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Biofilm-mediated wastewater treatment: a comprehensive review

Sonia Saini,[a] Sanjana Tewari,[b] Jaya Dwivedi*[b] and Vivek Sharma *[b]

Global industrialization has increased and so has the pollution generated by industries. Although all types of pollutions are a major concern for the environment, wastewater treatment has always intrigued the interest of researchers globally. Wastewater contains multiple contaminants, which can be treated *via* physical, biological, and chemical methods. Biological wastewater treatment technologies are seen as modern alternatives to conventional techniques that play a crucial role in improving human health and water quality. Organic pollution is one of the primary culprits of several phenomena, including eutrophication, decline in dissolved oxygen, and build-up of toxins, which impact the quality of water. Therefore, it becomes a necessity to eliminate organic pollutants from wastewater. The use of bioremediation as an environmentally benign method for the decontamination of harmful contaminants for environmental sustainability and safety has gained popularity due to the several disadvantages (expensive, operation, efficiency, and start-up) of traditional wastewater treatment plants. Bioremediation has been used to eliminate, mineralize, degrade, and neutralize many inorganic and organic contaminants from wastewater and contaminated environments using microbial communities (bacteria, fungi, and algae). The biofilm-mediated remediation method is one of the bioremediation techniques, which has been regarded as an economical and environment-friendly choice for environmental clean-up. Biofilms are aggregates of single or mixed microbial cells that adhere to a living or inert surface in an aqueous environment. In contrast to free-floating planktonic cells, biofilm-forming bacteria can compete for nutrients, show greater tolerance to contaminants, and offer a protective environment for cells. They show remarkable survival rate under extreme environmental parameters. Due to a properly regulated gene expression pattern mediated by quorum sensing, biofilm communities are therefore apt for the sorption and metabolism of organic contaminants and heavy metals. They are being used for wastewater treatment since the past few years due to their commendable ability to remove pollutants. Biodeterioration of materials such as metals, plastics, and concrete is accelerated by metabolic processes mediated by microorganisms living in biofilms. Electronic databases such as Scopus, Google Scholar, Science Direct, ACS, Wiley, Web of Science, Springer Link and, PubMed were used to collect all the appropriate information available in previously published literature related to biofilms. In this review, we briefly discuss the different types of biofilms used for wastewater treatment, factors affecting the development, structure, and function of biofilms and their use as potent biological remediation tools to accelerate the breakdown of environmental contaminants, types of bioreactors based on biofilms, and their application in removing various contaminants such as nitrate, bromate, and petroleum waste. In addition, the challenges faced while using biofilms in wastewater treatment and future perspectives have also been discussed.

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1 Introduction

Although water covers more than 70% of the planet, only 3% of it is safe for human consumption, with the remaining 97% being salty water.[1] Globally, water scarcity affects around 4 billion people for at least one month in a year.[2] Across the world, water supplies are becoming more limited as a result of rising imbalance between freshwater supply and its demand, thus making access to safe and clean water one of the primary concerns of modern society. Due to hazardous and recalcitrant chemicals, wastewater created from numerous industrial and urban activities greatly threaten public health and environment. Wastewaters containing inorganic and organic pollutants from different types of industries are dumped in the environment,

[a] *School of Earth Sciences, Banasthali Vidyapith, Newai, Rajasthan-304022, India*
[b] *Department of Chemistry, Banasthali Vidyapith, Newai, Rajasthan-304022, India. E-mail: viveksharma6696@gmail.com, jayadwivedi@yahoo.co.in*

thereby contaminating numerous ecosystems in the process. As a result, timely treatment of urban and industrial wastewater is an important research field for developing technology to improve wastewater quality to meet environmental regulatory requirements.[3] Many researchers have focused their research toward creating innovative wastewater treatment processes in response to the environmental challenges created by water contamination.[4] To date, physical methods, namely, membrane filtration, adsorption, and coagulation/flocculation, and chemical methods, namely, oxidation, ozonation, and biological treatments have been developed to reduce wastewater discharge and manage pollutant hazards (Fig. 1).[5] However, the chemical and physical methods need great energy and have substantial running expenses. The employment of biological agents, such as plants, fungi, and bacteria, is an inexpensive, environment-friendly alternative to the currently used chemical and physical approaches for environmental restoration. Bacteria may be employed for wastewater treatment in a variety of ways, including dead or alive, and immobilized or suspended.[6] Ugya *et al.* investigated the efficiency of microalgae to remediate the polluted water of River Kaduna that results in certain physicochemical factors and contaminants having substantially reduced efficiencies such as Pb (71.0%), Ni (74.0%), Cd (70.0%), BOD (33%), COD (24%), nitrate (42.9%), TSS (66.7%), TDS (9.9%), chloride (11.5%), alkalinity (62.5%), sulfate (37.5%), conductivity (9.8%), and turbidity (71%).[7]

Bioremediation has been known as an environmentally acceptable procedure for the decontamination of harmful contaminants for the safety and preservation of environment due

Sonia Saini

Sonia Saini hails from Jammu, Jammu & Kashmir, India. She has received her MSc in Environmental Science from Central University, Jammu in 2018, qualified NET, and subsequently joined Banasthali Vidyapith, Rajasthan. She is currently a PhD scholar at Banasthali Vidyapith. Her core area of research is nanotechnology, and currently her research work is focused on the synthesis of nanocomposites for water remediation and sensing applications.

Sanjana Tewari

Sanjana Tewari has grown up in Uttarakhand, India. She has pursued her bachelors and masters in chemistry from Banasthali Vidyapith, Rajasthan and received her MSc in 2018. She is currently a PhD student at Banasthali Vidyapith. Her core area of research is nanotechnology, and currently her research work is focused on the synthesis of novel nanocomposites for the effective sensing and removal of heavy metal ions in aqueous medium.

Jaya Dwivedi

Dr Jaya Dwivedi is working in the capacity of Professor and Head, Department of Chemistry and Dean, School of life sciences at Banasthali Vidyapith, Rajasthan. Her area of specialization is organic synthesis, material development, and natural products. She has published more than 125 research papers in journals of international/national repute. She holds six patents to her credit and has authored various book chapters. She has actively contributed in uplifting the departmental research infrastructure through resource mobilization from various funding agencies, viz., UGC, WTP- DST, DST-CURIE, and FAST-MHRD, India and developed 'State of Art' instrumental facility at the department. In addition to institutional projects, she has successfully handled four individual projects. She has supervised 25 PhD research scholars under her direct supervision. She has delivered various keynote lectures in International/National Conferences.

Vivek Sharma

Vivek Sharma is currently working as Assistant Professor in Department of Chemistry at Banasthali Vidyapith, India. He received his PhD in Chemistry in 2017 from the Indian Institute of Technology Roorkee, under the supervision of Prof. Paritosh Mohanty. His current research interest is the development of multifunctional nanomaterials for energy and environmental applications. He has authored/coauthored 13 research papers published in international reputed journals and a number of book chapters.

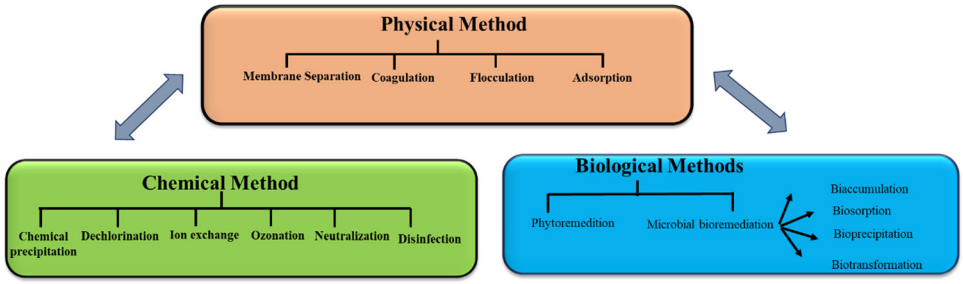

Fig. 1 Water treatment methods.

to several limitations (start-up, cost, efficiency, and operation) of traditional wastewater treatment plants.[8] Most of the time, microbial populations including algae, bacteria, and fungi are neutralized, mineralized, degraded, and often remove various inorganic and organic contaminants from contaminated environments and wastewater as a part of the bioremediation process.[9] Biological remediation is a broadly used remediation method that exploits the metabolic abilities of a broad variety of bio-inoculants for the amelioration and alleviation of contaminants present in environment. It is effective, economically feasible, compatible, and environmentally safe. Diverse advanced environmental studies are being conducted based on the remediation approach and the existing contaminants to create sustainable *ex situ* and *in situ* biological remediation procedures for the effective removal and reduction of hazardous pollutants. *Ex situ* bioremediation treats dangerous pollutants offsite, while *in situ* biological remediation treats the pollutant on-site. Compared to *ex situ* procedures, *in situ* techniques are more beneficial and valuable as they cause less site disruption and have cheaper transportation costs for the amelioration and alleviation of environmental pollutants.[10]

2 Biofilm

A biofilm is a well-structured group of microorganisms immobilized in a synthesized matrix generated by microorganisms called extracellular polymeric substances (EPS). EPS protects from pollutants, protozoa with predatory nature, and environmental challenges on biotic or abiotic surfaces. Proteins, lipids, polysaccharides, nucleic acids, humic compounds, and surfactants are all components of EPS (Fig. 2).[11]

Water (97%) is a key element of biofilms and has a significant impact in nutrient flow within the matrix of the biofilm.[12] The biofilm layer is roughly 10 to 30 mm thick with the EPS matrix layer thickness of about 0.2 to 1.0 mm. EPS makes up majority of the biofilm (65–95%), with bacteria accounting for the rest; biofilms majorly consist of polysaccharides and proteins, as evidenced from the Fourier Transform Infrared (FTIR) spectra.[13] The composition of EPS is largely determined by environmental growth circumstances, bacterial strain, and nutrient supply.

One of the many strategies, biofilm-mediated remediation, has been acknowledged as an affordable and ecologically

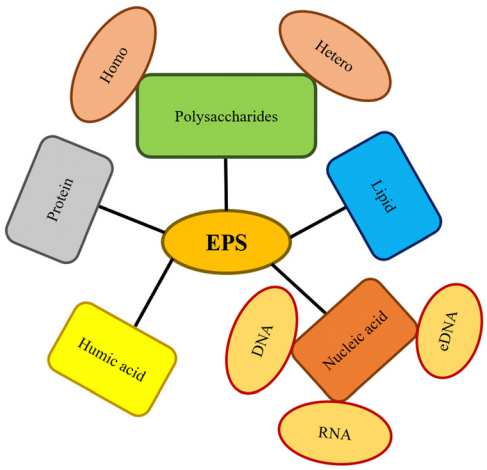

Fig. 2 EPS composition in biofilms (changes from microorganism to microorganism and is impacted by their surroundings).[11]

friendly alternative for cleaning up the environment. Because of their remarkable flexibility, functionality, genetic diversity, and cellular simplicity, microbial communities offer enormous promise for the repair of any damaged environment. Indigenous microbial communities and microorganisms have the capacity to detoxify dangerous metal pollution through biomineralization, biotransformation, biosorption, and bioaccumulation. When compared to the use of planktonic microorganisms, among the different biological remediation techniques, biofilm-mediated remediation has been regarded as a secure, competent, and organized option for the removal/refinement of pollutants. It is currently being used as an innovative remedial alternative for the biological degradation of environmental contaminants.[14]

In comparison to chemical and physical procedures, remediation techniques based on biofilms are less expensive and simpler to use. In the case of physiochemical approaches, the cost of recovering materials from waste is once again included. However, most research on biofilm-based remediation have relied on monospecies cultures, but almost all biofilm communities in nature that are connected to successful bioremediation include a range of microorganisms.[15]

