendly, and thus holds great potential for waste water 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 provides a comprehensive overview of microbial approaches employed in wastewater treatment, including physical, chemical, biological methods and membrane bioreactors. Microbial technologies employed are advanced oxidation, biodegradation and activated sludge. 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 explains different strategies employed for biodegradation, along with their efficacy, recent developments and challenges faced in implementation and commercialization of biodegradation practices.

Keywords- wastewater treatment, pollution, microorganisms, bioremediation, Sustainability

Introduction

Water pollution and its treatment have become major global concerns. Contamination of water is one of the key aspects of environmental pollution. The contaminants are mainly released from industries (fertilizers, mining, pesticides) 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 low- and middle-income group [1] .To further emphasize, World Health Organization (WHO), in its 2024 report, highlights that water contaminated with fecal matter that is consumed by over 2 billion people globally has been attributed to nearly 485,000 diarrheal deaths every 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 of a wide range of contaminants - from nutrient overloads (eutrophication) to emerging pollutants namely pharmaceuticals, microplastics, and personal care products [3] .These alarming statistics necessitate urgent efforts to develop sustainable and efficient wastewater treatment (WWT) solutions. Recent developments in biological WWT encourage the researchers to improvise microbial bioremediation technologies to ensure the availability of purified water [4,5]The method that integrates microbial bioremediation not only offers an eco-friendly and cost-effective alternative but also 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 process of bioremediation, contaminated soil and water are treated with potential microbiological species of yeast, fungi, or bacteria. Bioremediation is described as 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 cause or multiple sources. Major primary sources are released directly into the stream. Effluents from industrial and municipal

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activities, run-off and leachate from sites of solid waste disposal, drainage and run-off from industries and vessel discharge are the common sources contaminating the environment. 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]. Most treatments typically involve seeding polluted water with competent microflora that can degrade hazardous material in order to hasten the bioremediation process [8].

Principle of Bioremediation

M. Robinson gave the method of bioremediation utilising microorganisms [9]. The principle of biological remediation relies on biodegradation [10].. The process is commercially feasible and involves green treatment, 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 a terminal acceptor of electrons, through modification of humidity and temperature in a variety of ways [14].

During bioremediation, microorganisms utilize these contaminants as a nutrient or energy source. Some native microorganisms may exist and act at the site, whereas several other microbes are introduced to the site of treatment via bioreactors in other circumstances [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 efficient degradation of toxicants in presence of necessary environmental surroundings. By converting waste into carbon dioxide, biomass, water or other non-toxic materials, bioremediation mineralizes waste and minimises the requirement for further treatment. [12]. **Figure 1** represents different stages involved in wastewater treatment, which are divided into preliminary, primary, secondary and tertiary.

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. Behaviour of several microbial species' is also a major factor involved in biodegradation of a contaminant [12,15].

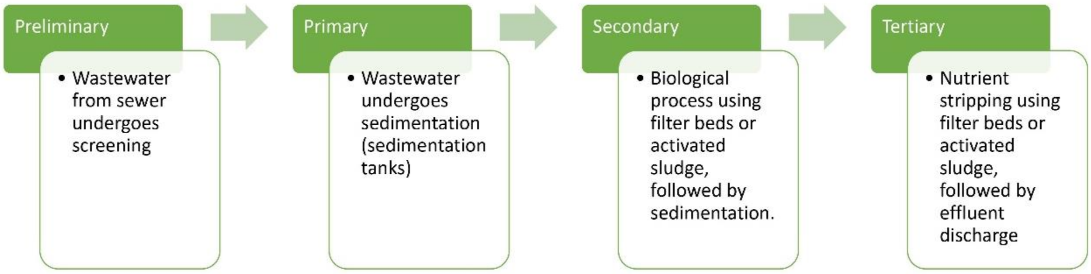

Figure 1: Stages of Wastewater Treatment

Sources of Water Pollution

Water contaminants comprise of domestic as well as industrial waste, which are briefly categorised into chemical pollutants, pharmaceutical contaminants, irrigation discharges. They constitute infectious agents, microbial toxins, and spores in water bodies that affect the day-to-day water requirements [16]. Some microbial pathogens accounting for water pollution, are responsible for causing water borne diseases. These organisms include fungi, bacteria, protozoa, viruses, roundworms or 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 water borne diseases [17,18].

