REVIEW ARTICLE

A Comprehensive Review of Biofilm Composition and Factors Affecting Efficacy in Microbial Bioremediation

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ABSTRACT

Biofilm-mediated bioremediation deploys the natural capabilities of microbial communities to remove environmental pollutants, offering a resilient and efficient means of pollutant degradation. This review highlights the critical role of biofilms in environmental pollution control, emphasizing their structural and functional attributes. Biofilms, complex assemblies of microorganisms, demonstrate superior capacity to absorb, immobilize, and degrade contaminants compared to planktonic cells. Key topics include biofilm formation, diversity, and the biochemical pathways utilized for pollutant breakdown. Applications include organic pollutant degradation, heavy metal detoxification, and the treatment of new pollutants such as microplastics and medications. Factors influencing biofilm efficacy, including environmental conditions and maturity, are examined alongside challenges such as resistance, stability issues, and limitations in large-scale applications. The utilization of genetically modified microbes, advancements in synthetic biology and biofilm engineering, and the combination of biofilm-mediated bioremediation with other technologies are the main topics of future research.

KEYWORDS: Biofilm, Bioremediation, Pollutant degradation, Heavy metal detoxification, Microbial enzymes, Emerging contaminants.

Highlights:

- Biofilms consist of microbial communities in a hydrated EPS matrix, which is crucial for ecosystems and industrial processes.
- Their formation includes adhesion, EPS secretion, maturation, and dispersal, with quorum sensing enhancing stability.
- Biofilms effectively detoxify pollutants, showing resilience and superior degradation compared to planktonic cells.
- Future advancements may involve engineered microorganisms and integrated methods, necessitating attention to regulatory and environmental issues.

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INTRODUCTION

Biofilms are microbial communities, including algae, bacteria, fungi, and protozoa, that bind to surfaces and are sink in a highly hydrated matrix of extracellular polymeric substances (EPS) (Flemming & Wingender, 2010) (Lewandowski & Beyenal, 2014). Over 90% of microorganisms on the planet are organized into biofilms, which inhabit ecosystems such as terrestrial environments, surface waters, lakes, and groundwater (Kallmeyer *et al*., 2012). They can also exist in drastic ecological habitats by surviving challenging factors, including extreme hydrodynamic, osmotic pressure, stress, temperature fluctuations, and exposure to extreme pH levels and chemicals (biocides and antibiotics). Biofilms play crucial functions in subaerial and marine microscale ecosystems (Herrling *et al*., 2019).

Biofilms can also prevail at non-ideal locations, exemplify clinical instruments, and lead to significant challenges as they can host pathogens responsible for diseases and infections (Costerton *et al*., 2005) (Hall-Stoodley & Stoodley, 2009). Biofilms can cause critical issues in technical systems also, biofouling of membranes in water purification procedures is one of notable examples. Conversely, biofilms facilitate beneficial technical applications, including the bioremediation of soils and groundwater, biological wastewater treatment, and the biotechnological production of chemicals and drugs (Maurya & Raj ,2020). They are the focus of interdisciplinary research that combines analytics, biology, chemistry, and engineering (Denkhaus *et al*., 2006; Morgenroth & Milferstedt, 2009). Even though biofilms are widely prevalent in both natural and artificial systems, in-depth study is still needed to fully understand the basic mechanisms, potential applicability, and advantages in environmental and technical processes. In the context of green chemistry, this thorough comprehension is also essential for taking material flows and general sustainability into account.

The process by which microorganisms (algae, bacteria, fungus, and so forth) break down, change, eliminate, detoxify, or immobilize different types of environmental pollutants, chemical and physical, is known as bioremediation. As a developing technology, bioremediation can be integrated with other treatment methods to manage multiple types of environmental contaminants thoroughly. This viable strategy to mitigate environmental pollution highlights the need for further research in the field. In order to sustain the effective and successful functioning and monitoring of a bioremediation process, it is crucial to establish a positive relationship between

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the environmental influence on the distribution and behavior of pollutants and the choice and execution of the bioremediation technique (Bala *et al*., 2022).

