

## REVIEW

# Biofilms: Friend or Foe? Unpacking the Ambiguity of Microbial Communities in Aquatic Systems

Pronob Das<sup>\*1</sup> | Dharmendra Kumar Meena<sup>2</sup> | Simanku Borah<sup>1</sup> | Soibam Khogen Singh<sup>3</sup>

<sup>1</sup>ICAR-Central Inland Fisheries Research Institute, Regional Centre, Guwahati-781006, Assam, India

<sup>2</sup>ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata-700 120, West Bengal, India

<sup>3</sup>Krishi Vigyan Kendra, ICAR- Research Complex for NEH Region, Manipur Centre, Ukhrul-795142, Manipur, India

## Correspondence

Pronob Das, ICAR-Central Inland Fisheries Research Institute, Regional Centre, Guwahati- 781006, Assam, India  
Email: [pronobjaan80@gmail.com](mailto:pronobjaan80@gmail.com)

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The authors assert that the manuscript was developed without any commercial or financial associations that could be interpreted as a potential conflict of interest.

## Authors Contribution

All listed authors have contributed significantly, directly, and intellectually to the work and have endorsed it for publication.

## Abstract

Biofilms are a crucial component of aquatic ecosystems, playing a significant role in the environment by colonizing various surfaces such as sand, rocks, and leaves. They supply energy and organic matter to the food chain, recycle organic matter, and contribute to water quality. However, biofilms can also have detrimental effects, particularly in man-made environments such as water distribution systems, where they can cause bio-fouling and lead to decreased water quality and pipe blockages. Biofilm formation in aquatic ecosystems is influenced by several environmental factors, including temperature, salinity, water flow, and nutrient concentration. The composition of biofilm on microplastics (MPs) is particularly affected by organic content in the water, salinity, and dissolved oxygen content. While biofilms can have negative impacts, they also serve beneficial purposes in natural environments. For instance, biofilms play a vital role in biogeochemical cycles and are essential for the basic chemistry of Earth's surface. They are also used in the treatment of drinking water, wastewater, and detoxification of hazardous waste. In conclusion, biofilms have both beneficial and detrimental effects on our world, and understanding their impact is crucial for managing aquatic ecosystems and man-made environments. Further research is needed to advance our understanding of complex, environmental, real-world biofilms and their interactions with different microorganisms within marine biofilms.

## KEYWORDS

Aquatic environment, Aquaculture, Biofilm, Microbes, Microplastics

## INTRODUCTION

Microorganisms play a critical role in the aquatic environment, contributing significantly to nutrient recycling, productivity, serving as food for other organisms, water quality and health management, and decomposition. Understanding microbial processes in aquatic ecosystems is of paramount importance due to their significant impact on the environment. Microbes can exist in two forms: planktonic, where they float freely in the water, and sessile, where they attach to solid surfaces. The latter has garnered considerable interest in the scientific community due to their ubiquitous nature. These microbial communities are known by various names, including *aufwuchs* (German for 'growth upon'), periphyton, epiphyton, or simply "biofilms" (Balareddy et al., 2002). Biofilms are complex assemblages of microbes, such as bacteria, algae, protozoa, and other microorganisms, embedded within an organic polymer matrix synthesized by the bacteria attached to surfaces submerged in the aquatic environment. In natural environments, the development of biofilms typically begins with primary colonization by bacteria, followed by the formation of diatom and other microorganism communities (Allison & Gilberg, 1992). Biofilms are a vital component of aquatic ecosystems, contributing to energy and organic matter supply to the food chain, recycling organic matter, and improving water quality. However, they can also have detrimental effects, particularly in man-made environments such as water distribution systems, where they can cause bio-fouling and lead to decreased water quality and pipe blockages. Recent studies have shown that biofilm formation on microplastics (MPs) is particularly affected by organic content in the water, salinity, and dissolved oxygen content. MPs are a growing concern in aquatic ecosystems, and understanding the impact of biofilms on MPs is crucial for managing these environments (Tu et al., 2021). Further research is needed to advance our understanding of complex, environmental, real-world biofilms and their interactions with different microorganisms within marine biofilms (Rummel et al., 2017).