Water Pollutants

(i) 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, industrial wastes, lead to addition of heavy metals to water [19].

Inorganic contaminants are not easily decomposed, which gradually settle into the aquatic environment and become hazardous for the aquatic life. The category of inorganic water pollutants are 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 arise from a variety of ecosystems, 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 board, colour dye production, and several other activities. Consequently, water is not accessible for use by common people [19,22,23].

(ii) Organic compounds

A few chemical contaminants found in wastewater include pesticides, herbicides, fertilisers, phenols, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), heterocyclic aliphatic compounds, agricultural runoffs, bacteria, sewage and effluents from food processing industry. Wastewater from industrial and agricultural processes have 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 (PBDE), food industry wastewater, and municipal wastewater. These organic contaminants in water pose a hazard to human health and environment [19,24].

Effects of Water Pollution

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

Certain waste types, 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 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 elimination of 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.

Wastewater Treatment Methodologies

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

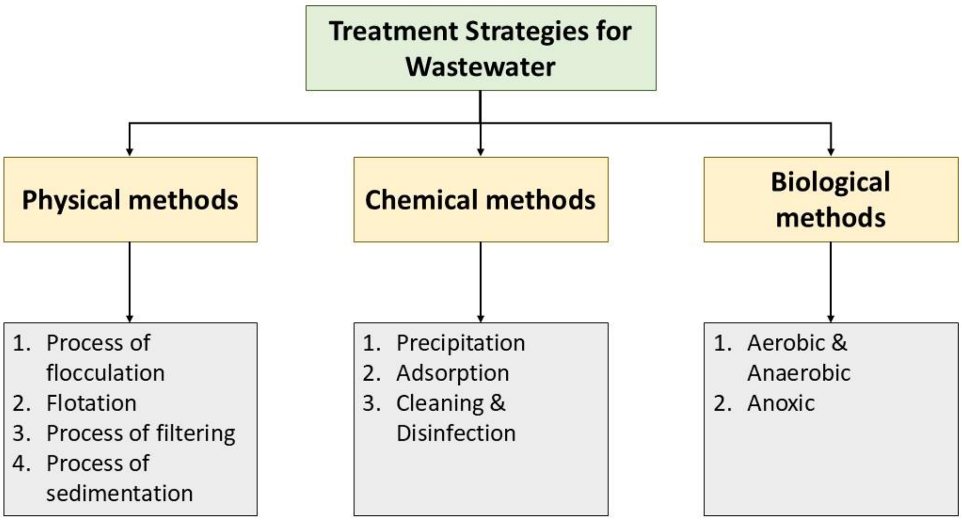

Figure 2: Strategies of Wastewater Treatment

Characteristic Features of Biological Wastewater Treatment

Biological WWT is eco-compatible and cost effective. It has a high metal binding potential of microbial consortium, can remove heavy metals from 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].

Need for Microbial Dependent Remediation of Polluted Water

In order to safeguard both human health and environment, microbial bioremediation rapidly and affordably immobilises or eliminates pollutants [33–35]. Exogenous, specialised microorganisms or genetically modified microbes are being studied in various ways to improvise the process [36]. A microbial remediation process is capable of efficient and cost-effective removal of contaminants, depending on a variety of spatial and temporal variables, including the pollutant, the hydrogeologic environment, the 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, bioremediation action through microbes are increased [37,38].

Therefore, in general, the bioremediation technique relies on locating the desired microorganisms at appropriate location for efficient degradation under requisite environmental conditions. Biological treatment procedures turning 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 population 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].

Microbial Activity in Treatment of Wastewater

Apart from the existing WWT techniques, microbial population plays a significant role in degradation of water pollutants. Factors enhancing the technology involves the community of microorganisms, their structures, adaptability to the environmental conditions and optimization of the biological systems. Potential of microbial WWT has become more stringent by development of cultivation-independent techniques and suite of molecular methods.