Due to its remarkable capacity to absorb, immobilize, or degrade toxins, combined with its biomass and adaptability, biofilm-mediated bioremediation holds great promise for eliminating environmental pollutants. Communities of single and mixed microbial cells form biofilms, which cling to surfaces in aquatic settings and exhibit exceptional resistance to severe environmental stressors. Biofilm-forming microorganisms are more resilient to contaminants and have an effective food competition than free-floating planktonic cells, which helps to create a protective habitat that increases their survival. Because of a well-regulated gene expression pattern triggered by quorum sensing, these biofilm communities can efficiently sorb and metabolize a variety of organic contaminants and heavy metals. Bioremediation, which converts different contaminants into less hazardous compounds, is an economical and environmentally acceptable process that uses biofilms. The immobility of microorganisms in a self-made matrix provides extra defense against external stressors, pollutants, and predatory species (Mishra *et al*., 2022). Biofilm-based bioremediation is a useful method for cleaning up contaminated soil and groundwater in industrial settings since it has been effectively used to remove pesticides, heavy metals, petroleum products, and explosives (Saini *et al*., 2023a).

This review article aims to present a thorough analysis of biofilm-mediated bioremediation as a practical method for mitigating environmental pollutants.

BIOFILMS

Process of Formation of Biofilm

Biofilms are intricate, syntrophic communities of microorganisms, such as fungi, bacteria, and protozoa, that stick to surfaces that are inert or living and are surrounded by a self-produced matrix-extracellular polymeric substance (EPS). The formation of biofilm is a complex multilevel process, including conditioning layer production, adhesion and growth of bacteria, and biofilm expansion (Fig 1). The adherent cells attach themselves to surfaces and one another, settling into a slimy matrix that is mostly made up of polysaccharides. In addition to giving the biofilm structural strength, this polymeric matrix helps the bacteria survive and thrive in a variety of settings (Kokare *et al*., 2009).

Biofilm can survive on different types of surfaces, such as soil particles, wood, tissue, plastic, glass, metal, medical implant material, and foodstuffs. Flagella, fimbriae, and pili help in bacterial attachment. EPS serves as a bridge that connects bacteria and the conditioning layer. Genetic studies report that multiple chemical, biological, and physical processes within complex microbial communities culminate in the production of biofilms (Kokare *et al*., 2009). To create a conditioning layer, planktonic microorganisms must first attach themselves reversibly to a surface that has already been conditioned. This connection becomes permanent when these microbes wrap themselves in a matrix of extracellular polymeric substances (EPS). The microbial survival in diverse conditions is facilitated by this matrix, which also preserves the biofilm's structural integrity.

When exposed to an aqueous media, this organic monolayer can form quickly and act as a docking site for the initial adhering cells. In addition to the presence of external appendages like flagella and pili that aid in surface contact, other factors that affect the initial adhesion include hydrophobic forces, van der Waals attraction, and electrostatic interactions. By secreting EPS, these germs become more bonded to the surface and eventually undergo permanent attachment. After secure adhesion, biofilms undergo a significant architectural development phase that begins with cell division and proliferation to form microcolonies. The biofilm acquires a distinctive three-dimensional structure during the maturation stage, with multiple layers that support a substantial nutrition exchange and intercellular communication. Quorum sensing (QS), a method by which bacterial cells communicate utilizing signaling molecules known as auto-inducers to regulate gene expression in response to population density, facilitates cell-to-cell interactions (Sedarat *et al*., 2023). The coordinated behaviors that improve stability and resilience are made possible by this signaling, which is essential for biofilm maturation.

Dispersal is the end phase of biofilm formation, during which cells split off to occupy new habitats. Daughter cell shedding, nutritional restriction, fluid dynamics, shear impacts,

