

Microbes for sustainable Wastewater Treatment: Nature's Cleanup Crew

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Abstract

Realizing the vitality of water for existence, the United Nations has identified water quality management as an urgent concern and included this in Sustainable Development Goal 6.3 in 2015. Microbe-based wastewater treatment is a viable and eco-friendly solution to the problems caused by industrial and urban wastewater. Numerous microorganisms, such as bacteria, fungi, algae, and archaea, are employed in this process because they break down organic contaminants, remove nutrients, and disinfect water. In addition, certain bacterial strains help in the process of denitrification, which turns nitrogen molecules into nitrogen gas that is safe for humans to breathe. Several advanced microbial-based sustainable techniques have been investigated thoroughly, including the use of biofloculants, microbial-based bionanoparticles, microbial fuel cells, etc. Other cutting-edge technologies include Aerobic Granular Sludge (AGS) technology, and an Up-flow Bio Electrochemical Filter Reactor (UBEFR). Enrichment of the reactor tank with Nitrogen-Removing Bacteria (NRB) further enhances the efficiency of the wastewater treatment system. The all-encompassing methods not only generate clean water that can be released or recycled, but they also take care of issues with pathogen inactivation and nutrient removal. To ensure a cleaner and healthier environment for present and future generations, research and development in the field of microbial wastewater treatment should continue to increase efficiency, lower energy consumption, and support sustainable water management practices.

Key words : Wastewater treatment, Sustainable, Microorganisms, Biofloculant, Bionanoparticles.

A sustainable approach to the importance for protecting the environment, treatment of wastewater is of the utmost conserving water resources, and ensuring

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public health. Sustainable wastewater treatment techniques seek to reduce environmental impact and enhance long-term viability, while traditional procedures can be resource- and energy-intensive.

In 2015, all United Nations Member States drafted and accepted a common roadmap for peace and prosperity of people and the planet, and prepared the 2030 Agenda for Sustainable Development. They targeted 17 Sustainable Development Goals (SDGs), which need an urgent call to action for all developed and developing nations in a global partnership. Goal 6 of the SDGs is to ensure the availability and sustainable management of water and sanitation for all by 2030. That is further bifurcated into 8 areas for the sake of convenience in achieving the goals. Goal 6.3 targets to enhance the quality of the world's water by reducing pollution, stopping dumping, limiting the discharge of dangerous substances, cutting in half the amount of untreated wastewater, and significantly increasing safe reuse and recycling worldwide by 2030. Using data from 140 nations and territories, it was anticipated that in 2022, 58% of home wastewater would be properly treated. When it comes to the goal of halving the percentage of harmful domestic wastewater discharges, trends point too little to no progress being achieved⁴⁷. This surges an acute need for an efficient sustainable system for wastewater treatment.

The process of treating wastewater is essential to protecting the environment and the general public's health. Even if conventional techniques have proven successful, it is becoming more and clearer that more sustainable solutions are required. Using microorganisms,

which are little living creatures with enormous potential to make wastewater treatment a more effective and environmentally friendly process, is one intriguing approach.^{14,43}

Role of Microbes :

Microorganisms such as bacteria (Aerobic, Anaerobic, and Denitrifying), Archaea (Methanogens), fungi, protozoa, bacteriophages, and microalgae are employed for the decomposition of organic materials and the elimination of impurities and harmful microbes from wastewater⁵⁰ (Table-1). Their metabolic processes can convert contaminants into innocuous metabolites, which helps to purify water. Because of their resilience and diversity, microorganisms are a good fit for treating wastewater sustainably²⁸. Wastewater treatment facilities may optimize their operations for efficient and long-lasting treatment results by having a thorough understanding of the traits and roles of these varied microbial communities. Several microbial species together in a well-thought-out treatment system contribute to thorough and effective wastewater cleanup⁵⁴. Microbes are excellent at cycling nutrients, particularly in removing phosphorus and nitrogen. Some bacteria can transform nitrogen molecules into nitrogen gas, and others help phosphorus precipitate, which lessens the need for chemical additions in treatment facilities^{39,53}. Microbial colonies are fed by the organic materials found in wastewater. Microbes cleanse water by dissolving complex organic substances into simpler forms through a sequence of metabolic reactions. This process of spontaneous deterioration lessens the need for energy-consuming mechanical treatment techniques¹⁰.