Using the culture-independent techniques (DGGE: Denaturing Gradient Gel Electrophoresis) [39], molecular methods (T-RFLP, Cloning, FISH) [5,40,41] described the structure of microorganisms 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, protein and amino acid derivatives or the metabolic products formed from aromatic compounds [45]. Key genera of microbes effective in wastewater treatment are summarised 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, Acrombacter, Alcaligenes, Pseudomonas veronii, Cinetobacter, Corneybacterium, Flavobacterium, Micrococcus, Sphingomonas, Rhodococcus, Nocardia, Mycobacterium, Bacillus cereus, Kocuria flava, Sporosarcina ginsengisoli, Vibrio, Lactobacillus plantarum, L. casei* and *Streptococcus lacti* (Lactic acid bacteria) and *Rhodopseudomonas palustrus, Rhodobacter spaeroide* (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 *Spirullina sp* are also involved in bioremediation [46–49].

Bacteria Dependent Bioremediation

Bacteria involved in degradation of pollutants for treating wastewater are predominantly aerobes, because of oxygen demand standpoint. However, momentary presence of facultative anaerobes and obligate anaerobes is also observed. 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 others involved enable breakdown of complex organic macromolecules into simple compounds [52–55].

Bacteria have a global application in waste water 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 m, depending on various shapes, like spherical, spiral, straight and curved rods. Depending on their shape, the bacterial cells are observed singly, in pairs, or 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, Archromobacter*, and *Alcaligenes* use organic materials. Heterotrophic bacteria are sub-classified further on the basis of their need for oxygen into:

(i) aerobic bacteria, which require free oxygen for breakdown of organic matter,

(ii) anaerobic bacteria, which grow in absence of oxygen to break down organic matter,

(iii) facultative bacteria, which disintegrate organic materials under both aerobic and anaerobic conditions.

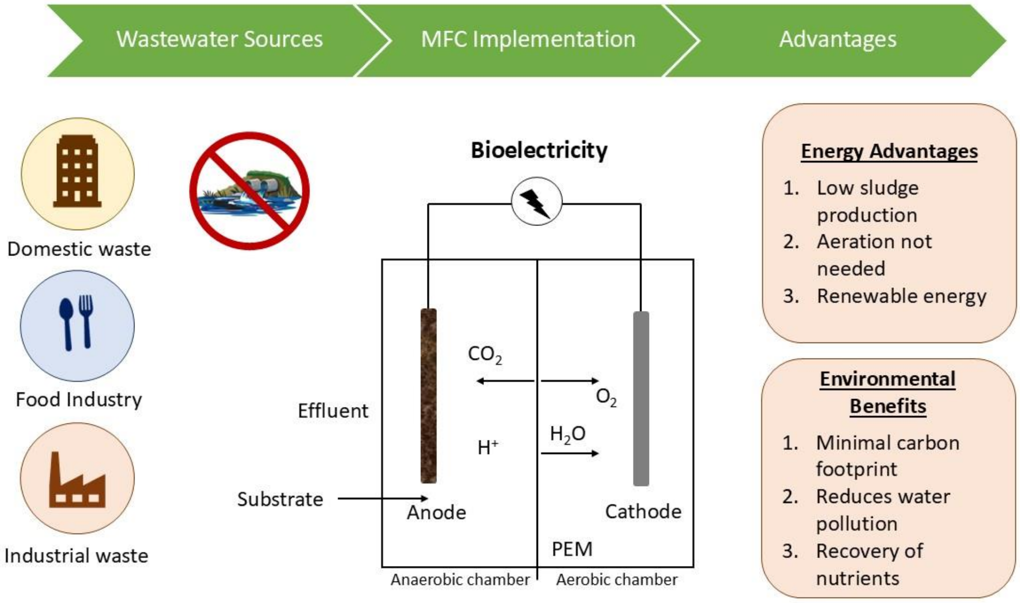

Figure 3: Workflow of Wastewater Treatment

Bioremediation by Aerobic Bacteria

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

$$\text{Organic waste} + \text{Aerobes} \longrightarrow CO_2 + H_2O + \text{Energy} \;\ldots\ldots\ldots\ldots\; (1)$$