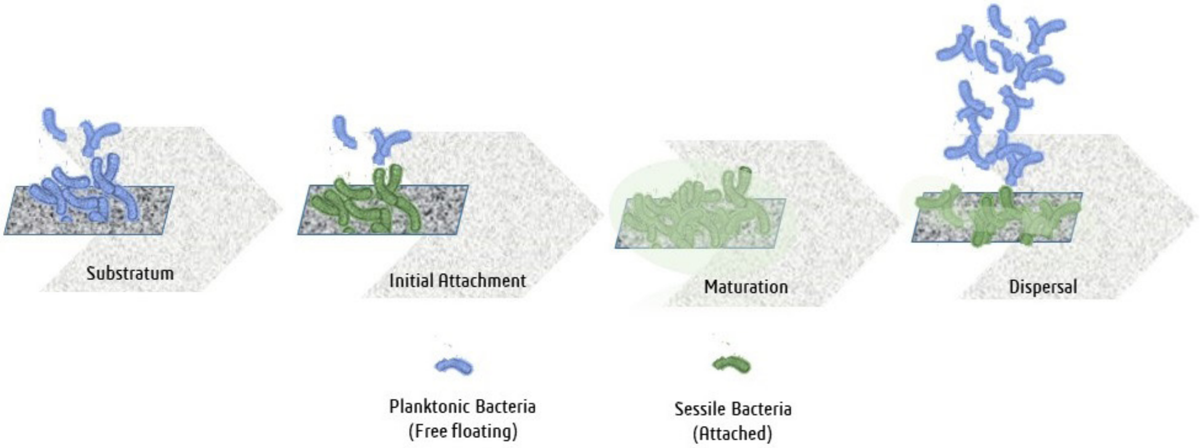

Fig. 1: Different stages of biofilm formation

and secretory proteins can lead to the process (Jojo *et al.,*2024). Abrasion, erosion, and sloughing are some of the detachment mechanisms. Abrasion is the loss of biofilm by suspended particles. Erosion is the ongoing removal of individual cells or microscopic fragments. Sloughing is the loss of a significant amount of biofilm biomass as a result of limited nutrition or oxygen. New biofilm production can then be triggered by dispersed cells, and this process is crucial for the transmission of diseases (Heather *et al.,*2017).

Structure

Biofilms are composed of various biopolymers, including polysaccharides, proteins, and extracellular DNA (eDNA) (Figure 2). These biomolecules provide microbial consortia protection and structural stability. As they strengthen adherence and serve as a framework for biological components, exopolysaccharides are essential for the stability of biofilms. (Flemming *et al.,* 2023) Several exopolysaccharides, such as colonic acid in *Escherichia coli* and alginate in *Pseudomonas aeruginosa* can change in response to environmental stressors (Limoli *et al*., 2015). Horizontal gene transfer (HGT) is the ability of bacteria to transfer genes between related and unrelated species. This ability helps the bacterial species to adapt to new habitats and pressures (Too & Masila, E.,2024).

Extracellular DNA is vital for the formation of biofilms as it is released through autolysis or active secretion and contributes to structural stability and resistance to antimicrobial treatments. Extracellular proteins also aid in the early adhesion and aggregation of cells, which stimulates biofilm formation and promotes microbial community dynamics. These elements work together to strengthen the ability of biofilm to withstand an array of conditions, underlining both their ecological significance as well as potential uses in biotechnology and bioremediation. Employing biofilms in a variety of applications, such as medical therapies and environmental management, requires an understanding of the complexities associated with their structure, function, and production (Besemer, 2015a).

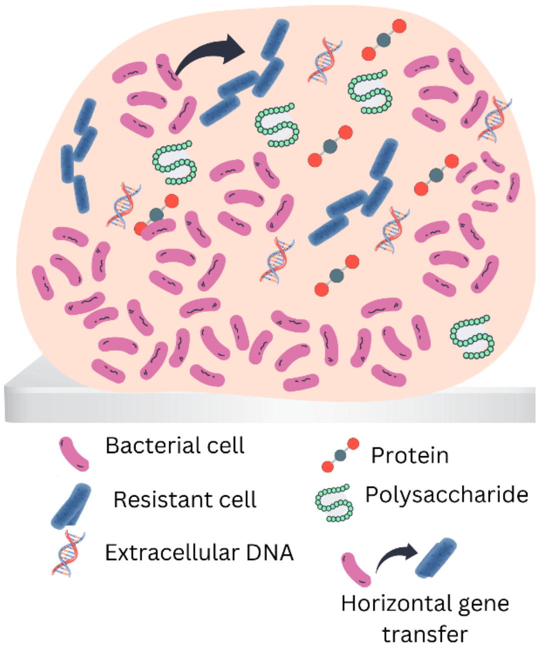

Fig. 2: Biofilm structure

Microbial Composition and Diversity in Biofilms

Biofilms are featured by a remarkable diversity of microorganisms, each contributing to ecological and biodiversity processes in aquatic ecosystems (Davey & O'toole, 2000). Bacteroidetes, Cyanobacteria, and Proteobacteria are the most common bacterial species found in freshwater biofilm. Among that, Beta-proteobacteria are frequently dominant in biofilms found in rivers, streams, and lake aggregates, reflecting their prominence in corresponding planktonic communities (Battin *et al*., 2001) (Simon *et al*., 2002) (Olapade & Leff, 2005) (Besemer *et al*., 2012). Remarkably, Alpha-Proteobacteria, which is commonly found in marine ecosystems, is more abundantly found in freshwater biofilms than Beta-Proteobacteria. Their habitats are varied and include diatom-aggregates in lakes, live or decaying plants, and stream biofilms in both epilithic and hyporheic conditions. They probably dominate as they can break down humid materials and develop morphologies that are grazing-resistant.