In treatment systems, microbes frequently develop biofilms as slimy coatings of microorganisms on surfaces. By offering a home for a varied microbial population that aids in the elimination of contaminants via a range of metabolic pathways, biofilms improve the treatment process³⁷. Such biofilms are synthesized in advanced systems for efficient wastewater treatment. The attachment of microorganisms to the carrier surface, their subsequent growth and reproduction in a certain environment, and their eventual development into a biofilm with a specific thickness and density may be summed up as the process of biofilm colonization on the carrier surface³¹. Selecting a biocarrier material with high breakdown rates and robust biofilm adherence is crucial. The most popular carriers are polyvinyl alcohol, polyurethane, polyethylene, and polypropylene³.

Role of Microbes in conventional Treatment of Wastewater :

Several wastewater treatment systems use microorganisms' metabolic processes to break down and eliminate contaminants^{4,6}. The following are the main phases of a typical microbial wastewater treatment process.

1. **Screening** : It is the first step for the removal of large debris and solids. Wastewater passes through screens to filter out large objects like sticks, leaves, and plastics.
2. **Primary Treatment** : Here initial separation of suspended solids and organic matter occurs. Wastewater enters a sedimentation tank where gravity allows heavy solids to settle at the bottom as sludge. Grease and lighter particles float to the surface as scum.
3. **Secondary Treatment (Biological Treatment)**: It reduces dissolved and suspended organic matter. For this activated sludge process or biofilm-based systems (such as trickling filters) are employed. Microorganisms, including bacteria, break down organic pollutants into simpler, less harmful substances.
4. **Activated Sludge Process Steps** : It consists of three steps;
 - a. Aeration: Wastewater is mixed with air to provide oxygen for microbial activity.
 - b. Flocculation: Microbial flocs form, capturing suspended particles.
 - c. Sedimentation: Flocs settle, and clarified water is separated from the settled biomass.
5. **Trickling Filter Steps** : It occurs in three steps;
 - a. Wastewater trickles over microbial-covered media.
 - b. Microbes in the media break down pollutants.
 - c. Clarified water is collected.
6. **Tertiary Treatment (Advanced Treatment)**: It is an Additional polishing step to remove remaining contaminants. Advanced treatment processes, including filtration, chemical precipitation, and disinfection, further improve water quality.
7. **Disinfection** : Here elimination of pathogenic microorganisms takes place. Chlorination, ultraviolet (UV) radiation, ozonation, or other disinfection methods are applied to kill or inactivate bacteria, viruses, and other harmful microorganisms. For the removal of pathogenic microbes,

four different types of decentralized waste water treatment systems are used namely; horizontal flow constructed wetlands (HFCW), vertical flow constructed wetlands (VFCW), biological sand filters (BSF), and biofilters (BF). While BSF showed a better ability to get rid of TC and E. coli, BF performed the best when it came to getting rid of germs for all the bacteria that were investigated. On the other hand, VFCW appears to be more successful in decreasing the number of intestinal enterococci, clostridia that reduce sulfur, and Bacteroides species¹⁶.

8. **Sludge Treatment and Disposal :** For management of the solid sludge produced during treatment thickening, digestion (anaerobic or aerobic), and dewatering to reduce volume and stabilize the organic content is carried out. The treated sludge can be used as fertilizer, disposed of in landfills, or incinerated.
9. **Nutrient Removal :** Additional treatment steps, such as biological nutrient removal or chemical precipitation, may be employed to meet regulatory standards and prevent nutrient-related environmental issues.
10. **Water Reuse :** Depending on the level of treatment, reclaimed water can be used for irrigation, industrial processes, or other non-drinking purposes, contributing to water conservation efforts. Depending on the particular layout and equipment employed in a wastewater treatment facility, these procedures may change. Further technological developments can result in the integration of novel techniques to improve the effectiveness and sustainability of microbial wastewater treatment^{17,18}.