Biofilms created from biopolymers may be used in the food sector, packaging, biomedicine, tissue engineering, and pharmaceuticals since they are biodegradable and have high biocompatibility. Because they are a source of clean energy, they

are made from renewable resources, and their raw materials are widely available.[16]

2.1 Biofilm formation

Biofilm formation is an important microbial proliferation process, which has different methods of development, and these are different from growing planktonic organisms in the environment.[17] Biofilm production occurs under a variety of conditions, but a majority of them necessitate the use of a wet surface, which can be either man-made, non-living, or natural/living. Biofilm groups that are beneficial have been discovered in a broad variety of habitats, including wastewater treatment plants, rivers, streams, and alimentary canals of mammals. Biofilms may be found in all three kinds of interactions in nature––solid/air, liquid/liquid, and solid/liquid. Hydrocarbons such as fuels, oils, and industrial coolants have been degraded using biofilms grown at liquid/liquid interfaces.[18] Physico-chemical bonding forces hold EPS components together to manage the architecture of the biofilm during its life cycle. Planktonic bacteria attach to the substrate *via* van der Waals forces and electrostatic forces during the onset of biofilm development. Then, to increase bacterial adherence, hydrogen forces or ionic interaction forces are used. The architecture of the biofilm is primarily comprised of four phases, beginning with planktonic bacterial adhesion to an unsterilized moist substratum and ending with separation. The 3-D structure of biofilm development and microcolony generation are also engaged between these processes.[19] Microcolonies are made up of a single or a group community of bacterial cells that are smooth or flat, rough filamentous, mushroom-shaped, or fluffy, and are surrounded by gaps filled with water.[20] The amount of EPS produced in a microcolony is determined by the number of bacteria present. Microcolonies have been discovered to contain 75–90% EPS content, with the remaining being cells. All stages are described in detail below (Fig. 3).[11]

2.2 Microorganisms adhering to the surface

An aqueous media such as blood and water initially favor bacterial adherence to the surface, which is thereafter coated by a layer of conditioning made up of the polymers from the medium. This organic conditioning layer forms within some minutes of contact and keeps expanding for a long time. At this stage of biofilm development, projections on the cell surface such as flagella and pili (structures made of fibers) encourage bacterial adhesion to the surface.[21] A variety of physical stresses, such as van der Waals contact, electrostatic forces contact, and various variables, play a role in bacterial attachment to the surface as well. The attachment of bacteria to a surface is called adhesion, while cell-to-cell contact is called cohesion. Flagella, in addition to pili and fimbriae, play a significant part in bacterial adherence to the surface. Another key aspect in the interplay between bacteria with surface attachment is surface hydrophobicity. Enhanced hydrophobicity of the surface causes less repulsion between the surface and bacteria during biofilm development. Biofilm production is aided by planktonic bacteria that naturally interact with the conditioning layer.

Bacterial adherence may be affected by physical surface features such as fibrousness, porosity, texture, and surface

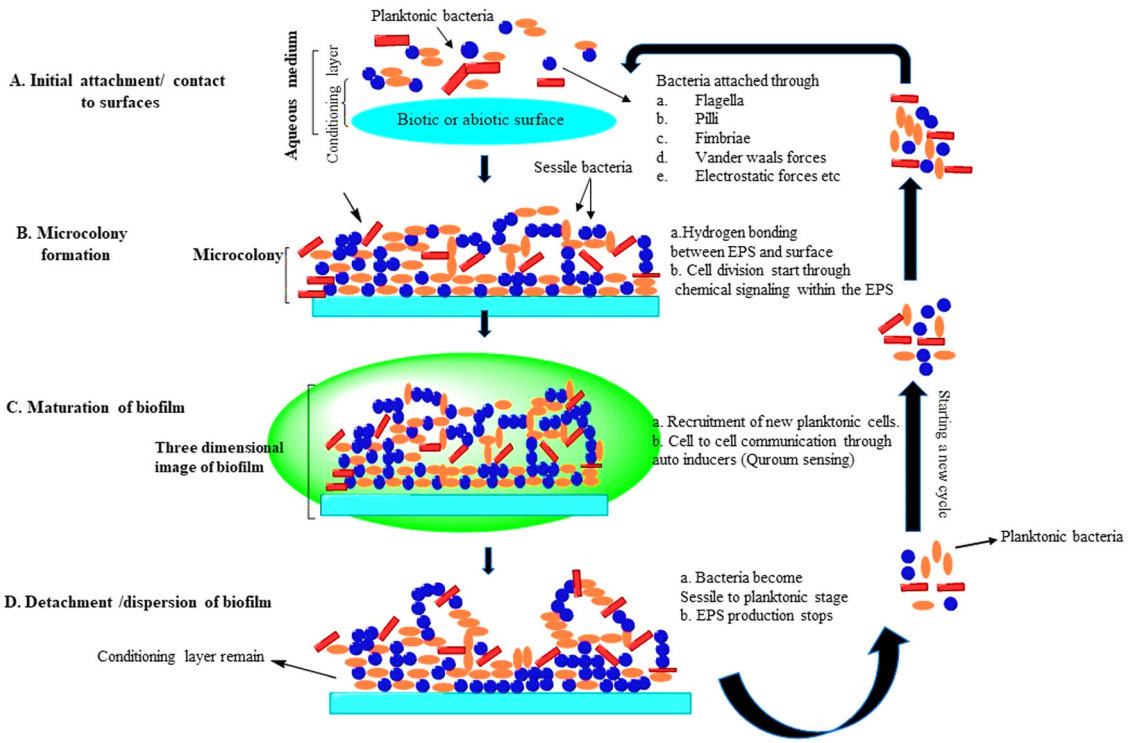

Fig. 3 Life cycle of a biofilm: (A) attachment of microbes, (B) development of microcolonies, (C) maturation of biofilm, and (D) detachment.[11]

roughness in addition to surface chemistry. In another investigation, the surface of HDPE coupons underwent physiochemical modification. It was shown that physical modification improved treatment effectiveness, live cell percentage, biofilm adhesion and development, contact angle, hydrophobicity, and surface roughness. The research that has been done to date shows that the formation of biofilms on polymer surfaces is a complex process that is influenced by extracellular proteins and polymers, initial bacterial concentrations, and the bacterias forming the biofilm.[22]

In a study, Zhou *et al.* assessed the effectiveness of the supporting media used in anaerobic attached growth wastewater remediation systems made up of polymethyl methacrylate (acrylic), polyvinylidene fluoride (PVDF), polypropylene (PP), polyvinylchloride (PVC), polycarbonate (PC), acrylonitrile butadiene styrene (ABS), and high-density polyethylene (HD). The results revealed that hydrophobic polymeric materials, such as PVDF and PP, promoted biofilm development (in less than 16 days) and initial cell attachment more effectively than hydrophilic materials, such as HDPE and ABS. The hydrophilic materials did, however, show greater mature biofilm volumes and improved COD elimination as a measure of wastewater treatment efficacy during longer-term and steady-state operation (81 days). These findings imply that the kind of polymer material has an important effect on the growth of biofilms, the variety of bacterial populations, and the efficacy of anaerobic fixed-film systems in treating wastewater. For the anaerobic wastewater treatment process, HDPE and ABS fared better than the commonly applied PVC in the industry and is considered as the most promising and appropriate medium.[23]

2.3 Formation of microcolonies

Bacterial cells proliferate and replicate to create a microcolony after adhering to an unsterilized surface.[27] In other words, the biofilm's intricate composition and purpose is created by the spatial arrangement and cell clusters of these microcolonies in connection to one another. Different micro-communities of bacterial colonies are often involved in biofilm construction. The distribution of nutrients, supply of metabolic products, and elimination of end products are all aided by this control.[24] For instance, in an anaerobic biofilm, three bacterial species break down a complex chemical substance into two end products, CH_4 and CO_2. In the beginning, fermentative bacteria break down complex organic substances to make alcohol and acid. These products are then used as substrates by acetogenic bacteria, and finally, methanogens produce CH_4 gas using CO_2, hydrogen, and acetate particles in the final stages of the reaction. Biofilms are a good habitat for forming syntrophic bacterial partnerships because it offers a complete environment for two or more bacterial species that vary metabolically to form a bond. Syntropy is the association between two or higher metabolically diverse bacteria that rely on one another for specific substrates to create energy. These polyhydroxy units in extracellular polymeric substances operate as a biofilm's anchoring and enhance bacterial contact to the surface with hydrogen. At this moment, it is difficult for the bacteria to detach from the surface. Mature biofilms have been found to remain in place until they reach the end of their development cycle.[25]

2.4 Biofilm architecture and maturation

The maturation of biofilms is the next stage just after biofilm creation, where auto-inducer signals are used by bacterial cells inside a biofilm to communicate with one another to achieve appropriate cell density.[26] Quorum sensing (QS) is aided by these auto-inducer signals in biofilms, making it easier to produce certain gene products that are required for EPS production at this stage of biofilm development. Because the EPS matrix is such an important feature of a three-dimensional biofilm, water-filled interstitial spaces grow in the extracellular polymeric substances. They operate in biofilms as a systemic circulation, assisting in metabolic product excretion from microcolony bacterial colonies and nutrient supply.[27] During the development phase, the biofilm's motive is to expand in the three dimensions. It is performed by recruiting new planktonic bacteria and collecting sand from the environment. During these stages, a biofilm develops due to reproduction, which happens at a regular basis in the microcolonies. In certain situations, biofilms may grow to reach several inches thick.[11]

2.5 Dispersion

Whenever detachment follows biological patterns, the biofilm's bacterial cell division and multiplication processes occur fast at the beginning of detachment, enabling sessile cells to become motile.[28] Some types of bacteria do not manufacture EPS and scatter or get mechanically strained as a result. Dispersion that is passive, such as sloughing and erosion, may occur in the aquatic environment due to shear or hydrodynamic forces and is not generally intentional. Biofilm layers may be shattered due to abrasion caused by nature, shortage of nutrients, and stress caused by hydrodynamics that results from the liquid's velocity.[29] A cellular layer, known as the conditioning layer, remains at the surface regardless of how dispersion happens, thus allowing the developmental process to continue or resume. Enzymes that break down sugar aid in the eradication of bacteria from the outer surfaces of the biofilm, and they are sometimes produced by bacterial populations. Some enzymes are beneficial to bacteria for colonizing a new environment, which might lead to the formation of a newly made biofilm and bacteria such as *Streptococcus equi*, *Pseudomonas aeruginosa*, and *E. coli* produce saccharolytic enzymes including hyaluronidase, alginate, lyase, and *N*-acetyl-heparosanlyase in ongoing detachment procedure for EPS matrix lysis. During this step of biofilm development, bacterial cells focus their efforts on increasing the expression of bacterial flagellum genes, which aids the bacterium in moving to a new site.[11]

3 Types of biofilms

3.1 Bacterial biofilms

Biofilms are microbial communities that are essential for their survival in harsh environments.[30] Bacterial biofilms are

described as a self-regulating community of bacteria that have colonized a surface and structured themselves into a community with a structure made up of a self-generated polymeric matrix to enhance their development in the environment.[31,32]

The competence of bacterial biofilms is influenced by surface proteins, extracellular proteins, capsular polysaccharides, adhesins, autolysin, anaerobicity, carbon dioxide level, glucose, osmotic levels, pH, temperature, ionic concentration, nutritional environment, and the presence of surfactants.[33] Mature bacterial biofilms are geographically, temporally, and dynamically heterogeneous communities that depend on a variety of topologies depending on the surrounding environment (osmolarity, pH, shear pressures, temperature, and nutrient availability) as well as on the composition of microbial consortia.[34,35]