Bacteroidetes are another key category in freshwater biofilms. They are known for their ability to utilize complex macromolecules, which probably accounts for their great abundance, particularly in aggregate-associated communities. They possess a significant role in degrading suspended particles, especially when easily degradable organic compounds are depleted, leaving refractory organic material as the primary component. Cyanobacteria are commonly found in large populations in biofilms that are exposed to light. Actinobacteria, Acidobacteria (in low pH conditions), Deinococcus-Thermus, Gamma-Delta-Proteobacteria, Firmicutes, Planctomycetes, and Verrucomicrobia are the other bacterial genera that are frequently found in biofilms(Yin, 2014).

Despite archaea playing a minimal role in river and stream biofilms, certain conditions can make them far more prevalent. For example, Methanogenic archaea can occasionally account for more than 10% of the relative abundance in the hyporheic microbial community, making them a prominent component. Microbial eukaryotes are also present in large numbers and have functional significance in biofilms. Algae like bacillariophyta and chlorophyta contribute substrates through exudates and lysis products and act as a major carbon source for heterotrophic biofilm microorganisms. Ascomycota is a fungal group that plays a vital role in the decomposition of submerged organic matter and can significantly structure biofilms. Protists, which include amoebae, ciliates, and flagellates, as well as viruses, hold a role in regulating the growth of biofilms and modifying their composition, structure, and activity (Table 1).

Factors Influencing Biofilm Development and Stability

The formation of biofilm is an intricate and dynamic process that holds a significant role in various environmental, medical, and industrial contexts (Puttamreddy *et al*., 2010). At first, the bacterial cells get attached to the substrate, followed by numerous physiological transformations, including the formation of microcolonies by the replication of adhered cells and biofilm maturation (Wei & Ma, 2013). Compared to other free-living or planktonic peers, bacteria associated with biofilms show distinct

Biofilms and their potential in bioremediation

Table 1: Various biofilms involved in contaminant removal with their source and the potential application in pollutant removal.

Biofilms	*Wastewater type*	*Contaminants*	*Reference*
Chlorella vulgaris	Wastewater collected from petroleum storage	Aromatic hydrocarbons	(Martin & Johnson, 2012)
Microalgal biofilm	Sewage from the city	Phosphorus and nitrogen	(Iman Shayan *et al*., 2016)
B. mojavensis M1+ *R. rhodochrous* BX2	Groundwater	Organic cyanide	(An *et al*., 2018)
Moving bed biofilm reactor (MBBR)	Sewage from the city	4-Nonylphenol, 17β-estradiol, naproxen, and diclofenac	(Abtahi *et al*., 2018)
Moving bed biofilm reactor (MBBR)	Sewage from the city	Medicines	(Polesel *et al.,* 2017)
Membrane bioreactors (MBR)	Wastewater with saline conditions	Ammonium	(Tchounwou *et al*., 2012)
Batch biofilm reactor using algae as a sequence agent	Sewage from the home	Phosphorus and Nitrogen	(Torresi *et al*., 2019)
Xanthomonadales, Flavobacteriales, Sphingobacteriales, and *Burkholderiales*	Sewage from the hospitals	Drugs	(Torresi *et al*., 2018)
Moving bed biofilm reactor (MBBR)	Sewage from the hospitals	Trimethoprim, propranolol, diatrizoic acid, clarithromycin, and azithromycin	(Gao *et al*., 2015)
Batch-dispersed biofilm reactor sequencing based on algal-bacterial interaction	Sewage from the home	Total phosphorous and total nitrogen	(Tang *et al*., 2018)
P. monteilii P26 and *Gordonia* sp. *H19*	Artificially created seawater	Crude oil	(Alessandrello *et al*., 2017)
Bacillus sp. *GH-s29*	Contaminated groundwater	Heavy metals	(Maity *et al*.,2023)

features such as physiological changes and strong resistance to immune system assaults and medications. Considering their resistance, biofilms remain an important contributor to long-term and persistent infections (Rossi *et al.,* 2016). The development from a planktonic to an adherent form is mainly induced by shifts in environmental parameters, including temperature, ionic strength, pH, and nutrition levels (Agarwal *et al*., 2011). These constituents are vital for the formation of biofilms.