## WHY BIOFILMS IS ESSENTIAL TO STUDY?

Over the past three decades, there has been a step-up of interest in understanding the complex dynamics of biofilms, particularly those found in aquatic environments. Researchers continue striving to manipulate biofilm processes to leverage them for beneficial purposes, including technological advances and ecological improvements. Recently, a meta-analysis by Simões et al. (2021) reported that global investments in biofilm research reached \$1.6 billion annually, highlighting growing recognition of the significance of these ubiquitous communities. There are situations where encouraging biofilm formation proves advantageous; for instance, they serve as sustenance for aquatic organisms like fish, facilitate wastewater treatment using biofilters, employ heat- or formalin-preserved bacterial biofilms as potent fish vaccines instead of single-cell forms, and modifying microbial relationships in natural habitats to generate efficient consortia. In line with these observations, the World Health Organization (2019) estimates that over 1 million tons of untreated human sewage enters freshwater sources daily, underscoring the potential utility of engineered biofilms in treating waste streams before discharge into vulnerable bodies of water. Conversely, cut back biofilm proliferation becomes necessary in circumstances adversely affected by their presence, such as reduced functionality of ship hulls and widespread mortality among fish and shellfish populations. An analysis conducted by Schultz et al. (2020) suggested that bio-fouling causes annual economic losses exceeding \$150 billion worldwide, demonstrating the urgent demand for effective anti-bio-fouling strategies. Additionally, biofilms provide exceptional environments for exchanging genetic material amongst diverse microbial communities. Such cross-species interaction bolsters microbial robustness and versatility, adding layers of complexity in managing biofilms. Recent findings by Wolska et al. (2019) emphasized the pivotal influence of horizontal gene transfer in molding microbial community composition, which contributes substantially to swift adaptation and evolution. Hence, acknowledging both favourable and detrimental aspects of biofilms allows professionals to tailor bespoke intervention strategies targeting particular problems related to biofilm activity. Understanding the multifarious nature of biofilms enables us to appreciate how they play indispensable roles in many domains, ranging from supporting aquatic life chains to advancing medical treatments. Continued investigation remains imperative to reveal novel insights capable of guiding informed decision-making regarding appropriate utilization and mitigation measures concerning biofilms.

## BIOFILMS FORMATION

The process involving microbial adhesion to surfaces in aquatic environments is highly intricate, contingent upon both abiotic and biotic factors. Abiotic factors consist of nutrient concentrations in water, surface charge, pH, temperature, electrolyte content, and material flux. Balareddy et al. (2002) noted that nutrient-deficient conditions tend to favour attachment to a surface as an adaptive strategy. Substrate features, such as surface charge and roughness, determine the initial phase of attachment. Common substrates include animal tissue/organs, aquatic plants, organic debris, and artificial structures. Biotic factors span numerous micro and macro-organisms and their interactions, including predation, commensalism, and mutualism. Initiation of attachment entails forming a 'conditioning film' on the substrate surface, attracting organic macromolecules and small hydrophobic particles from the surrounding water (Balareddy et al., 2002). Adsorbed organic matter offers potential energy sources for bacteria settling on the surface, influencing the physiological ecology of attached microbes. Chemooorganotrophs dominate the initial colonization stages. After just a few hours of submersion, bacteria adhere to the surface via extracellular polymer secretions. Primary colonizers subsequently modify substrate properties, enabling secondary microbe colonization. Rod-shaped bacteria primarily populate the earliest layer in seawater-immersed substrates, succeeded by additional genera like *Caulobacter*, *Hyphomicrobium*, and *Saprospira*. Specific flow rates, trophic status, and microbial physiologies dictate the identity of initial colonizers on newly emerged solid substrata. Initial colonizers can either stay immobile or reproduce to augment biomass and thicken biofilms, creating lower anoxic zones. Some biofilm bacteria synthesize various macromolecules and exhibit altered cell morphology. Exemplified by *Pseudomonas* spp. in turbulent flows, filamentous structures emerge. Interactions between diverse microorganisms differ considerably in biofilms versus natural free-living communities owing to increased population density and extended cell residency times. Certain strains of *Bacillus* sp. and *Pseudomonas* sp. release substances impeding specific cyanobacteria. Biofilm establishment leads to algae growth and heightened protist predation within the microbial community. Higher population densities, intensified metabolic activity, and sophisticated interactions between microbes render biofilms a dynamic microenvironment displaying complex organizational patterns under native conditions.

## Significance of biofilms

The real significance of bacterial biofilms has gradually emerged since their first description and recognition, because of their potential benefits and detrimental effects.

### Beneficial effects

The role of microorganisms as food for aquatic organisms needs no further emphasis. It is very well recorded that the particle-bound sessile bacterial aggregates in the form of biofilms in association with algal communities are preferred source of food compared to their planktonic free living counterpart by zooplankton and fish such as carps, tilapia, milkfish and mullets (Azim et al., 2001). This is because of their easy exploitation and high nutritive value. The biofilm cell density is generally 100-1000 times greater than the free living cell population in the aquatic environment. Recent studies indicate that the use of agricultural products (sugar cane bagases, paddy straw, etc.) and aquatic plants in aquaculture ponds enhance food production through biofilm development, which is considered to be one of the cheapest ways of enhancing food in the pond ecosystem for planktivorous fishes.