The structure of polysaccharides influences the fundamental shape of bacterial biofilms. It is made up of biomolecules such as DNA, protein, lipids, and organic compounds in addition to polysaccharides. Andersson *et al.* investigated biofilm formation and adhesion characteristics of thirteen bacterial strains in pure and mixed cultures.[35] Turki *et al.* studied the structure and diversification of bacterial communities in a semi-industrial test facility employing rotating bio-disk approach (RB). They reported that by determining the dominance of bacteria, the existence of useful and beneficial species that may play a vital role in the wastewater purification process was identified. *Cronobacter sakazakii*, *Enterobacter agglomerans*, and *Pantoea agglomerans* were found in a majority of biofilm samples, and these species are well known for their bioremediation potential. *Salmonella* community detection is frequent, suggesting that the RB system had little effect on *Salmonella*.[36]

3.2 Algal biofilms

Algal biofilms are algae-dominated biofilm ecosystems that colonize illuminated surface areas in the presence of moisture and nutrients.[37,38] Algal biofilms like bacterial biofilms may form a colony on a surface and separate from the surface in clumps or as a single colony and respond to changes in the environment. Despite their extensive occurrence in the environment, algal biofilms have been studied largely for their structural implications,[39] largely for safety and economic reasons, with the purpose of restricting or preventing their expansion.[37] However, the need for wastewater treatment solutions for alternative biofuel feedstock, efficient and inexpensive biomass harvesting, and nutrient control methods has recently rekindled interest in algal biofilms.[40,41]

Producing algal biofilms as a substitute for removing nutrients from wastewater might provide both nutrient remediation and a source of algal biomass for the development of bioproducts.[42,43] However, the development of algal biofilm-based wastewater treatment systems faces problems owing to a lack of knowledge on biofilm area requirements, algal growth demands, standard operating procedures, and nutrient removal efficiency for field- or bench-scale testing and operations.[44]

Li *et al.* employed microalgal biofilm to extract zinc from the effluent and leachate from a mine dump. They employed *Halochlorella rubescens* (CCAC 2069 B) and the trebouxiophyte green alga *Stichococcus bacillaris* to make microalgal biofilms (strains CCAC 1898 B and CCAC 1896 B). *S. bacillaris* strains were selected for this work due to their effectiveness as heavy metal bioadsorbents,[45–47] whilst *H. rubescens* has previously been employed effectively in two-layer PBRs for treating wastewater.[48,49] The results showed that the *S. bacillaris* strain CCAC 1898 B exhibited the highest zinc resistance. *Halochlorella* and *Stichococcus* CCAC 1896 B were found in quasi environments; however, this strain was discovered at a metal waste site's drainage stream at Decazeville, France. It is commonly known that when microalgae are exposed to toxic substances, such as in polluted environments, they might acquire a degree of tolerance to them.[50] Furthermore, earlier research has shown that algal strains isolated from polluted settings are capable of removing more heavy metals.[51,52]

3.3 Fungi biofilms

Fungi, like bacteria, adhere to the basic requirements for biofilm development, which include adhesion to a surface or to one another and encapsulation in EPS. Filamentous fungi have recently been discovered to have hydrophobic protein of amphipathic type, which aids in fungal biofilm growth by allowing the fungus to cling to the surface.[53] Hydrophobins act as morphogenetic type cues, allowing fungal spores to adhere to surfaces that repel water (artificial and natural). Eight cysteine residue hydrophobic pattern is secreted by *Aspergillus fumigates*, creating significant attachment forces during spore adherence with the surface.[54] *Candida albicans*, *Penicillium rubrum*, *Fusarium species*, *Acremonium*, and *Neocosmopora* are examples of fungal species that can create biofilms.[55] However, in diverse microbial biofilm in clean water delivery methods, fungi's variation is lower than bacterial variation. Howell and Shepperd compared *C. albicans* and *Candida fumigates* biofilms to bacterial biofilms to acquire a better knowledge of their biological function, biosynthesis, and structure.[56] Despite the fact that the sequence homology of glycan materials and fungi and bacteria have different biosynthetic enzymes, both are able to construct antibiotic resistance strategies involving immune evasion and biofilm formation. Despite a range of treatment techniques, enzyme-based therapy procedures to suppress and deconstruct biofilm components are emerging as potential future options for better treatment of skin diseases.[56]

3.4 Symbiotic biofilms

Physiological efficiency has been consistently shown in complex multispecies biofilms.[57] Biofilms with multicultures produced 70% higher cellulase than biofilms containing just one species.[58,59] Because successive gastrointestinal processes are helped by the superposition of multiple species that work together within a multilayer biofilm matrix, multispecies synergy is particularly important in the case of solid substrate breakdown.[60]

3.4.1 Fungi and bacterial biofilms. Fungi and bacteria biofilms have the potential to increase biological delignification. In nature, there is a lot of synergy between fungi

and bacteria. Bacteria and fungi are the only species that can degrade large plant polymers such as chitin and lignin, and break down cellulose into small molecules that biota may absorb.[61] Fungi are much more effective than bacteria in utilizing resources available through enzymatic hydrolysis and invasion of leaf material and lysed hyphae; hence, there is generally more fungal biomass on leaves than bacterial biomass.[62,63] Bacterial activity supplies growth factors to fungi in return; for example, nitrogen-fixing bacteria enhance the quantity of nitrogen available for fungal development.[64,65] Bacteria consume fungal compounds and synergy develops, thus allowing the activity of fungal enzymes to increase. It has been proven that a large number of tiny molecules induce fungi to produce enzymes that need to be suppressed, which enhance severe bacterial infection due to significant reduction in metabolite concentrations to avoid this kind of feedback regulation.[60,66]

3.4.2 Protozoans and bacterial biofilms. Biofilm architecture is influenced by protozoans not only *via* their own structures but also through their feeding activities on biofilm microorganisms. The spatially patched feeding activity of protozoans may increase the temporal and spatial heterogeneity of the bacterial biofilm.[67,68] Amoebae's behaviors (feeding on diatoms, migration between biofilm and substrate) were thought to be crucial to biofilm disintegration and sloughing. Because of their abundance and distinct characteristics, protozoans may have a significant influence on biofilm architecture.[69] Because of the intricate entanglement generated by their stalks, Cereceda *et al.* highlighted the critical role of peritrich ciliates in sustaining the layered biofilm structure in a full-scale treatment plant.[70]

Protozoans can constantly modify the 3-dimensional structure of biofilms because they have extracellular structures including loricae, shells, and stalks that can be present in biofilms long after cell death. Protozoans may improve the movement of biochemically essential solutes into and through the biofilm, in addition to their impact on biofilm architecture. Benthic ciliates have been shown to increase extracellular solute transport in sediment biofilms by a factor of 1.1 to 10 due to their cilia motility.[71,72]

3.4.3 Microalgal and bacterial biofilms. Water treatment may be aided by microalgal-bacterial consortiums,[73,74] providing enhanced nutrient removal, minimal maintenance, and self-sustaining oxygen.[75,76] Su*et al.* used microalgae and activated sludge to obtain a synergistic connection to enhance wastewater treatment, achieving nitrogen and phosphorus reductions of 91 and 93.5%, respectively, using a 5 : 1 algae-to-sludge ratio.[77] Fig. 4 illustrates the interaction of microalgal-bacterial consortia and the structural development of microalgal bacterial biofilm.

A xenic culture fluid was employed in another study to enhance *Chlorella vulgaris* flocculation; it was discovered that bacteria connected to microalgae increased floc size for better microalgal flocculation, resulting in increased sedimentation and better microalgae harvest.[78] Using a symbiotic microalgal bacterial consortia in a photobioreactor to treat municipal wastewater, TKN (Total Kjeldahl Nitrogen) and COD removal efficiency was reported to be 87% and 98%, respectively.[79] Gou *et al.* built an efficient reactor system that increased the COD and rate of removal of ammonium to 90% in a 12 h retention time.[80]

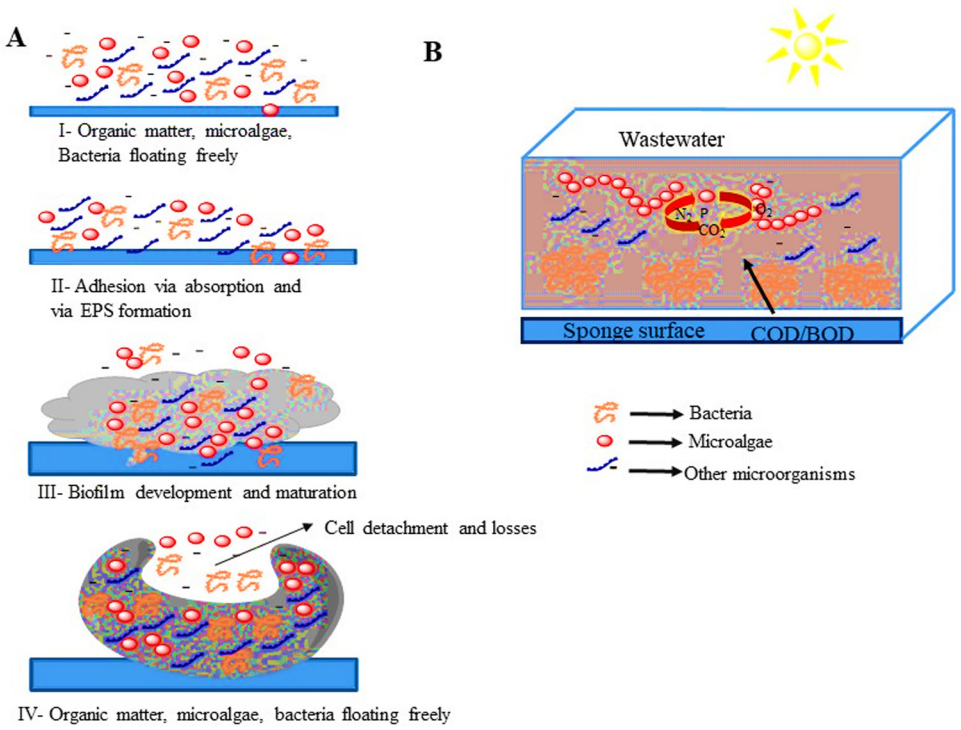

Fig. 4 (A) Microalgal bacterial biofilm structure and development. (B) Main biological interactions between microalgal-bacterial consortia.[86,87]

Microalgae for raising oxygen and nutrient reduction (organic compounds, phosphorus, and nitrogen) in polluted water may be less expensive than typical treatment procedures. Several ranges of water quality indicators, such as DO (dissolved oxygen), temperature, and pH, have been acknowledged for wastewater remediation employing a bacterium and mixed microalgae culture.[74] DO profiles may affect the symbiosis between bacteria that use microalgae and oxygen that create it. For example, Mhedhbi *et al.* discovered that DO varied from 2–11 mg L^{-1} when the microalgae first started generating oxygen and gradually decreased over the dark phase.[81] Microalgal growth (mixotrophic microalgal culture and/or CO_2 absorption by autotrophic) and/or excretion from organic matter biodegradation elevated the pH from 7.8 to 10.0, according to Mirquez *et al.*[82] Depending on the type of bacteria and microalgae, microalgae cultures can develop at temperatures ranging from 10 to 45 °C, with ideal conditions between 20 and 25 °C,[83] while bacterial cultures may thrive at temperatures ranging from 0 to 49 °C.[84] Several studies have employed immobilized biofilms of microalgal-bacterial consortia as a secondary treatment for wastewater; however, additional study is required to increase surface attachment.[85]

4 Types of bioreactors based on biofilms

The progression of biofilm-based bioreactors as a technology for wastewater remediation processes has shown potential. Biofilm-based systems provide a number of benefits, including improved pollutant removal effectiveness, minimal sludge generation, shorter hydraulic retention times (HRT), presence of EPS, high concentrations of active biomass, and high diversity. As a result, a variety of biofilm-based bioreactors have been widely applied to remove pollutants from highly contaminated wastewater.[88] Biofilm reactors are primarily made up of five compartments, with certain additional components that are specific to a particular reactor type: (1) containment structure; (2) influent, which refers to wastewater having a specific level of the contaminant of concern; (3) biofilm substratum or carrier, which refers to the material used for microbial cell attachment and growth; (4) system for collecting wastewater; and (5) mixing system or aeration for carrier distribution and agitation.[89]

4.1 Membrane biofilm reactors

Membrane biofilm reactors (MBR) have long been hailed as a viable biotechnology for the removal and/or recovery of contaminants from water. It fundamentally entails the breakdown of biological waste components *via* physical separation and a system based on biofilms *via* a membrane module that replaces the secondary settlement. It is used to treat a number of urban and industrial wastewater, demonstrating excellent eradication of both inorganic as well as organic matter.[90] It has specific advantages over other treatment systems.