They facilitate the breakdown of organic matter. Such bacteria operate as autocatalysts and decompose organic matter under aerobic conditions. Based on pH, temperature, and the biological reaction involved, a series of aerobe concentrations are used, and the highest bacterial concentration is used by the activated sludge process. For converting a significant volume of feedstock in aerobic WWT, activated sludge procedure is a straightforward and economically viable practise. Anaerobic bacteria have a substantially slower metabolic rate than aerobic bacteria. However, major limitation of the process in aerobic condition is production of excessive biomass, often known as clarification sludge. In addition, it is quite cumbersome, managing and disposing 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 fertiliser for agriculture, necessitating its processing and treatment before final placement on land [58]. Moreover, dumping sludge in landfills can result in the leaching of hazardous metals and organic pollutants into soil and groundwater sources nearby, which in turn causes secondary pollution [59]. Several AGT (Advanced Green Technology) techniques are currently being applied either alone or in conjunction with conventional WWT techniques.

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 immobilised media bed, which is composed of enough surface area for 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 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 WWT, through membrane filtration to distinguish various suspended solids rather than sedimentation or settling down. 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. In order to accelerate 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.

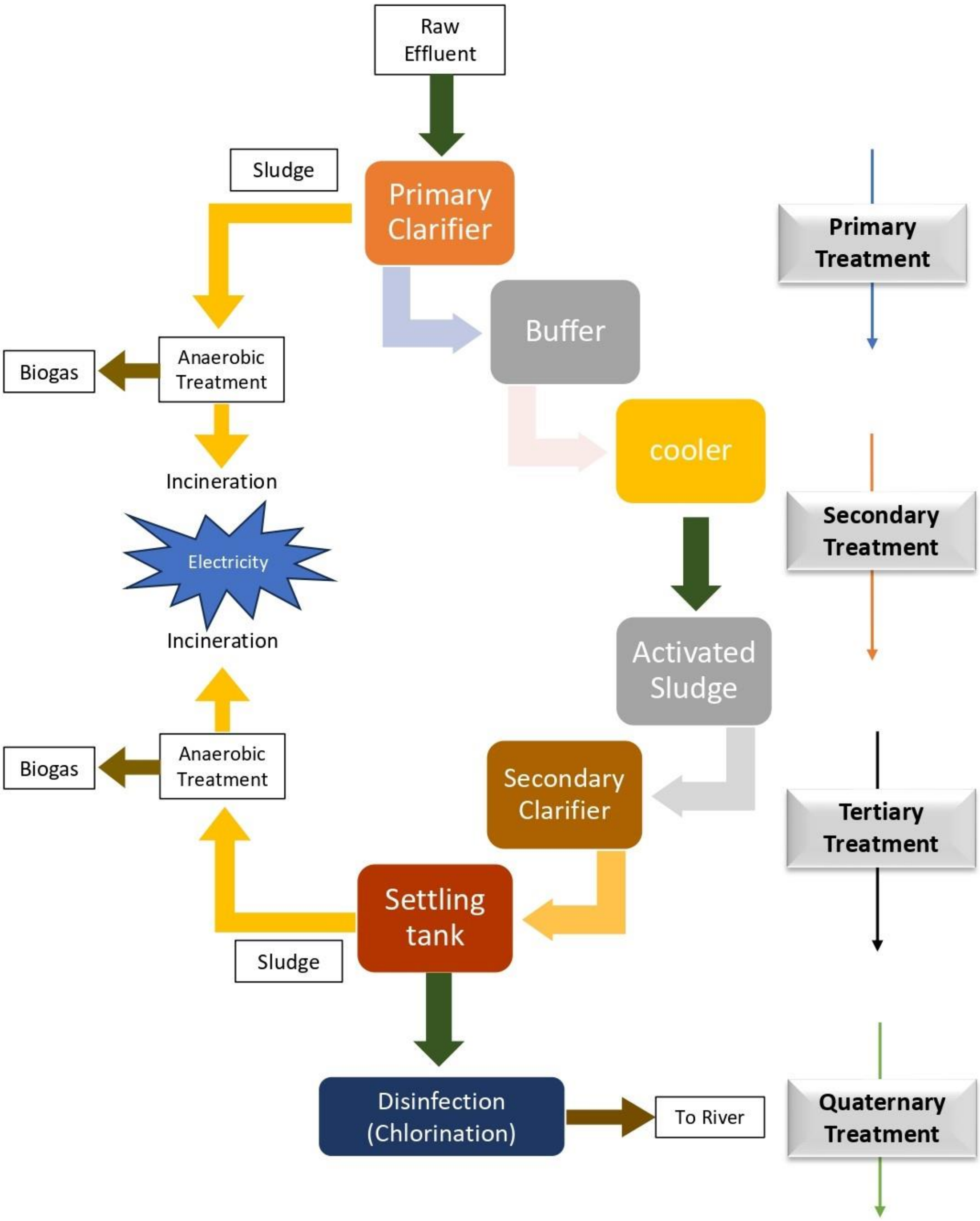

Figure 4: Schematic representation of wastewater treatment plant

Bioremediation by Anaerobic Bacteria

Due to strict environmental regulations and policies, anaerobic treatment has significantly increased in popularity in spite of the drawbacks of aerobic treatment, like high expenses for disposal of energy 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):

Organic waste + Nitrate $\longrightarrow$ $CO_2 + N_2$ + energy (bound to oxygen in nitrate) ……..(2)

Organic waste + sulphate $\longrightarrow$ $CO_2 + H_2S$ + energy (bound to oxygen in sulphate) ……….(3)

These anaerobic reactions have a sluggish 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 O_2, 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 maximise the positive effects of both aerobic and anaerobic procedures, there is more emphasis on their precise merging. To obtain the desired result, many modifications have been tried by fusing the operations of the two processes [70]. A good example is a combination 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 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. In order to effectively generate electricity from wastewater released by paper, agro-based and dye industries, a number of 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 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].