Microbes Based Advanced Techniques

Bioflocculants :

The conventional process includes the use of a chemical coagulant in the wastewater tank, which can interact with the suspended impurities and convert them into a neutralized mass to form floccules that sediment in the later stage²¹. These chemical coagulants are either metal-based or polymer-based. Studies have shown that these chemical coagulants severely harm the ecosystem due to their harmful impact on plants, microbes, animals, and even human health. Several diseases including Alzheimer's disease have been reported due to the accumulation of Aluminum in the brain⁴¹.

Differently structured flocculants can be made using microorganisms. These polymers are compounds that are biodegradable, safe for the environment, and possess flocculation properties^{30,32}. They show significant flocculating activity (FA > 70–90%), contingent upon the strain and operating settings. Depending on the flocculating materials and the parameters of the wastewater, these biopolymers have been demonstrated to dramatically reduce suspended solids (SS), turbidity, chemical oxygen demand (COD), total nitrogen (NT), dye, and heavy metals. Removal percentages have been found to approach 90%⁸. A polysaccharide-based bioflocculant BP50-2 from banana peel waste has shown good performance in the removal of pigment, adsorption of heavy metals, and flocculation of particles³⁸, while a polysaccharide-based bioflocculant isolated from the marine actinobacterium *Streptomyces* sp., was efficiently used in the recovery of microalgae after wastewater treatment⁴⁵.

A bioflocculant HCB2, isolated from a marine bacterium *Alcaligenes faecalis* has been identified for the successful removal of BOD, COD, and Sulfur from coal mining wastewater³⁴. From untreated maize stover, a biomass-degrading bacterium called *Pseudomonas* sp. GO2 created an inexpensive bioflocculant. To create a bioflocculant, GO2 directly used untreated biomass as a carbon source, as demonstrated by the gram's iodine staining and BRT analysis. Under optimum circumstances (fermentation period 130.46 h, beginning pH 7.46, and maize stover 0.64%), an ideal flocculating efficiency of 99.8% was achieved. At a dose of 12.5 mg L⁻¹, the extracted bioflocculant had a maximum flocculating effectiveness of 94.7%, demonstrating a great resistance to pH and temperature. Moreover, two green microalgae may be efficiently harvested by the inexpensive bioflocculant at a low GO2 fermentation broth/algal culture ratio⁴¹. A bioflocculant isolated from *B. subtilis*, named CSM5 displayed a variety of functional groups (hydroxyl, carboxyl, and amine), for the high flocculating activity (92 %) at a low dose (0.6 mg/mL). It was shown to be non-toxic and efficient in reducing contaminants in coal mine effluent^{33,43}. Chemical research of a polysaccharide bioflocculant BM2, obtained from *Bacillus megaterium*, exhibited excellent pH and heat stability, cation independence along with substantial flocculation with kaolin clay, Congo red, and Pb²⁺.⁴⁰

Biocoagulants and bioflocculants offer a sustainable alternative to chemical coagulants in water and wastewater treatment, yet their broader application faces key challenges and opportunities. Selection of low-cost, abundant raw materials such as agricultural or microbial byproducts is crucial for economic viability.

Simplifying extraction and purification processes can enhance scalability and reduce production costs. These biopolymers must be tailored to treat varying water chemistries effectively. Industrial-scale implementation requires consistent performance and process standardization. Importantly, biocoagulants enable eco-friendly sludge generation, which holds potential for recovery and reuse in agriculture or bioenergy. Advancing research and innovation is essential for commercial success²⁹.

Bionanoparticles :

Nanoparticles are highly efficient for a wide range of applications due to their unique size, shape, and surface area. The removal of extremely persistent and xenobiotic water pollutants such as cationic dyes, acid dyes, azo dyes, and other contaminants is critical for wastewater treatment.