MBRs, unlike traditional filtration membranes, do not separate liquids from solids. Rather, a gaseous substrate is transmitted across the membrane, such as oxygen or hydrogen. On the outer membrane surface, a biofilm grows naturally, triggering desired reactions. Exogenous substrates such as gaseous substrates can be ideal because they leave nothing behind in the bulk and are often affordable or produced on-site. Unfortunately, the traditional gas delivery method of aeration or bubbling is energy intensive and limited by their low solubility. Furthermore, a majority of the gas is lost to the atmosphere. Compared to suspended processes, MBR has the same advantages as other biofilm reactors (*e.g.*, IFAS, MBBR)––higher biomass concentrations in the tank, support for slow-growing microorganisms, and generation of fewer solids (*e.g.*, activated sludge). Infact, it does produce high effluent quality and lower sludge generation. It also has a lot of flexibility when it comes to disinfection efficiency, tremendous volumetric loading, and influent fluctuation.[91,92]

It is possible to use it in two ways, namely, the exterior or side stream configuration and second, the submerged or immersed configuration. Yamamoto *et al.* proposed the submerged configuration in 1989, which involves the membrane module being immersed in the reactor directly.[93] Negative pressure on the permeate side or pressurization of the bioreactor provide the driving force in this configuration. This configuration has a number of benefits, including lower energy usage, cheaper running costs, and fewer cleaning processes. The development of this system has been aided by these benefits. Outside the bioreactor, the membrane is put in the external configuration, allowing a mixture of spirits to be recirculated. This setup makes membrane replacement and control simple. In this setup, the bioreactor's high cross-flow velocity (CFV) serves as a driving force.[94,95] MBR systems have been employed successfully for a range of industrial effluents to date because of their numerous advantages, the most notable of which is their decreased energy usage. The creation of air-lift side stream MBRs has recently emerged as a new configuration.[92] The basic concept behind this configuration is to use the side-stream airlift principle to make use of all of the abovementioned benefits of MBRs.[96,97] For the remediation of urban and many types of industrial wastewater, this concept is highly effective and much work is still being done to improve its applicability.[98]

To promote the growth of biofilms, a medium may be added for moving or fixed-bed arrangements, or aerated membranes can be added to the bioreactor, which could be used to facilitate biofilm processes in MBRs. For biofilm support, a variety of materials are available. Granular activated carbon (GAC), sponge, cord media, plastic media, and RBC media have all been used in full-scale systems commercially to date.[99] This approach has been shown to be successful in the removal of a variety of contaminants and it allows a degradation efficiency of more than 90%.[100,101] In subsequent testing, it has demonstrated good elimination of COD and inorganic nitrogen.[102]

Membrane biofilm bioreactors have emerged as a popular and effective treatment solution for home and industrial wastes. However, membrane fouling and clogging layers, as well as the repercussions for plant maintenance and operating

expenses, limit its broad implementation. The demand for immense energy associated with air scouring and the high cost of membranes further limit its widespread implementation. Membrane fouling is a typical occurrence in membrane-based systems, especially MBRs, which is mostly caused by soluble and colloidal organic content, such as suspended particles, EPS, or biopolymers or physical qualities.[99] Controlling membrane fouling mechanisms and discovering economical membrane materials have been the focus of many studies to address these drawbacks. It also aimed to reduce energy usage, making this system more realistic and trustworthy than activated sludge and other traditional technologies.[89,99] Based on the application, we can classify MBRs into 3 types, as discussed below.

4.1.1 Oxygen supplying MBRs. MBRs are well-suited for high-strength biological oxygen demand (BOD) oxidation due to their high gas transfer rates of air or oxygen. Due to the high yields and rapid growth rates of heterotrophic bacteria, excessive biofilm formation is a key problem in these systems. A pilot-scale study found that regular biofilm washings successfully cleaned high-strength brewery effluent.[103] When BOD and ammonia are supplied *via* the bulk liquid, oxygen-based MBRs support unique biofilm communities capable of concurrent BOD oxidation and complete nitrogen removal. Nitrifying bacteria live near the membrane, where oxygen is plentiful, and oxidize ammonia to nitrite and nitrate. They produce anoxic conditions for fast-growing, denitrifying bacteria that live in the outer biofilm layer by devouring oxygen. BOD from the bulk liquid and nitrate from the nitrifying bacteria are consumed by the denitrifying bacteria. The layered microbial community structure of this biofilm has been validated by a number of experimental investigations and modelling approaches.[104–107] Researchers have also shown that oxygen concentrations may be manipulated to encourage the formation of nitrite rather than nitrate by changing intra-membrane pressure.[108] Thick biofilms, unfortunately, can disrupt the community structure. If the denitrifying layer is too thick, mass transfer resistance prevents ammonia from reaching the nitrifying biofilm, resulting in poor activity or loss of nitrifying bacteria.[109] Nitrification occurs along the membrane surface in the aerobic inner sections of the biofilm, while anaerobic ammonia oxidation occurs at the outside borders.[110,111]

4.1.2 Hydrogen-supplying MBRs. Hydrogen is a powerful electron acceptor. Its advantages include reduced biomass yields, low toxicity to humans, and extensive utilization as an electron donor by a varied population of bacteria.[112] Low solubility issues and the risk of a flammable headspace have limited the number of full-scale gas sparging installations.[113] MBR provides a safe method of delivering hydrogen while also increasing gas transfer rates. Hydrogen is used within the biofilm before reaching the bulk liquid, which is supplied at high quantities by pressured membranes. Hydrogen can be produced on-site using electrolysis or methane reforming, which is a bonus.[91]

MBRs based on hydrogen have been shown to be effective against a wide spectrum of inorganic and organic pollutants. Perchlorate, chlorate, bromate, chromate, selenate, arsenate, and dichloromethane are among the inorganic oxidizing pollutants that can be reduced by hydrogenotrophic biofilms using nitrate or oxygen as the major electron acceptor.[114–118] Hydrogen can also engage in nitro reduction and reductive dehalogenation for the treatment of chlorinated solvents, chlorophenols, and chloronitrobenzenes under anaerobic circumstances.[119,120] For these applications, nitrate or sulphate could be used as the principal electron acceptor.

4.1.3 Novel MBRs. MBRs have been studied with methane and carbon dioxide, two inexpensive and abundant gases, in addition to hydrogen and oxygen. Methane-based MBRs support methanotrophic bacteria, which can co-metabolize organic molecules and act as an electron donor by releasing decay products.[121,122] To support methane gas production, Ju *et al.* supplied an MBR with a mixture of carbon dioxide and hydrogen (ratio 1 : 4).[123]

The extractive membrane reactor, for example, transports contaminated gas or liquid across the membrane's lumen. This configuration prevents the biofilm from being exposed to toxic chemicals in the waste stream by avoiding air-stripping of volatile organic compound contaminants, spatially separating competing substrates (one in the bulk liquid, the other in the membrane lumen), and preventing the biofilm from being exposed to toxic chemicals in the waste stream.[91]

4.2 Moving bed biofilm reactors (MBBR)

The MBBR is a wastewater treatment method based on biofilms that is currently being used in over fifty countries. It was invented in Norway between 1980s and 1990s.[124] The MBBR plants have been a huge success and are employed for municipal and industrial wastewater treatment.

This technique is based on combining the greatest aspects of both the activated sludge and biofiltration processes. Aerobic, anoxic, and anaerobic processes can all be carried out in this reactor. In this method, the carriers that function as biofilm growth housing move about freely within the tank capacity. The movement of the carriers is driven by the unrest created by the gas in aerobic processes, whereas mechanical mixing keeps the carriers moving in anaerobic and anoxic processes. K1 is the most often utilized biofilm carrier in MBBRs, which are continuous-flow reactor units. They take the form of a cylinder made of polyethylene with a high density (0.95 g cm^{-3}) featuring ''fins'' on the outside and a cross on the inside. With these carriers, the bioreactor does not have a filling portion; it can range from 25% to 70% of the whole volume of the tank. It is, however, suggested that it can be maintained below 70% (for K1, the effective specific area is 350 m^2 m^{-3}). On the inside of the plastic containers, biofilms flourish, where they are sheltered abrasion from the outside. The use of movable beds has the positive effect of decreasing congestion and allowing the bioreactor's entire volume to be utilized. In the reactor, there is turbulence caused by forces of shearing, which ensures not only proper compound diffusion with the biofilm but also a thin biofilm. Furthermore, this method removes or lowers the requirement for biomass recirculation, which is a major concern in activated sludge systems

and fixed bed biofilms. The extra biomass must only be removed from the solution.[99]

Because of the carrier materials, a moving-bed biofilm reactor has larger accessible surface for growth and adhesion of microbes than a fixed or fluidized bed biofilm reactor. Furthermore, it provides effective mixing within the reactor, which favors biogas liberation and the distribution of volatile acids in an aqueous medium.[125] Combining moving-bed biofilm bioreactors with other treatment procedures has already been explored for different treatment processes. Indeed, the use of this method in conjunction with flotation and coagulation for higher-rate secondary treatment has been previously documented. MBBR has been shown to be a credible, resilient, and compact wastewater treatment reactor that may be used for nitrification, denitrification, carbon oxidation, and as a single stage or in combined systems,[126,127] with satisfactory operating results at both lab and larger scales. To benefit the reactor components, the carrier must be removed, which is a recognized drawback of these systems.

In MBBR, unlike most biofilm reactors, for biomass growth, the full tank capacity is used. It also has a lower probability of head loss. It does not require sludge recycling, unlike the activated sludge reactor. It is accomplished by biomass production on carriers that float freely in the reactor's water volume and a sieve structure keeps them confined at the reactor's output. As shown in Fig. 5, the reactor can be employed for aerobic, anoxic, or anaerobic operations.[128]

The movement of biofilm carriers in aerobic processes is generated by the turbulence created by air, whereas in anaerobic and anoxic processes, the carriers are kept moving by a mixer (often a horizontal shaft mounted banana mixer). A specific coarse bubble aeration mechanism has been devised for aerobic reactors. Special filter arrangements have also been designed to keep the biofilm carriers within the reactors. It can be rectangular mesh sieves positioned vertically, but more recently, the sieve has been formed as a cylindrical bar sieve mounted horizontally or vertically. For the MBBR process to perform optimally, the design of aeration grids and sieves is important.[128]

4.3 Fluidized bed biofilm reactors

Fluidized-bed biofilm reactors (FBBRs) employ tiny carriers to build a bed within a column that is maintained in fluidized movement by running wastewaters, and the bed increases as a result. A recycling line is utilized inside this system to keep up with the vertical hydraulic velocity of the constantly introduced wastewater. Aeration occurs when influent wastewater combines with effluent recovered from the top of the bed during recycling. It is possible to introduce air into the recycling stream; however, this has been demonstrated to generate turbulence within the reactor, which might shear the adherent biofilm off the carriers.