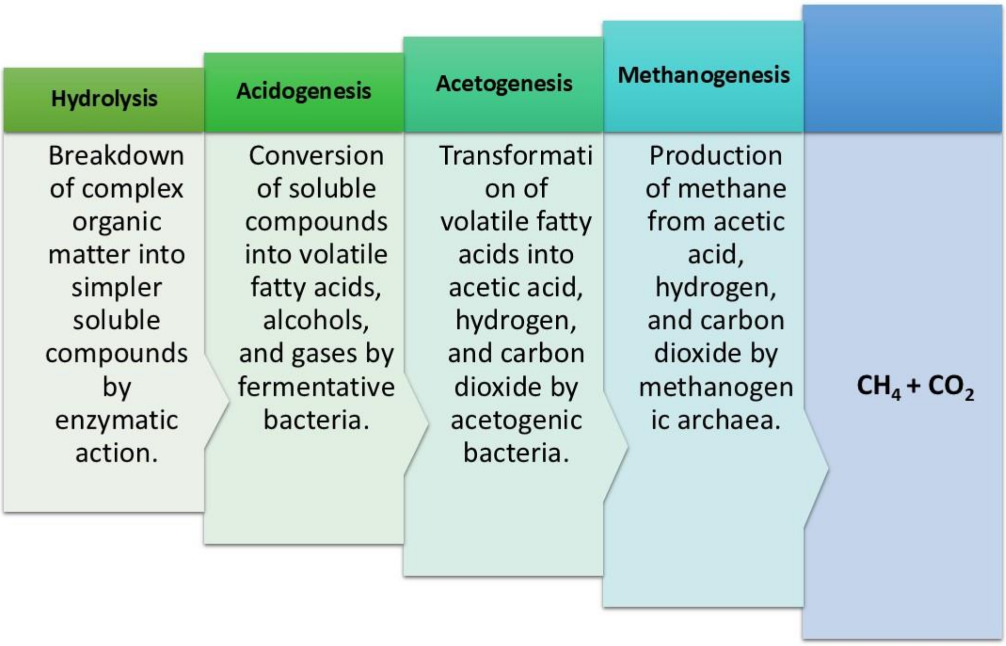

Figure 5: Showcasing stepwise anaerobic digestion

Fungal Dependent Bioremediation

Fungi have unique metabolic skills to break down and eliminate a variety of contaminants hence, fungal bioremediation offers a viable and sustainable solution to environmental contamination. Laccases, peroxidases, and hydrolases are among the few of the many enzymes that fungi possess and facilitates the degradation of heavy metals, complex organic compounds, and xenobiotics into less toxic forms. Because of their adaptability, fungus can be used in a variety of environmental settings, such as soil, water, and air remediation **(Table 1b)**. Recently the major phylum of fungi includes:, *Ascomycota, Basidiobolomycota, Basidiomycota, Calcarisporiellomycota, , Chytridiomycota, Entomophthoromycota, Entorrhizmycota, Glomeromycota, Kickxellomycota, Monoblepharomycota, , Mucoromycota, Neocallimastigomycota, Olpidiomycota, and Zoopagomycota* [75–77].

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

Archaea-

Combination of resource recovery and energy production into a clean water production process, Archaea-involved technology is crucial for wastewater treatment. Archaea play a vital role in the transformation of contaminants into sustainable resources. The traits and contributions of archaea are still poorly understood, nevertheless, in contrast to bacteria that are extensively researched in wastewater treatment systems. Insufficient literature is available that explains about the metabolisms of a few significant archaea and the ecological patterns of archaea in a complex wastewater microbiome. Infrastructure for FAging: 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].

Microbial removal of inorganic and organic constituents from wastewater

(i) Removal of inorganic constituents

a) Nitrogen Removal

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

b) Phosphorus Removal

Concentration of phosphorous in water bodies give rise to eutrophication and affects environmental conditions. The biological processes involved for removal of phosphorus containing waste involve enhanced biological phosphorus removal (EBPR), and putative polyphosphate-accumulating organisms (PAOs). Microbes' deposit 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].

(ii) Organic Matter Removal