Different biological organisms, including bacteria, fungi, algae, viruses, yeast, and plants, can be used to create a wide variety of Bio Nano Particles (BNPs)^{4,25}. Every entity uses a different biochemical processing strategy to create BNPs, such as using enzymes to oxidize or reduce metallic ions, sugars, proteins, polyphenols, aldehydes, and carboxyl groups. BNPs have been used in wastewater treatment plants to remove pesticides, refractory organic micro-pollutants, hazardous textile dyes, pigments, pharmaceutical and personal care products (PPCP), halogenated recalcitrant pollutants, and pathogenic microorganisms⁹. Because of their tiny size, nano-sorbent compounds are difficult to separate and recover from polluted water. Magnetic nanoparticles may be separated and recovered from the system using an external magnetic field. As a

result, they have been effectively employed as sorbent materials to remove different heavy metals from wastewater systems⁴⁵. The microorganisms have also been immobilized on such magnetic nano-based carriers for producing biocatalysts for environmental pollution management¹⁹. This is a relatively advanced technique facilitating quick and easy recovery of the catalyst from liquid phase⁴⁶. In a study, the gold nanoparticles with surface proteins derived from the fungus *Cladosporium oxysporum* AJP03 have shown a significant increase in the adsorption of rhodamine-B organic dye from wastewater⁴⁷. Microbial nanoparticle synthesis is a safe, ecologically friendly, and sustainable process that uses renewable resources to reduce metals and stabilize nanoparticles²³. Nanomaterials produced by bacteria can be employed as a pollution control technique because they include many functional groups that can readily target contaminants for potent biological cleanup and restoration of the environment^{35,49}.

Microbial Fuel Cells :

To make the entire process self-sustaining, wastewater treatment plants can be connected with energy generation (bioenergy) and resource recovery (N, P, and K fertilizers and molecular intermediates as value-added products)^{10,20}. Microbes can be used in creative ways to produce power by employing microbial fuel cells⁵. This offers a dual advantage that is consistent with sustainable practices; it treats wastewater in addition to producing electricity^{11, 12}. Several microbes, including bacteria (*Proteus*, *Gluconobacter*, *Klebsiella*, *Micrococcus*, *E. coli*, etc.), fungi (*Saccharomyces*), and photosynthetic microalgae⁵⁰ have been

successfully investigated for this dual purpose¹⁸. The use of nanomaterials in conjunction with microbial fuel cells may be the solution to waste and energy-related issues. Based on optimized process parameters, MFC might perform better when treating any effluent from industrial processes²². To optimize the process several factors affecting energy production should be standardized²². For generating the maximum power possible, microbial metabolism and electron transport processes are also important. In theory, it is challenging to strike a balance between harvesting efficiency and system upscaling²⁰. Furthermore, the advancement of anaerobic technologies aided by bioelectrochemistry has gained significant traction as a substitute technology that may be included in municipal wastewater treatment facilities^{36,54,56}. Wastewater treatment through Microbial Fuel Cells (MFCs) and Microbial Electrolysis Cells (MECs) represents an innovative and sustainable method not only for wastewater remediation but also for bioenergy production, particularly hydrogen gas and recovery of value-added products^{6,7}.

Other Advanced Technologies :

Both artificial and natural wetlands are effective methods of treating wastewater because they use microbial communities to break down pollutants. These systems demonstrate how microorganisms may flourish in a variety of settings and provide long-term therapeutic results¹³. Another advanced technology is **Aerobic Granular sludge (AGS) technology**, an emerging technique for treating wastewater from homes and businesses using aerobic processes. Aerobic

Table-1. Microorganisms employed in Wastewater Treatment Plant as per the Requirements depending on the Composition of Waste

S No.	Categories of Microbes	Names of Microbes	References
1	Activated Sludge Bacteria	<i>Proteobacteria, Bacteroidetes, Chloroflexi, Acidobacteria, Chloroflexi, Saccharibacteria, Planctomycetes, Nitrospirae Acinetobacter, Pseudomonas, Aeromonas. Zooglea</i>	51,52
2	Denitrifying Bacteria	<i>Paracoccus denitrificans, Thiobacillus, Pseudomonas denitrificans, Bacillus subtilis, Pseudomonas stutzeri, Rhodococcus sp., Bacillus licheniformis</i>	55, 24
3	Anaerobic Bacteria	<i>Clostridium, Bacteroides, Methanosaeta, Actinomyces, Bifidobacterium, Clostridium, Propionibacterium and Peptostreptococcus</i>	12
4	Methanogenic Archaea	<i>Methanobacterium, Methanosarcina, Methanococcus, Methanosaeta, Methanoculleus, Methanospirillum, Methanobrevibacter</i>	48,38
5	Fungi	<i>Trichoderma, Acremonium, Talaromyces, Paecilomyces, Cladophialophora, Saccharomyces, Aspergillus, Penicillium</i>	9
6	Algae	<i>Chlorella, Spirulina, Microcystis, Scenedesmus chlorelloides, Chlamydomonas, Chlorella vulgaris, Oscillatoria spp.</i>	11
7	Protozoa	<i>Amoeba, Ciliates (Paramecium.) Flagellates (Euglena)</i>	13
8	Phages (Bacteriophages)	T4 Phage, MS2 Phage, PhiX174 Phage, Coliphages	15
9	Bioaugmentation Microbes	<i>Bacillus, Pseudomonas putida, Rhodococcus, Aeromonas, Acinetobacter, Klebsiella.</i>	42
10	Microbial Biofilm Formers	Microalgae, Bacterial sp.	44