In general, medium particles in FBBRs are distributed in a size gradient from the bioreactor's top to bottom. Depending on the size of the particles that have expanded, the bed is characterized as inflated or fluidized. There are several biofilm-supporting materials available such as silica-based compounds,[129] zeolite, and GAC,[130] which have been employed in this system. In pilot-scale studies, small materials (<1 mm) were used to improve the active surface area, which is a key aspect of this approach. The microscopic particles get suspended and separated when the driving power of the flow rate within the bioreactor is greater than gravity (30–50 m h^{-1}).[131]

In comparison to fixed biofilm reactors, FBBR is commonly found to have greater mass transfer properties. In the instance of municipal wastewater treatment, it has demonstrated its efficacy for tertiary denitrification. FBBRs are used in wastewater treatment to remove oxidized pollutants.[132]

The advantage of fluidizing the media particles is that it increases the surface of contact between effluents and microbial cells. This also helps in mass transport and, as a result, efficiency of treatment. A modest extent of the bed's extension, on the other hand, is encouraged since it reduces flow velocity, saves energy, and enhances the amount of biomass that is effective. The number of connected FBBR microbial cells is critical since it indicates a high level of microbial diversity. This setting allows the system to quickly recover from changes under unstable circumstances.[133,134] Despite this, due to the high biomass concentration, it increased volumetric oxygen biomass. Puhakka *et al.* utilized *Rhodococcus* sp. and *Pseudomonas* sp. in a lab-scale fluidized-bed biofilm reactor to remediate chlorophenol-contaminated groundwater. The treatment allowed for considerable mineralization of chlorophenols effectively at chlorophenol rates with loading of 1000 mg L^{-1} d^{-1} and hydraulic retention periods of less than 1 h (above 99.9% removal efficiency of pentachlorophenol, 2,4,6-trichlorophenol, and 2,3,4,6-tetrachlorophenol).

The actual idea behind the procedure is to pump wastewater upward through a densely packed particle bed at a high enough speed to fluidize or move the particles. As the wastewater rises through the biological bed, extremely concentrated organisms living on the surface of the bed particles devour the biodegradable waste pollutants in the wastewater. A basic unit of the process is depicted in Fig. 6 as a fluidized bed reactor with wastewater moving upward through the bed, fluidizing the particles in the liquid. A clear water zone exists above the bed, where the liquid and the particles separate.

The associated microbes on the suspended particles could be any of the aerobic, facultative, or anaerobic organisms commonly found in trickling filters and suspended growth treatment systems from a biological standpoint. The dominant

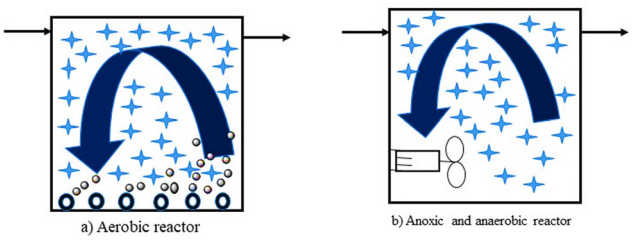

Fig. 5 Principle of biofilm carriers in aerobic and anaerobic reactors.[128]

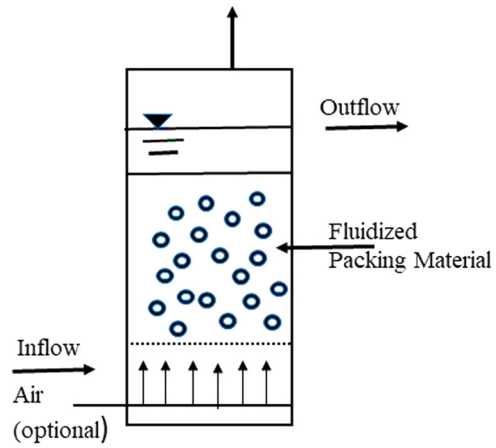

Fig. 6 Fluidized bed biofilm reactor.[135]

species would be determined only by the waste pollutant consumed, whether an aerobic or anaerobic environment is maintained, and other parameters affecting biological growth.[135]

4.4 Microbial fuel cells

Microbial fuel cells (MFCs) have the potential to solve a number of issues concerned with traditional wastewater treatment. They lower the energy input and superfluous sludge production while enabling energy recovery from wastewater. The chemical energy stored in biological materials is converted into electricity in this method, which provides for the simultaneous resolution of two current global challenges—wastewater depollution and green energy production.[136]

MFCs are made up of a certain microbial species that can contribute to current generation using an electrode to exchange an electron network. Electroactive biofilms (EAB) are formed when microbial cells develop a biofilm on the electrodes' surface. MFCs have been successfully used with pure cultures of several bacterial species in a number of articles. Mixed culture MFCs, on the other hand, have outperformed pure culture MFCs, generating significantly higher power densities.[137] MFCs can contain a wide variety of microorganisms, according to community analysis; however, algal and bacterial species are the most used.[138] MFCs are available in a variety of configurations. An anodic and cathodic MFC is made up of two chambers connected by a conductive substance including a resistor or operated under load (Fig. 7). Separate electrochemical and biochemical reactions take place in these chambers. The organic matter in the anodic chamber (negative terminal) is oxidized by the microorganisms that make up the biofilm, leading to the creation of protons and electrons. After that, the electrons move toward the anode and travel to the other side of the MFC through an external connection, which contains the cathode. Using shuttles or electron mediators, electrons can be delivered to the anode through bacteria-produced nanowires or direct membrane-associated electron transfer.[139] Oxygen serves as an electron acceptor in the cathodic chamber. Electrons and protons diffuse from the anode to a separator, where they interact with oxygen under aerobic conditions to form water molecules.[140]

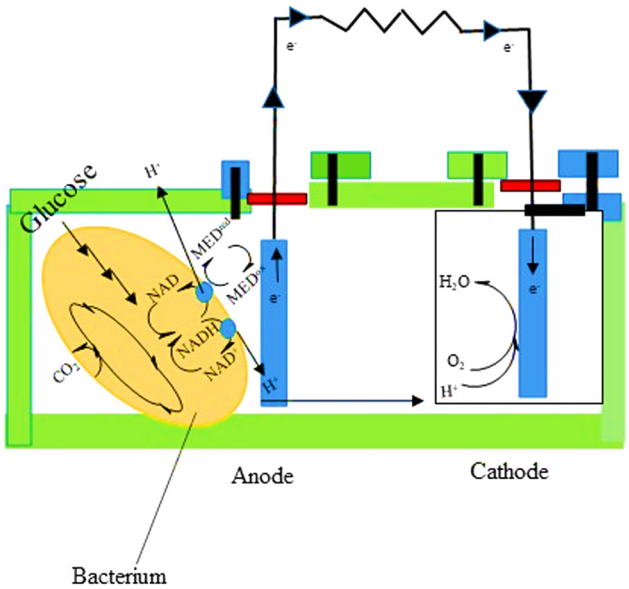

Fig. 7 The principle of MFC.[139]

Presently, MFCs are utilized to generate power and treat organic and inorganic wastes. Important influencing aspects such as design, construction materials, and voltage generating processes are currently receiving great attention.[141–143] MFC devices have made significant breakthroughs in terms of the generated power density,[144] which has risen dramatically after numerous research studies from a baseline less than 0.1 W m^{-2} to nearly 7 W m^{-2}.[145,146]

MFC technology has demonstrated its efficacy in the treatment of a wide range of contaminants. Many industrial wastewaters, such as food-processing effluents[147] and refinery wastewater, have been employed as inoculums in anode chambers.[148] Indeed, heavy metal-rich wastewaters (with a great possibility for reduction in potential) might be utilized as an electron acceptor alternative. As a result, the inorganic stuff in the container is reduced to non-hazardous or less toxic forms. It also demonstrated its excellent capacity to denitrify without the use of a supplier of exogenous electrons as the electrons released for the oxidation of organic substances are passed to the cathodic chamber, aiding nitrate reduction.[149] Furthermore, the MFC technique is suited for the treatment of high-strength wastewaters due to the anaerobic conditions in the anode chamber (COD greater than 8000 mg L^{-1})

Microbial fuel cells are touted as a viable biotechnology for electricity generation and removing contaminants owing to their energy efficiency, low biomass production, and COD elimination without further oxygenation. However, significant obstacles remain in the way of its practical use. To improve MFC performance, it was suggested that the reactor capacity should be increased; nevertheless, larger MFCs produced disappointing results.

These constraints are primarily encountered when laboratory-size experiments are scaled up to pilot scale. As a considerable fact, maintaining the generated power density in larger systems is difficult. With the growth in the MFC size, the volumetric power

density tended to drop, but a minimum threshold volumetric power density of 1 kW m^{-3} should be maintained.[150] This can be attributed to ohmic losses and low conductivity of wastewaters. However, there were some outstanding achievements. Indeed, the larger reactor size gave a twofold volumetric power density to the significant cathode specific area.[151] These findings demonstrated that the power density may be maintained in MFC scale-up with careful control of the key elements influencing the system performance. Indeed, increasing the cathode specific area using electrodes with a three-dimensional structure showed its capacity for improving the MFC performance and overcoming this constraint. This technique proposes increasing the accessible surface area for microbial adhesion while keeping the MFC volume the same.[152] For MFC scale-up, the parallel connection of microbial fuel cell in sequence is commonly used. The shortcomings of the previous method including voltage reversal and operating instability were demonstrated.[153,154] Furthermore, the voltage loss caused by the substrate cross-conduction effect may severely limit full-scale operation. When two independent stacks were serially coupled with both hydraulic and electrical connections, this phenomenon was discovered.[155]

4.5 Trickling filter

For more than 60 years, many types of biological trickling filters (BTFs) have been employed to treat wastewater. The development of BTFs in their early stages was done on the basis of empirical knowledge of treating wastewater.[156] It is a three-phase biofilm reactor with a pump station for recirculating influent, a clarification unit, and a TF (Fig. 8). It usually consists of (1) a wastewater distribution system, which is used to introduce wastewaters into the reactor, (2) a containment system, (3) a medium for support, (4) a drainage system under the ground, and (5) a system for ventilation. The removal of suspended solids from wastewater treated with TFs necessitates further liquid-solid separation since the TF treatment produces total suspended solids. Secondary clarifiers, either circular or rectangular, are commonly used for this step.[157]

For influent distribution, the rotatory distributor is ideal because it enables periodic wastewater application as well as better substratum soaking. These parameters prevent dry pockets and odour emission, as well as allow the biofilm to rest and serve as a process aeration mechanism.[131] Natural ventilation may be provided by the temperature differential between air inside the trickling filter and ambient air. When there is a temperature differential between the two, the oxygen dose delivered is insufficient. In this example, the air supply could come from an underdrain system that collects the treated influent in a chamber beneath the trickling filter.

The ability of TFs to satisfy the treatment goals in terms of nitrification and carbon oxidation has been demonstrated. For carbon oxidation, the biofilm on the filter medium that is being sustained needs oxygen in the form of air. When a solid separation is included in the treatment train, TFs are ideal for carbon oxidation and combined carbon oxidation and nitrification. With a combined oxidation and nitrification, good nitrification outcomes have been reported, with ammonia concentrations less than 3 mg NH_4^+ N L^{-1} and BOD concentrations less than 10 mg L^{-1}. When nitrification is the primary treatment goal, the ammonia concentration is significantly lower.