Bacterial Adhesion and Surface Properties

Bacterial adhesion is potentially influenced by several factors, like surface characteristics - roughness and hydrophobicity, environmental elements - nutrition availability, temperature, pH, and hydrodynamic conditions (Oder *et al*., 2017). A single microbe can possess a competitive advantage over others in a mixed microbial community due to cell surface characteristics like extracellular appendages like flagella and fimbria presence, cell-to-cell communication interactions, and EPS production (surface-associated polysaccharides or proteins). Although hydrophobic bacteria are more likely than hydrophilic bacteria to bind to surfaces, rough, hydrophobic surfaces covered in a surface conditioning layer are ideal for the easy attachment of biofilms. Environmental factors, including pH, temperature, and nutrient levels, can also affect the physicochemical characteristics of the substratum, including its texture (rough or smooth), hydrophobicity, and charge (García-Gonzalo & Pagán, 2015). In aquatic environments, the process of microbial adhesion can be accelerated by raising the flow velocity, water temperature, or nutrient concentration as prolonged as these factors remain beneath critical thresholds.

Cyclic-di-GMP

Cyclic-di-GMP (cyclic dimeric guanosine monophosphate) is a secondary messenger that performs a key role in controlling the formation of biofilms. This chemical regulates the development from a motile to a sessile biofilm-associated planktonic life (Sisti *et al*., 2013). The high intracellular concentration of c-di-GMP supports bacterial adhesion, EPS synthesis, and biofilm formation, whereas low concentration increases motility, biofilm deconstruction, and virulence pathway activation (Toyofuku *et al*., 2016).

The intracellular concentration of c-di-GMP is controlled by the antagonistic activity of diguanylate cyclases (DGCs), which produce c-di-GMP, and phosphodiesterases (PDEs), which degrade it (Cruz *et al*., 2012). During the biofilm maturation process, for instance, c-di-GMP controls the formation of extracellular polysaccharides like Pel and Psl in *Pseudomonas aeruginosa.* Furthermore, c-di-GMP is involved in the regulation of biofilm dispersion. For example, exposure to chemicals that release nitric oxide (NO) in *P. aeruginosa* biofilms may result in dispersal by elevating PDE activity, which subsequently decreases c-di-GMP levels (Zhao *et al*.,2013).

Hydrodynamic Conditions

Biofilms in diverse habitats are exposed to various hydrodynamic conditions, which can significantly impact their formation and structure (Lembre *et al*., 2012). These factors exert an impact on the availability of oxygen and nutrients and also the forces of compression that influence cell adhesion to surfaces (Gomes *et al.,* 2014). Fluid hydrodynamics controls the rate at which bacterial cells, nutrients, and oxygen are transported from the bulk fluid to the biofilm. It can also affect the strength and density of biofilm. Biofilms in diverse ecosystems confront various hydrodynamic conditions, which can significantly affect the formation and structure of biofilms (Purevdorj *et al.,* 2002).

Environmental Conditions

Environmental factors, such as oxygen and nutrient availability, temperature, and pH, play a crucial role in the formation of biofilm (Besemer, 2015b; Sabater *et al.,* 2002). The pH level can influence microbial attachment (Oder *et al.,* 2017; Pompilio *et al*., 2008). Some bacteria, like *Staphylococcus epidermidis,* exhibit a strong dependence on pH for surface binding and biofilm slime formation (Nostro *et al.,* 2012). Temperature influences the vital biofilm stability measures like bacterial growth and the physical properties of EPS (García-Gonzalo & Pagán, 2015). The availability of oxygen is necessary for energy production and biofilm development (Ahn & Burne, 2007; Keleştemur *et al.,* 2018; Toyofuku *et al*., 2016). It may vary in the case of bacteria. Some bacteria require oxygen, while others can produce biofilms in anaerobic environments. Nutrient concentration is another significant factor for biofilm formation. It may have varying effects based on the microbial species and nutrient type, such as glucose activating or inhibiting biofilm formation in different bacteria.

FACTORS AFFECTING BIOFILM EFFICACY IN BIOREMEDIATION

Environmental factors, including nutrition availability, oxygen content, temperature, and pH, can influence the efficacy of biofilms in bioremediation. The development of biofilm stability and degrading capacity depends on the existence of divalent cations, biofilm maturity, and the genetic adaptability of the microbial community, particularly through horizontal gene transfer.