granules can develop a specialized layered structure, with anaerobic species in the anaerobic or anoxic inner core and an aerobic outside layer containing nitrifying organisms and coexisting denitrifying phosphate-accumulating organisms¹⁴.

An Up-flow BioElectrochemical Filter Reactor (UBEFR) was constructed to treat actual household wastewater, without giving importance to positive aeration. A high level of efficiency was achieved in the removal of total nitrogen, ammonia, and chemical oxygen demand (COD). It was observed that specifically, the anode and cathode of the UBEFR were enriched in nitrogen-removing bacteria (NRB) such as *Nitrosomonas*, *Ignavibacterium*, *Thiobacillus*, *Dokdonella*, *Comamonas*, *Sterolibacterium*, and *Flavobacterium*. These NRBS helped remove nitrogen through nitrification and denitrification¹⁵.

Other Sustainable Approaches for Wastewater Management :

Several sustainable approaches are being used for wastewater treatment, like the reuse of treated wastewater for non-potable purposes, such as irrigation or industrial processes, to help conserve water resources. Additionally, recovering valuable resources from wastewater, such as nutrients (nitrogen and phosphorus), can contribute to a circular economy. Decentralized Treatment Systems, in which rather than relying on large centralized treatment plants, decentralized systems distribute treatment processes across various smaller facilities. This can reduce energy consumption and infrastructure costs, especially in remote or rural areas. Further, utilizing

energy-efficient technologies and practices, such as renewable energy sources (solar, wind), and energy recovery from biogas generated during treatment, can reduce the overall environmental impact of wastewater treatment^{26,27}. The use of advanced treatment technologies, like membrane filtration, ultraviolet (UV) disinfection, and advanced oxidation processes, can efficiently remove pollutants and pathogens^{1,2}. They also require less space and energy compared to conventional methods. The application of smart sensors and monitoring systems can optimize the performance of treatment processes, ensuring they operate at peak efficiency, leading to energy savings, reduced chemical usage, and improved overall system reliability. Incorporating green infrastructure elements, such as permeable pavements, green roofs, and rain gardens, can help manage stormwater runoff, reducing the burden on wastewater treatment systems and improving overall water quality. Employing natural processes to treat wastewater, such as constructed wetlands and ponds, can be more sustainable than conventional methods. These systems use plants, microorganisms, and natural filtration processes to remove contaminants from the water^{20,21}.

By integrating these strategies, communities can work towards developing sustainable wastewater treatment solutions that balance environmental, economic, and social considerations.

In the quest for sustainable and environmentally friendly wastewater treatment, researchers and engineers are turning to the microscopic world for innovative solutions.

Microbes have proven to be a boon

for the treatment of wastewater because they break down organic debris, remove nutrients, and lessen the amount of dangerous pathogens. In wastewater treatment operations, a variety of microorganisms are used, each of which contributes to a distinct step of the treatment cycle. Wastewater treatment facilities may optimize their operations for efficient and long-lasting treatment results by having a thorough understanding of the traits and roles of these varied microbial communities. Several microbial species together in a well-thought-out treatment system contribute to thorough and effective wastewater cleanup. Further research is needed to prepare an effective consortium of microbes specifically formulated according to the composition of wastewater to be treated in a particular locality.

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