The selection of filter media is an important factor to consider while designing TFs. For a long time, the most often used materials in this system were gravel and stones. However, these materials restricted air movement in the filter, limiting the amount of oxygen available for microbial biofilm formation. This issue limited the amount of treated wastewater available, as well as the specific surface area available for microbial attachment that can support the reactor's BOD load. Furthermore, while handling high organic loads, stone bed trickling filters were limited by void space blockage caused by increased microbial cell development. However, at low organic loading (*i.e.*, 1 kg BOD_5 d^{-1} m^{-3}), rock-media TFs were able to give excellent treatment performance.[89]

Other materials, such as plastic rings, zeolite, ceramsite, sponge, and others, have been employed to circumvent these constraints.[158] These tiny particles improved oxygen transmission and biofilm thickness management as compared to the rock medium. TFs using plastic modules have specific surface area between 89 to 102 m^{-2} m^{-3} and are suitable for carbon oxidation and combined carbon oxidation and nitrification.[131] The trickling filter, on the other hand, has the drawback of not being a volume-effective system. Table 1 illustrates various bioreactors used for the treatment of various pollutants.

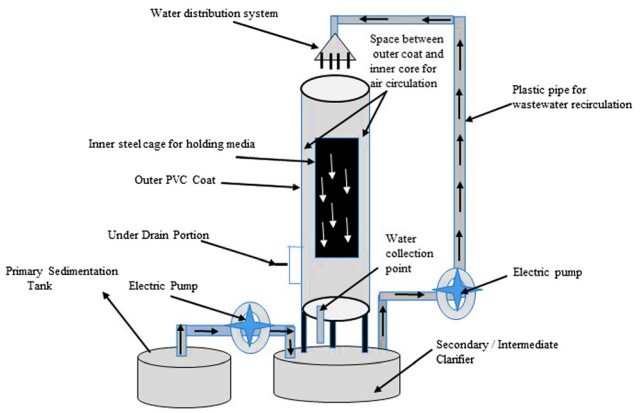

Fig. 8 Setup of a biological trickling filter (BTF) for wastewater treatment in a laboratory-sized area.[157]

5 Wastewater treatment using biofilm

5.1 Pollutants of organic origin

Because of their large microbial bio-mass and immobilizing capabilities, bacterial biofilm communities are much resilient and show stronger resistance to organic contaminants. Thus, by creating extracellular enzymes *via* the carefully regulated gene expression profile controlled by the cell communication signalling mechanism known as quorum sensing (QS), biofilm communities may participate in the sorption and metabolism of heavy metal contamination and organic contaminants.[167]

Table 1 Traditional bioreactors for the removal of different pollutants

Contaminants	Bioreactors	Ref.
NO_3^-	Methane-based MBR	159
BrO_3^-	Methane-based MBR	160
Tetracycline	Hydrogen-based MBR	161
Sulfur-containing organic pollutants	Membrane aerated biofilm reactor (MABR)	162
Petroleum wastewater	Moving bed MBBR	163
Industrial waste water	Fluidized bed biofilm reactor	164
Organic contaminants	Microbial fuel cells	165
p-Chloronitrobenzene	Hydrogen-based MBR	166

Many human activities including natural gas production, crude oil extraction, and mining have resulted in a significant increase in environmental pollution. Dyes, oils, pesticides, heavy metals, herbicides, phenolic compounds, and polyaromatic hydrocarbons (PAHs) are among the most harmful and refractory xenobiotic wastes generated by these activities. Because of their negative effects on the environment and human health, these compounds are classified as toxic and carcinogenic.[168] This contamination extended across 5 million places on Earth, with petrochemical chemicals polluting about 67% of them.[169]

Industrial wastewater contains acetonitrile, a water-soluble hazardous chemical that is metabolised to poisonous HCN and C_2H_4O compounds in living creatures, posing a serious health risk to aquatic and terrestrial species. Li *et al.* utilized a mixture of three biofilm-forming bacterial strains, *B. subtilis* N4, E2, and E3, along with an acetonitrile degrading bacterium to reduce acetonitrile toxicity (*Rhodococcusrhodochrous* BX2). In a similar investigation, the recombinant bacterium capable of generating biofilms (*B. subtilis* N4-pHT01-nit) was found to be extremely capable of degrading higher concentrations of acetonitrile (800 mg L^{-1}) over the next 24 h when used in conjunction with MBBR.[170] The degradation of diverse organic and inorganic contaminants is very significant to biofilms utilized in bioremediation and wastewater treatment operations (Tables 2 and 3). As a substrate, biofilm effectively decomposes or degrades organic substances. Furthermore, enzymes involved in the breakdown process can be kept in the biofilm EPS near the producing cell, increasing the likelihood that both the product and the enzyme will not be separated and utilized by other cells.[28]

Biofilms have greater biomass than suspended cells, which defends cells from chemical damage. Furthermore, biofilm was found to be more efficient at degrading toxicants as the physicochemical structure of the biofilm matrix optimizes compound availability and sorption while allowing aerobic and anaerobic bacteria to coexist.[185] It was discovered that flocks (associated biofilms) of anaerobic denitrifiers and oxygen-dependent nitrifiers coexisted in biofilm by occupying distinct levels and efficiently removed nitrogen from wastewater.[186,187] Anaerobic ammonium oxidation (anammox) bacteria were found to have a similar relationship in the establishment of micro-environments with the existence of aerobic ammonia-oxidizing bacteria. Due to gradient niches, reactions with various oxygen and substrate needs can occur in the same biofilm in this form of interaction. When compared with single-species biofilms, multi-component biofilms exhibited more efficient bioremediation. This has been noticed during the breakdown of PAHs employing multispecies biofilms and it has been linked to the addition of emulsifier makers. Emulsifiers are well-known for lowering the surface tension between surfaces, compounds, and cells, resulting in increased substrate availability on cell surface adhesion.[188] The genes that code for emulsifier synthesis are found on plasmid DNA, which is routinely passed between bacteria in PAH breakdown by multispecies biofilms.[189] Yoshida *et al.* observed that when Burkholderia sp. NK8 was cocultured in a biofilm with *P. aeruginosa* PAO1, the breakdown of 3-chlorobenzoate was increased. Despite its inability to degrade 3-chlorobenzoate, *P. aeruginosa* aids in the production of *Burkholderia* sp. NK8 biofilms,[190] and as a consequence, deterioration was tenfold

Table 2 Biofilm-mediated bioremediation of inorganic micropollutants and organic contaminants from wastewater

Biofilms	Wastewater type	Contaminants	Ref.
Gordonia sp. H19 and *P. monteilii* P26	Artificially created seawater	Crude oil	171
Batch-dispersed biofilm reactor sequencing based on algal-bacterial interaction	Sewage from the home	Total phosphorous and total nitrogen	172
MBBR	Sewage from the hospitals	Trimethoprim, propranolol, diatrizoic acid, clarithromycin, and azithromycin	173
Sphingobacteriales, *Flavobacteriales*, *Xanthomonadales*, and *Burkholderiales*	Sewage from the hospitals	Drugs	174
Batch biofilm reactor using algae as a sequence agent	Sewage from the home	Phosphorus and Nitrogen	175
MBR	Wastewater with saline conditions	Ammonium and COD	176
MBBR	Sewage from the city	Medicines	177
MBBR	Sewage from the city	4-Nonylphenol, 17β-estradiol, naproxen, and diclofenac	178
B. mojavensis M1+ *R. rhodochrous* BX2	Groundwater	Organic cyanide	179
Arthrobacter sp.	—	Chromium(VI)	180
B. atrophaeus CN4	—	Naphthalene	181
Trichosporonasahii B1, *Candida tropicalis* TH4, and *Candida viswanathii* TH1	—	Pyrene, anthracene, naphthalene, and phenol	182
Microalgal biofilm	Sewage from the city	Phosphorus and nitrogen	183
Chlorella vulgaris	Wastewater contaminated with aromatic hydrocarbons that was collected from petroleum storage	Removing nutrients	184

Table 3 Traditional biofilms for the removal of different contaminants

Biofilms	Contaminants	Ref.
Algal-bacterial biofilm	Nitrogen Phosphorous	215
Kaolin-supported bacterial biofilms	Cr(VI) Zn(II)	216
Algal-bacterial biofilm	Nitrogen	217
Algal biofilm	Sulfonamides	218
Fungi biofilm	Pharmaceuticals contaminants	219
Microbial biofilm	Polycyclic-aromatic hydrocarbons	220
Fungal–bacterial biofilm	Crude oil	221
Bacterial biofilm	Hydrocarbon	222
Fungi biofilm	Mercury	223
Fungi biofilm	Cr(VI)	224
Fungal–bacterial biofilm	Cd^{2+} and Pb^{2+}	225
Fungal–bacterial biofilm	Cr(VI)	226

enhanced when compared to monospecies biofilms, demonstrating that multispecies biofilms may be used for biocatalysis and bioremediation.[191] Another research discovered that biofilm process for energy-efficient carbon removal from wastewater was dominated by a glycogen-accumulating organism that was passively aerated.[192]

In the cytoplasm, EPS, and cell walls, biofilms feature distinct and variable sorption processes and binding sites (anionic and cat-ionic). Even in low concentrations, a diverse group of pollutants, including hormones, phenolics, medicines, and poisonous chemicals, can be bounded and stored for possible ingestion by biofilm cells.[193–195] Despite the lack of obvious lipophilic binding sites, slightly hydrophilic chemicals such as xylene, toluene, and benzene can develop in the EPS matrix. The sorbed chemicals disintegrate or persist in the biofilm till decomposition. Higher toluene concentrations express a greater amount of anionic carboxyl groups inside the EPS of *P. putida* biofilm, resulting in an increase in its cation binding ability. Other ions, such as phosphate ions, also collect within the dental plaque biofilm to improve the biofilm's mechanical resilience.[196] Suspended biodegradable materials, in addition to ions, can be settled in EPS and exploited as the nutrients' source. Extracellular signalling, metabolic heterogeneity, metal immobilization, complex reactivity with siderophores, phenotypic variations, and genetic alterations are used by biofilms to detoxify metals.[197] The entire EPS has a net positive charge due to the net negative charge of the sludge, which may produce flocculation.

Because of their aromatic ring structures that contain carbon, one of the most dangerous pollutants in the environment is PAHs having mutagenic and carcinogenic consequences in humans.[198] For the bioremediation of PAHs, many bacterial species were used such as pollutant trapping inside EPS through biosorption, usage as a nutrition source for their development, and stimulating biofilm growth to guard against PAH toxicity. Atrazine herbicide may be digested and used as a nitrogen source by *Pseudomonas* sp. strain ADP bacterium.[199] In one investigation, the selective concentration of PAH was discovered to help *P. aeruginosa* N6P6 marine bacteria produce biofilms.[200] A range of insecticides and herbicides have been successfully bioremediated using biofilm-mediated reactors, including 2-propionic acid, diclofop, 2,4-dichlorophenoxyacetic acid, diclofop, 2-propionic acid, carbendazim, choropropham, methomyl, diazinon, carbamate compounds, carbaryl, and carbofuran.[201–203]

5.2 Pollutants of inorganic origin

Inorganic contaminants, similar to organic contaminants, are a source of pollution in wastewater. In general, wastewater includes inorganic pollutants such as synthetic colors and toxic heavy metals, which pollute aquatic bodies and endanger human health. Furthermore, bacterial biofilms play a key role in the elimination or bioremediation of inorganic pollutants from wastewater (Table 3).