Degradation of organic waste from contaminated water enhances in presence of filamentous bacteria. These bacteria are specifically added to biological wastewater plants and bioreactors for waste removal. The removal is facilitated by formation of bioflocs (is a technology to improve the effectiveness and utilization of fish feeds and maximizes aquaculture productivity) [87] in activated sludge. They perform well in adverse conditions of reduced chemical oxygen demand or under substrate limiting circumstances. The active role of filamentous bacteria in 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 over growth is defined as bulking, which gives rise to deterioration in settleability of bioflocs. As a result, efficacy of the process is reduced therefore, insufficient separation of pollutants in the final effluent [5,88].

(iii) Carbon Mineralisation

Anaerobic digestion breaks down complex carbon compounds. The predominant genera involved in this method are archaea, which are specifically added to wastewater plants to reduce pollutants. 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].

(iv)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 categorised as microbial fuel cells (MFC). The operation principle is based on electric performance, the electron and ion transport mechanism. Diversity in microbial population 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.

Table 1(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 *Nitrosoccocus mobilis*, which is a beta-proteobacterium)	Ammonia oxidation
	Aerobic nitrite bacteria (NOB) - - Alphaproteobacteria *(like Nitrobacter*2014*)*, - Gammaproteobacteria (like *Nitrococcus*)	Nitrification

	- Nitrospirae (like *Nitrospira*)	
	- *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.
Organic Matter Removal	Filamentous Bacteria - Alphaproteobacteria (similar to '*Nostocoida*'), Gammaproteobacteria (eg *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.

Table 1(b): Significant Fungi in Treating Wastewater [77,93]

Water Pollutants	Fungi Species	Mechanism of Action
Heavy metals (e.g., 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

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		and peroxidase, which break down complex hydrocarbons into simpler compounds.
Dyes (e.g., azo, anthraquinone dyes)	- *Trametes versicolor* - *Pleurotus ostreatus*	Laccase and manganese peroxidase enzymes degrades the dyes.
Pesticides	- *Phanerochaete chysosporium* - *Trametes versicolor* - *Bjerkandera adjusta* - *Pleurotus sp*	Hydrolysis and oxidation through enzymatic pathways.
Pharmaceuticals (e.g, antibiotics)	- *Pleurotus ostreatus* - *Aspergillus fumigatus*	Enzyme base 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.
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.