Environmental parameters

Nutrient Availability

Nutrient levels have a major impact on biofilm formation. Bacterial cells frequently change from a planktonic (free-swimming) phase to a biofilm state and then to denser biofilms in nutrient-rich settings. In contrast, the separation of biofilm cells from surfaces can be triggered by the lack of nutrients. This adaptation to a sessile life in the absence of nutrients points to the formation of biofilms as a survival tactic that boosts nutrient uptake from surfaces (Rochex & Lebeault, 2007).

pH

The environment pH possesses a major role in the formation of biofilms. Variations in pH cause microorganisms to modify the way they function on a cellular level; particular pH values influence microbial adherence to surfaces. As the ideal pH varies based on the species, biofilms are generally more strong and resilient at neutral pH (about 7). Abnormally high or low pH values might hinder the production of biofilms and impact the activity of enzymes (Saini *et al.,* 2023b).

Temperature

Microbial activity and the formation of biofilms are directly impacted by temperature. By accelerating enzyme reaction rates, the ideal temperature promotes bacterial growth and the formation of biofilms. Temperatures over the ideal range can inhibit the development of biofilms and limit bacterial growth. Many bacteria prefer a temperature of about 40°C, especially in cold water systems (Samrot *et al.,* 2021).

Oxygen Availability

The growth of biofilms depends on oxygen availability, which influences the bacterial energy generation process. Inadequate oxygen can lower metabolic activity, which leads to active dispersal or detachment from the lower layers of the biofilm. Some bacteria, like *P. aeruginosa*, can survive anaerobically, while others, like *E. coli*, require oxygen to form biofilms (Samrot *et al.,* 2021)

CHALLENGES AND LIMITATIONS

Biofilm-based bioremediation faces numerous obstacles and restrictions that affect its effectiveness and applicability. Biofilm resistance to environmental stresses and antimicrobials is a significant concern that can result in difficult-to-treat chronic diseases (Mirghani *et al.,* 2022). The structural complexity of biofilms hides the microbial community, making it harder to eliminate embedded bacteria, especially in medical instruments, where this could result in chronic illnesses requiring long-term antibiotic administration (Mi *et al.,* 2018). Environmental factors that impact biofilm formation, stability, and functionality include pH, temperature, and oxygen levels. Maintaining ideal conditions in large-scale bioremediation projects can be difficult as variations in these elements can either help or hinder biofilm formation. The possibility of biofilm instability and separation further complicates application in various sectors. To overcome these barriers, a deeper understanding of the ecological and genetic processes inside biofilms is needed to develop bioremediation techniques that are more resilient and flexible.

FUTURE PERSPECTIVES AND INNOVATIONS

Several significant advancements are the main focus of future perspectives in biofilm-based bioremediation. The utilization of genetically modified microorganisms (GMOs) in biofilm engineering and synthetic biology provides an intriguing potential for improving the efficacy and selectivity of bioremediation treatments. Pollutant decomposition can be further optimized by combining biofilm technology with other bioremediation techniques like phytoremediation or chemical treatments. Nonetheless, policy and regulatory factors continue to be crucial, especially when it pertains to the utilization of GMOs and nanomaterials that can be harmful to the environment and public health. In addition to tackling issues like biofilm formation management, material durability, and the economic viability of large-scale applications, more research is required to produce biofilm-based treatments that are affordable, energy-efficient, and sustainable (Maqsood *et al.,* 2023).

CONCLUSION

In conclusion, biofilms, which are made up of intricate microbial communities and extracellular polymeric materials, are essential to industrial and environmental processes. Biofilm-mediated bioremediation is an effective and adaptable method for tackling the increasing environmental problems caused by pollution. A wide range of contaminants, including heavy metals, persistent organic pollutants (POPs), and newly developing pollutants like nanomaterials and microplastics, can be effectively degraded and removed by biofilms. Thanks to their powerful EPS and varied microbial populations. The potential of biofilms in bioremediation processes is highlighted by the complex interactions occurring between biofilms and contaminants, and by the biochemical pathways involved in degradation. Bioremediation procedures can be made more selective and efficient by utilizing the inherent capabilities of biofilms and improving genetic and molecular engineering methods. This strategy provides long-term environmental management with sustainable and environmentally friendly solutions and reduces environmental pollutants.

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AUTHOR CONTRIBUTION

Author 1, Ligi Lambert D Rosario

Conceptualized the study, conducted the literature review, wrote the initial draft, and coordinated the overall review writing. Conducted data analysis for the case studies, contributed to the writing of specific sections on biofilms and reviewed the final manuscript.

Author 2, Dr. Sona S Dev

Assisted in the literature review and contributed to the writing and editing of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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