The polysaccharides in the EPS matrix, in particular, have the ability to chelate heavy metals from contaminated locations.[180,198] Many bacterial biofilms, such as *Pseudochrobactrummendocina* NR802, *Pseudochrobactrumsaccharolyticum* LY10 sp., and *Arthrobacter* sp., have been used to eliminate harmful heavy metal ions and xenobiotic chemicals.[181,204] Furthermore, certain isolates were able to tolerate larger concentrations of manganese and zinc heavy metal ions (2000 mg L^{-1}); thus, the advantage of their effectiveness allows for the establishment of a biofilm capable of removing the greatest quantity of heavy metals.[205]

To counteract the toxic effects of heavy metals, indigenous microorganisms from heavy metal-contaminated locations evolve a variety of adaptive mechanisms such as heavy metal chelation, biotransformation, and metal efflux from cells. Despite these advantages, biofilms acquire resistance against heavy metal stress by forming an EPS barrier that may absorb, entrap, or immobilize metals surrounding their cell population. When it comes to some metals, EPS may act as a diffusional barrier that stops them from entering the cell.[206]

In all cases, a biofilm of pure bacterial species or a consortium of *Cellulosimicrobium* sp.[207] and *Halomonasaquamarina* TA-04[208] grown on diverse matrices (sand, stone, polyvinyl chloride, and rubber) may reduce 500 g mL^{-1} of chromate by more than 90%. It was discovered that the *B. subtilis* biofilm may accumulate many heavy metal ions, including Cu(II), Zn(II), Fe(II), Fe(III), and Al(III).[209] The metal adsorption characteristics of biofilm have been employed in biotechnology to remove uranium from the groundwater.[210]

Periphyton-based biochar biofilm systems have also been found to remove 95.4% of 2 mg L^{-1} of As(III). The functional groups (aCQO and aOH) on the surface of the calcite, as well as the periphyton biofilm, were both responsible for As(III) contamination removal and binding.[211] The EPS produced by the periphytic biofilm's phototrophic and heterotrophic community of microbes has the greatest potential for heavy metal sorption, which also includes arsenic.[212] Metal fixation is enabled by the ionizable functional groups of EPS, while the microbial surface's anionic charge provides binding sites for several metals.

5.3 Micropollutants removal

Electronics, pharmaceuticals, and cosmetics industries, in addition to inorganic and organic pollutants, discharge dangerous micropollutants in the environment, creating water

pollution. Bacteria and biofilms are two of the most promising solutions for this issue. Microbes in biofilms are beneficial for micropollutant bioremediation because they may absorb or degrade a wide range of severe contaminants *via* a variety of catabolic routes.[198] According to Polesel *et al.*, when predenitrifying MBBR was exposed to different concentrations of organic contaminants, it eliminated severalmantibiotic microcontaminants including erythromycin, trimethoprim, acetylsulfadiazine, sulfadiazine, sulfamethizole, sulfamethoxazole, metoprolol, and atendol.[177] Torresi *et al.* investigated the sorption of several micropollutants in MBBR carriers with varying biofilm thicknesses, and only eight micropollutants such as roxithromycin, clarithromycin, erythromycin, venlafaxine, citalopram, propranolol, metoprolol, and atenolol showed significant binding and sorption *via* interactions between carriers and electrostatics.[213] Biofilm microbial cells have proven to be much more adaptive and resilient to stress than planktonic cells in numerous bioprocesses.

Titanium dioxide nanoparticles can be found practically in all cosmetics, paints, and a variety of other household items. As a result, a substantial number of these nanoparticles end up in wastewater discharged from residential areas all over the world. Titanium dioxide nanoparticles may represent a major hazard to the microenvironment because titania element is known to be photocatalytically activated. To minimize the toxicity of nanoparticles, biofilm-mediated absorption and removal of these particles were studied. The remediation of nanoparticles was simple due to the presence of humic and surfactants compounds in the wastewater.[214] By modulating the physical trapping and diffusion transport of the substances, contaminant adhesion and absorption are regulated by the EPS matrix. The uptake of contaminants by biofilm microorganisms is influenced by nutritional, temperature, and osmotic pressure conditions, in addition to diffusion transport. The dissolved and organic matter components in wastewater act as a support for micropollutant absorption.

6 Factors affecting biofilm formation

Changes in environmental factors are known to cause a shift from planktonic toward sessile phenotype.[227] The variables that aid production of biofilms are discussed below.

6.1 Environmental factors affecting biofilm formation

Biofilm production can be influenced by environmental parameters such as nutrition levels, temperature, ionic strength, and pH.[228]

6.1.1 Effects of nutrients. Biofilm formation is affected by nutrient concentration, which may vary from very excessive to just about unnoticeable. They are more common and denser in a nutrient-rich habitat since it stimulates bacterial cells to transition from planktonic toward biofilm states, while a lack of nutrients leads biofilm cells to disengage from surfaces.[229] Bacterial biofilms are nourished in a number of ways: (i) using extracellular polymer to concentrate minor organics onto surfaces, (ii) repurposing waste from secondary colonialists, and (iii) combining biochemical resources using variety of enzymes to decompose the food supply.[230]

Several studies have found that living in a nutritionally deficient environment speeds up the transition from planktonic toward sessile lifestyle.[231] This finding revealed that biofilm development could be a survival strategy in a nutrient-limited environment, as surface colonization would give a number of benefits, including greater uptake of nutrients that could otherwise be absorbed to surfaces. Because the growing medium's nutritional content has been discovered to influence the establishment of biofilms by other organisms.[232,233]

The impact of nutrient levels on the production of bacterial biofilm has been studied in a number of ways. High nutrient concentrations, for example, increased cell counts inside the biofilms in drinking water distribution systems.[234,235] The pace and quantity of *P. putida* biofilm in a paper mill was increased as nutrient levels increased.[229] The addition of glucose as a carbon source to the medium has been observed to improve biofilm formation in several bacteria, such as *E. coli*[236] and *P. putida*.[229] Adding glucose to various medium, on the other hand, was reported to inhibit biofilm formation in numerous *Enterobacteriaceae* species, including *K. pneumoniae*, *Citrobacter freundii*, and *Salmonella enterica*.[237]

6.1.2 Effects of pH. The pH of the surrounding environment can have a significant impact on the production of biofilms.[228] Since changes in pH have the potential to overwhelm numerous processes and have fatal or negative effects on microorganisms, they have a substantial influence on bacterial growth and development as well as biofilm formation. Bacteria swiftly adjust the synthesis and activity of proteins linked with many cellular functions in response to external or internal pH variations. Some cellular functions, such as the excretion of exopolymeric compounds or polysaccharides, are less adaptive to pH changes.

This factor has been demonstrated to influence microbial adherence to surfaces, which is the first stage in biofilm development.[238,239] The pH is considered a key variable in the first attachment to surfaces of bacteria such as *Staphylococcus epidermidis*.[240] Furthermore, the pH of the medium has been demonstrated to alter the development of bacterial biofilm slime, which can affect enzyme performance because each enzyme has an ideal pH.[241] *S. aureus* biofilm development was found to be reduced at highly alkaline (pH 12) pH *and* acidic (pH 3) levels than at pH 7.[242]

Each species has a different preferred pH for polysaccharide synthesis; however, it is about 7 for most bacteria.[243] Exopolysaccharide synthesis helps biofilms protect themselves from environmental stressors such as pH.[244] As a result, bacterial cells within the biofilm are more resistant to pH fluctuations than free-floating cells.[245] Under severely acidic conditions, for example, the gel-like structure of the bacterial biofilm can aid in reducing fast ion diffusion and allowing the establishment of a pH gradient within the extracellular matrix. Under alkaline circumstances, however, poorly organized and very

thin biofilms have been seen as a result of biofilm maturation impairment. Some bacteria, such as *S. epidermidis* and *S. aureus*, are resistant to adhesion.[241]

6.1.3 Effect of temperature. Microbial activity is strongly influenced by temperature. The ideal temperature for bacterial growth is correlated with an increase in food consumption.[246] The presence and response rates of enzymes that control the development of several biochemical and physiological systems in bacteria are known to have an impact on food metabolism, and as a consequence, the appropriate temperature favors bacterial growth, resulting in fast biofilm creation.[246] When the temperature is raised above the optimum, bacterial growth is hampered due to a decrease in response rates, and biofilm production may be hampered as a result. The physical features of substances inside and surrounding cells may be influenced by environmental temperature in addition to enzymes.[247] This is related to a reduction in the rate of bacterial enzyme reaction. The ideal temperature for many bacteria found in cold water systems is about 40 °C.[248]

6.1.4 Effect of oxygen availability. The ability of bacteria to cling to submerged surfaces and form biofilms is influenced by oxygen availability.[249] Oxygen availability can also influence bacterial energy production, which can have an impact on biofilm development; for example, inadequate oxygen supply can reduce bacterial biofilm metabolic activity.[250] As a result, oxygen supply is regarded as a critical environmental component that might influence biofilm composition and development.[251,252] Active dispersal, which is crucial for the biofilm's life cycle, is mainly triggered by lower oxygen availability.[253] Bacterial cells in the biofilm's base were discovered to get less oxygen than those at the top, allowing separation from the biofilm's greater depths.[254] Sloughing has also been reported in biofilms growing in low-oxygen environments.[255] The presence of oxygen is necessary for the formation of biofilm in some bacteria, such as *E. coli*, because a lack of oxygen can signal separation.[256] Biofilm has been observed in other bacteria, such as *P. aeruginosa*, when grown anaerobically.[257]

6.1.5 Divalent cations. One of the influences that bacteria may experience during biofilm-related development is calcium because divalent cations such as Ca^{2+} are common in aquatic and terrestrial habitats. According to recent research, eDNA chelates divalent cations, which aids in the alteration of bacterial cell surface characteristics and hence enhances biofilm resistance to antimicrobial agents and detergents.[258] By bridging negatively charged sites on extracellular polymers, divalent cations, such as calcium, play a vital role in the early attachment of microbial aggregates of activated anaerobic sludge granules, sludge flocs, and biofilms.[259] Recent research has found that adding more divalent cations to a biofilm increases its thickness, making it more mechanically stable and denser.[260] Calcium has been discovered to play a role in alginate control, cellular extracellular product production, biofilm virulence, and cell signalling in addition to acting as a cofactor for specific proteins.[261]

6.2 Biological factors affecting biofilm formation

6.2.1 Quorum sensing (QS) and gene regulation. According to a study, the downregulation and upregulation of numerous genes are involved in the earliest attachment of cells to the substratum. During the development of *P. aeruginosa* biofilms, about 22% of genes were upregulated and 16% were downregulated.[262] Furthermore, in *P. aeruginosa* biofilm development, rpoS, algU, algD, and genes driving polyphosphokinase synthesis were upregulated.[263] In *S. aureus* biofilms, genes encoding enzymes involved in the fermentation or glycolysis, such as alcohol dehydrogenase, triphosphate, and phosphoglycerate mutase, were upregulated.[264] QS, or cell-to-cell signalling, has recently been shown to play an important role in biofilm cell attachment and detachment. The creation and proliferation of biofilms on diverse surfaces is triggered by a density dependent chemical signal released by bacterial cells firmly packed with an EPS matrix. QS activates target gene expression by combining a transcriptional activator protein with small autoinducers (AIs) signalling molecules, resulting in chemical behavior changes. This sort of intercellular communication is utilized to coordinate bacterial cell growth, morphological differentiation, and gene expression responses when a large number of AIs have been gathered.[265]

6.2.2 Extracellular polymeric substance production (EPSs). Extracellular polymeric substances (EPSs) are a complex combination of microorganism excreted adsorbed organic compounds from wastewater, hydrolysis products, lysis, and high molecular weight polymers (M_w 10 000). Humic acids, phospholipids, DNA oligomers, glycoproteins, proteins, and polysaccharides in general have been discovered in EPSs.[20] Because of their capacity to hydrogen link large quantities of water into their structure, EPSs are also very hydrated. EPSs are thought to contribute to the formation of a gel-like network that holds bacteria together in biofilms through bridging with hydrophobic contacts and multivalent cations. In addition to granulation, flocculation, and biofilm adherence to surfaces, EPSs help bacteria to acquire nutrients from the environment and protect bacteria from harmful environmental conditions. Various biofilms create different amounts of EPSs, and the quantity of EPSs produced rises as the biofilms mature.[266]