Table 1(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 facilitated by oxidative enzymes and incorporation into metabolic pathways.
Pesticides (e.g.,	- *Scenedesmus dimorphus,*	Biotransformation using

atrazine, lindane)	- *Ankistrodesmus sp.*	detoxification enzymes.
Organic load (BOD, COD)	- *Spirulina maxima* - *Chlorella ellipsoidea* -	Reduction through oxygenation and microbial symbiosis enhance 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.

Mechanism of action

Bioremediation involves eukaryotes as well prokaryotes in 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 physiochemical interactions of microbes and pollutants. Factors affecting biosorption are pH, concentration of biomass, temperature, and size of particles [4]. Both dead and alive biomass is made available in biosorption, which is not dependent 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 adsorption of the contaminants [31]. Therefore, algae act as biosorbents and produce less or negligible toxic substances [1]. 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, encapsulation.

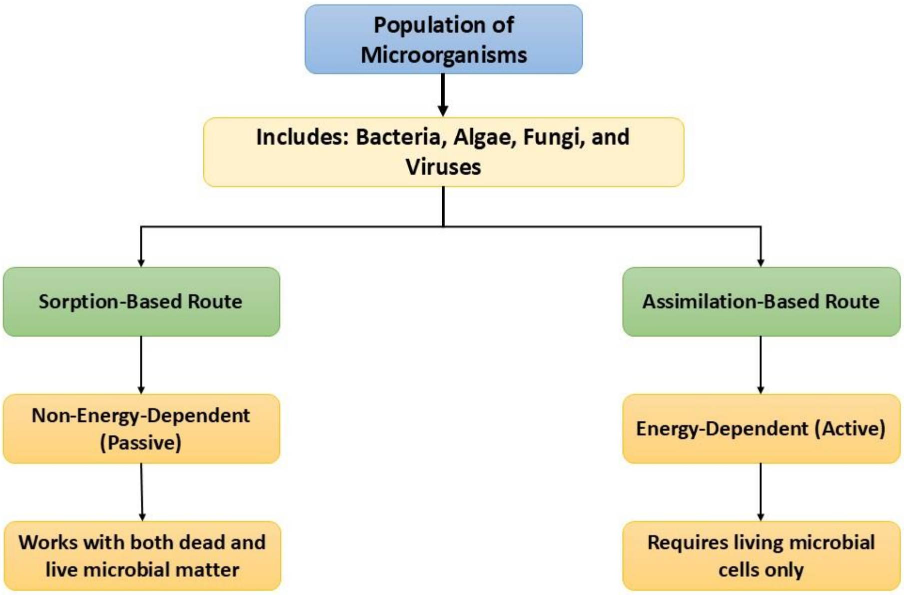

Figure 6: Schematic representation of degradation of wastewater by microbes

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 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 genomes 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].

Genetics

The study of bioremediation bacteria has given emergence to a new area of genetics. This method is predicated on microorganisms' capacity to thoroughly examine their genetic material inside of 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.

Table 2: Bacteria used in the treatment of wastewater

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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 90.46% reduction in COD of waste water.	111
2.	*Bacillus aryabhattai DDN*	*Bacillus aryabhattai* is a *rhizobacterium* that promotes plant growth, which colonizes plant roots.	pH 8-8.7	Sewage Water Pollutants	The obtained result showed reduction in BOD (65.81%) an COD (58.02%) af 21 days	112
3.	*Pseudomonas aeruginosa*	Gram-negative, aerobic, rod-shaped bacterium	-	Dairy wastewater	The obtained result showed 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 hr	Organic pollutant	The obtained result showed reduction in BOD from 352.18 to 32.56mg/L COD from 125.12 to 74.28 mg/L of waste water.	115
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 hour in microaerophilic conditions.	116
7.	*Bacillus aryabhattai* B8W22	-	pH 8.0/ 30 °C	Phenol in waste water	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%	118