6.2.3 Extracellular DNA (eDNA). Extracellular DNA (eDNA) has been discovered to be a substantial component of biofilms from both multispecies and single species. eDNA, often known as naked DNA, is a component of bacterial self-produced extracellular polymeric substances (EPSs) that shares chromosomal DNA's fundamental sequence.[267] It is important for biofilm development at all phases, including early bacterial adhesion, aggregation, and the production of microcolonies that help with wastewater treatment. Biofilms benefit from eDNA because it strengthens them, protects them against detergents, antibiotics, and physical stress, and offers a plentiful source of nutrients for biofilm development.[268] eDNA may also be employed in biofilm engineering for beneficial purposes such as pollution remediation and power or fuel generation in bioreactors or bio-electrochemical systems.[230]

6.3 Physical factors affecting biofilm formation

6.3.1 Surface topology. Surface topography has a large impact on microbes' capacity to cling to a surface. Surface roughness at

the nanoscale and microscale levels improves bacterial adhesion to substrates during the early stages of colonization by increasing the surface area available for cell adhesion. Surface roughness reduces the shear stress on bacterial cells and communities found in moving liquids at high flow rates, such as water pipes in industrial facilities. Polymers from the medium will unavoidably condition or coat a material surface exposed in an aqueous medium, and the consequent chemical alteration will affect the rate and degree of microbial adhesion. Microbial adhesion is also influenced by other parameters such as elasticity, hydrophobicity, and charge.[263]

6.3.2 Turbulence, hydrodynamics, and velocity. The boundary layer is the region where there is zero turbulent flow away from the surface. This location flow velocity has been shown to be inadequate for eliminating biofilms. Outside of this layer, there is a lot of turbulent flow, which has an impact on cell adherence to the surface. The water flow velocity determines the size of the boundary layer. At high speed, the boundary layer decreases, exposing the cells to a higher amount of turbulence. Hydrodynamic conditions may affect metabolic activities, mass, thickness, EPS production, structure, and biofilm growth.[269]

7 Advantages of biofilm-based wastewater treatment

Low space requirements, operational flexibility, resilience to changes in the environment, reduced hydraulic retention time, high active biomass concentration, enhanced ability to degrade recalcitrant compounds, and a slower microbial growth rate, which result in lower sludge production, are all advantages of wastewater treatment with biofilm systems.[230]

7.1 Operational flexibility

Wastewater treatment systems based on biofilms are quite adaptable in terms of operation. When it comes to reactors such as MBBR, they are relatively simple to run and have a straightforward design. Other treatment technologies have been utilized in conjunction with the MBBR method.[270] Schneider *et al.* investigated how oil refinery effluent may be treated in a hybrid system that included a biologically-activated carbon column, an ozone reactor, and an MBBR.[271] This demonstrates that biofilm-based reactors can operate in a hybrid environment.

7.2 Low space requirements

Varied biofilm-based water treatment reactors require different amounts of area to function. MBBR utilizes the entire tank volume for biomass growth, unlike other biofilm-based treatment technologies. The biofilm that grows on carriers has aerobic outer layers and anaerobic/anoxic interior layers. Consequently, nutrient removal may be accomplished in a single reactor, minimizing the size of the wastewater treatment facility.[272]

7.3 Reduced hydraulic retention time

At an organic loading rate (OLR) of 4.9 kg COD m^{-3} sponge per day and a hydraulic retention time (HRT) for 15.3 h, the experiment demonstrated a COD removal efficiency of over 98% on a trickling filter reactor.[273] For the simultaneous removal of bromate and nitrate, the CH_4-MBR employed by Lai *et al.* has a working capacity of 65 mL. For an influent bromate concentration of 800 g L^{-1} and 40 h hydraulic retention period, the biofilm process removed bromate (driven by denitrifiers) at a maximum rate of 40.7 g L^{-1} min^{-1}.[274]

7.4 Resilience to changes in the environment

Bacteria have complex mechanisms that enable them to adjust to small pH changes in their surroundings. EPS outflow may be an adaptive response to overcome pH oscillations since cellular activities do not respond as quickly to pH variations.[247] Heavy metal chelation, biotransformation, and metal efflux from cells are among the adaptation processes found by Teitzel and Parsek in indigenous bacteria from heavy metal-contaminated environments.[206]

In bioremediation, biofilm-forming bacteria that have been found in nutrition and oxygen competition as well as resistance to contaminants have been examined. Biofilm-forming bacteria found in nutrition and oxygen competition as well as contaminant resistance have all been studied in bioremediation. In biofilms found in profoundly polluted areas, indigenous bacteria can live, endure, and handle tough environmental conditions such as predation, nutrition, salinity, pH, changeable and high temperature, UV exposure, and pollutant concentrations.

7.5 High active biomass concentration

Various studies have revealed that the usual biomass content, when estimated on reactor volume, is in the range of 2–5 kg SSm^{-3}, which is high when compared to other biological water treatment procedures.[275–277]

7.6 Low sludge production

Two pilot experiments of an immersed membrane activated sludge process were given by Cote *et al.* and it was discovered that the sludge production rate from a submerged MBR (0.25 kgSS kg^{-1} COD removed) was nearly half that of an extended aeration activated sludge process.[278] Another French pilot investigation of series MBR indicated a sludge production rate of 0.23 kgSS kg^{-1} COD removed, backed the low sludge production.[279]

8 Challenges in biofilm-mediated removal of contaminants

8.1 Biofilm thickness/architecture

The thickness and cell density of a biofilm are significant variables to consider while assessing it, according to Mathur *et al.*[214] Another crucial factor is the matrix porosity, which influences the biofilm's ability and structure. According to study, thin biofilms contain fewer holes and a greater cell

density than thick biofilms. Biofilm may use these perforations as a solid–liquid partitioning substrate. Consequently, the biofilm's ability to break down phenol is related to its thickness; therefore, thickness is suggested for usage as an effective bioremediation strategy.[280]

8.2 Quality of pollutants/effluent nature

Positively-charged micro-contaminants (greater than 90%) have exhibited significant sorption potential in activated sludge as compared to neutrally or negatively charged micropollutants compounds at pH 7.5.[213] The pH has been found to play a role in determining the charge and sorption potential of micropollutants. The protonation of the macrolides amino group causes a strong ionic contact between the negatively-charged cell surface and positively-charged macrolides.

9 Future perspectives

More research is required to create and execute economically efficient processes due to the significant use of energy and carbon sources. For the treatment of nitrogen-rich water that is related to concentrated waste streams produced by various industries, biofilm-based treatment solutions provide a viable substitute. Several biofilm-based wastewater treatment technologies have been developed during the last several decades.

Since biofilms are superior at protecting the environment, they are employed in many industrial applications, including the packaging of commercial items and food. Numerous research noted certain drawbacks of employing biofilms, including their poor mechanical qualities, susceptibility to bacterial and fungal infections, susceptibility to air permeability, and water sensitivity. Recent research has however shown that these problems may be avoided using plasticizers, nanomaterials, *etc.* In addition, the use of plasticizers and nanoparticles enhances the products' structural, thermal, and chemical qualities. The use of preservative and antibacterial nanomaterials in food packaging biofilms carries a risk since human health may be adversely affected by nanoparticle movement, injection/inhalation, and exposure to food surfaces. Contemporary researchers are confronted with the significant challenge of creating regulatory standards and thorough toxicological analyses on nanomaterials. When compared to synthetic polymers, the cost of producing biofilms is excessively high. New kinds of bio-based films with simple processing that use affordable, renewable resources, and have high antibacterial, chemical, physical, and thermomechanical properties are needed to replace conventional materials. Currently, research is focusing on antibacterial edible packaging materials, but in the next few years, antibacterial nanoparticle-reinforced biofilms will become a key trend. Even though this field has seen a lot of study, there are still numerous obstacles to overcome, including gas permeability, control over drug release, adverse effects on humans, drug loading, and encapsulation ability. Therefore, in the near future, it is important to produce mechanically and chemically superior edible biofilms that have the potential to be employed in the packaging and biomedical sectors.

There is still a need for more research: (1) recovering aged biofilm using economical and ecofriendly regulatory techniques. In addition, novel biofilm reaction technologies, and principles continue to pique people's interest to meet the rising demands for lower energy consumption and pollution removal; (2) uncovering the attributes of biofilm from a more microscopic viewpoint through *in situ* monitoring methods and molecular biology technologies (*i.e.*, metagenomic and macroproteomics approaches); and (3) specific and practical methods for shortening biofilm formation in refractive processes.

The use of this technique for the treatment of industrial wastewater is constrained by the lack of techno-economic information on biofilm dependability, sustainability, and system performance. In addition, certain components of effluent wastewater, including suspended particles, have a detrimental impact on the functioning of the biofilm by obstructing the diffusion of oxygen and substrate into the biofilm. In addition, the high cost and biofouling of membranes are the main obstacles to the development of the membrane biofilm process.

10 Conclusion

With increase in number of toxic xenobiotics in the wastewater, different bioremediation approaches have been introduced to eliminate them. Biofilm-mediated reduction of pollutants from wastewater is one of the potential approaches that have been used widely since the past few years. In this approach, bacterial cells exchange signalling molecules, genetic materials, and metabolites, and this interaction between bacterial cells of various strains depends on quorum sensing. The understanding of bacterial diversity in biofilm by utilizing metagenomic approach, *i.e.*, 16s rRNA is much essential to understand the bioremediation of contaminated water. Globally, the usage of microbiome-based bioremediation approach has tremendously increased, and the fabrication of genetically-modified biofilm thus increases the elimination of pollutants. In industries, quorum sensing technology is applied to remove contaminates from industrial wastewater, it regulates the synthesis of EPS, fabrication of biofilm, and synthesis of biosurfactants, which could significantly remove organic substances and heavy metals from wastewater. In wastewater treatment plants, quorum sensing and quorum quenching are responsible for biofouling, granulation, aggregation, colonization, metabolic of organic substances, and removal of nutrients. In a nutshell, the omics approach would provide a much-needed understanding of how the potential to form biofilms is influenced by a variety of physiological, environmental, and mutational variables, as well as how these factors interact with one another in a predictable way. The development of a database where biofilm signatures are analyzed and recognized may follow from this. However, before starting such a venture, it will be necessary to choose a high-throughput, reliable, and adaptable biofilm growth apparatus as well as the proper techniques for biofilm

analysis. Although strategies similar to metabolic engineering are very necessary for controlling metabolic flux, the underlying biofilm architecture should not be disregarded. The effectiveness of any system is substantially impacted by each of the biofilm's constituent parts as well as the biofilm's overall form. Currently, the improvement of bioremediation involves the use of genome editing tools such as clustered regularly interspaced short palindromic repeats (CRISPR-Cas), transcription activators such as effector nucleases, and zinc finger nucleases. *Escherichia coli* and *Pseudomonas* are two bacteria that often employ the CRISPR-Cas system, which consists of guide RNA connected crisper generated RNA and transacting antisense RNA. In addition, the *Achromobacter* sp. HZ01, Comamonas testosterone, and *Rhodococcusruber* TH utilize the CRISPR system to express genes necessary for bioremediation. A new direction for the study of biofilm-mediated bioremediation may be opened up by genome editing, which improves the ability of bacteria to survive in hazardous environments when used with bioinformatics techniques and *in silico* databases.

Conflicts of interest

There are no conflicts to declare.

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