					COD, and 53.6% BOD.	
9.	*Bacillus subtilis*	-	-	Pharmaceutical wastewater	The Result obtained showed COD reduction 150 mg/L from 395 mg/L initial raw wastewater value and with removal efficiency of 62.03 % after 14 days. BOD was reduced to 45 mg/L after 14 days with 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 50mg/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 eg for COD (90±100%) and NH4+-N (85±100%) removal was	121

					observed.	
12.	*Serratia marcescens Abhi 001*	A Gram-negative, rod-shaped bacterium, which produces a red pigment at room temperature	18 hr	Phenolic compound (P cresol) in waste water	The obtained result showed 85%degradation of phenols in waste water.	122
13.	*Bacillus sterothermophilus* ABO11	*Bacillus stearothermophilus* also known as *Geobacillus stearothermophilus*/ Prefer 30–75°C temperature/Gram positive/rod shaped/spore forming	Maximum growth was observed at 40˚C, pH 8 and using NH_4Cl as nitrogen source	Removal of phenol from waste water	The result obtained showed 100% of degradation after 10 days.	123

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].

Transcriptomics

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. A thorough understanding of this process throughout the human genome is offered by transcriptomics. DNA microarray analysis is a potent technique in transcriptomics for figuring out the amounts of mRNA expression [107].

Proteomics and Metabolomics

Proteomics pertains with 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 involves in bioremediation become more practical and has greater potential than genomics. On other hand, metabolomics studies used to analyze biological systems. Implementing these approaches, the identification and recovery of a large number of metabolites in the sample, produce immense quantity of data that can be further utilized to demonstrate variations in the

Comparative Analysis of Microbial Dependent Remediation [104]

Bioremediation approach has pros and cons of its own. Few are summarised below.

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 the life forms or environment.
ii. It requires minimal work and is frequently performed on-site on a regular basis 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 in a cost-effective manner. 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 natural process there no hazardous chemicals are used. Fertilizers in particular are added to nutrients to promote rapid and vigorous microbial growth. The toxic compounds are totally eliminated due to bioremediation, which converts them into innocuous gasses and water.
v. Because of their inherent role in the environment, they are easy to use, less labour-intensive, and inexpensive.

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 gasses that are contaminants. It frequently takes longer than alternative treatment options like incineration or soil excavation and removal.

Limitations of Microbial Dependent Remediation [124]

Only biodegradable chemicals can be used in bioremediation. This process is prone to quick and total breakdown. In the environment, biodegradation products could be more hazardous or persistent than the parent molecule.

i. **Specificity-** Biological processes depends on 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-** In order to develop novel engineer 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 excavating and removing soil from a contaminated site, bioremediation takes longer time.

Future Perspectives and Conclusion

The commercial application of microbial WWT depends on various factors like ecology microbial population, implementation, mechanism of action, sensitivity and specificity. Microbial treatment of wastewater is involved in both existing and conventional techniques, but outcome is boosted by better understanding of the microbial diversity, their metabolic and biological processes. Therefore, the future prospects can be enhanced by involving the branch of 'omics.' Genetic engineering, development of novel microbial species using recombinant DNA technology is a promising tool in bioremediation. These provide new insights over 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 in progress to attain the SDG goals that will be reliable and cost effective. In order to overcome the existing gaps execution of Artificial Intelligence (AI) is anticipated to enhance the process of bioremediation [125]. Novel techniques like CRISPR (clustered regularly interspaced short palindromic repeats) can be utilised to easily fit these data into simulation and numerical modelling. 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 Chemicals
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

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Dr. Tanushri Chatterji: conceptualization, writing original draft; **Tripti Singh:** conceptualization, writing original draft; **Namrata Khanna:** Analysis, review and editing**:** ***Tanya Bhagat*** conceptualization, review & editing; **Disha Tyagi:** conceptualization, review & editing; **Riya Totlani:** review & editing

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Conflicts of Interest

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