Increased concern over water quality management and limited availability of good water quality water for aquaculture have led the treatment of used and waste waters by bio-filtration systems. The biofilms of *Nitrosomonas* and *Nitrobacter* are important in metabolising the nitrogenous metabolites and toxic substances into simpler nutrient and non-toxic components. The negative charge of organic polymer matrix of biofilms traps the organic molecules and particles circulating in the vicinity, thereby adding efficient oxidation of metabolites by biofilm bacteria. The principle is being better exploited in biofilters, bioreactors, biodrums and bioreefs, where the biofilm bacteria are developed on suitable solid surfaces. Many organic pollutants, especially synthetic surfactants, absorbed onto available solid surfaces in natural and engineered environments. The biofilms consortia on surfaces contribute to heterotrophic activity and biodegradation at a much faster rate in comparison to free planktonic cells of the same

species, which are slow or often fail to degrade. This is because the biofilm maintain a large surface area, genetic diversity and metabolic versatility. This has led to bioremediation, bio-augmentation and biodegradation process manipulation in the aquatic environment.

The biofilm of pathogenic bacteria is a promising area of application in the field of vaccination (Balareddy et al., 2002). Because of their ability to resist digestive process in the gut, the biofilms provide an excellent mode of antigen delivery to antigen to certain specific sites in the gut. Encouraging result has been achieved by immunising Indian Major Carps through heat activated biofilm cells of *Aeromonas hydrophilla*. The immunised specimen showed increase antibody titres compared to controls which were immunised with heat killed free living planktonic cells.

The general material exchange between free living bacteria in aquatic habitats is normally limited by their low population densities. Subsequently higher population densities of biofilms combined with greater physiological activities where nutrient are continuously replenished in flowing systems, mainly lead to greater opportunities in various forms of genetic material exchange within the populations of the biofilm. This help in designing many ecological and technological processes. However, extensive studies are necessary to comprehend genetic processes within the biofilm environment.

### **Detrimental effects**

Aquaculture systems encounter several challenges stemming from biofilms, notably those containing pathogenic microorganisms. Problematic species inhabit solid surfaces, such as container walls, internal sections of water inlets and outlets, aerator components, and other accessible spaces in the water column. These biofilm cells can detach, becoming planktonic cells able to propagate quickly under optimal nutrient conditions, potentially inducing acute infections in fish and shellfish stocks (Lehtola et al., 2006). Eliminating these tenacious biofilm bacteria is challenging since they demonstrate remarkable resistance to typical biocidal agents, rendering conventional control measures insufficient (Pickett et al., 2019). For instance, *Vibrio harveyi* is a prominent culprit behind severe mortality episodes in shrimp hatcheries globally. Despite rigorous application of prophylactic measures, outbreaks still occur, attributable to the species' innate ability to persist as part of multi-species biofilms (Tendencia et al., 2020). Other notable species of concern include *Streptococcus agalactiae*, *Edwardsiella tarda*, *Tenacibaculum maritimum*, and *Photobacterium damsela* sub sp. *damsela*, each presenting varying degrees of threat depending on locality and host susceptibility (Toranzo et al., 2005; Austin & Austin, 2016). Parallel threats originate from other biological entities besides bacteria. Microbial colonization includes fungi, filamentous blue-green algae, and protozoans such as *Vorticella* spp. These invasive organisms afflict eggs of various aquatic taxa, including fish and crustaceans, thereby reducing hatching percentages and occasionally precipitating massive mortality events within hatcheries (Alippi & Romei, 2007). Susceptible species extend beyond finfish, ensnaring decapod crustaceans like lobsters, shrimps, and crabs, which suffer similarly debilitating fates linked to bacterial and filamentous algal colonizations (Ottah & Hepburn, 2000). Beyond threatening farmed organisms, biofilms established by bacteria and fungi disrupt water circulation infrastructure, hampering requisite flow rates in hatcheries. Metabolic by products generated by biofilm constituents increase the total suspended solids burden and impart disagreeable odors to the recirculating water (Chung et al., 2008). Disrupted water chemistry carries implications extending beyond physical parameters, as documented shifts in nitrogen cycling kinetics suggest potential ripple effects on overall system stability and sustainability (Defoirdt et al., 2011). Addressing these pervasive issues requires contemporary facilities to implement stringent sanitary regimes alongside integration of cutting-edge technologies designed explicitly to counter microbial accretion, thereby safeguarding productive and lucrative aquaculture ventures.

### **MEASURES TO REDUCE THE PATHOGENIC BIOFILMS**

Eradicating biofilms from surfaces presents a major hurdle in maintaining cleanliness and hygiene, given the protective glycocalyx covering that renders the films immune to customary disinfection and sterilization processes (Marshall et al., 2012). Regrettably, rising thickness and age of biofilms fortify their defence mechanisms, reinforcing resistance to chemical assaults (Costerton et al., 1999). Mounting evidence accentuates the urgency to circumvent biofilm formation altogether, as opposed to grappling

with its ramifications post-formation. Previously mentioned tactics, namely disinfecting surfaces exposed to probable biofilm formation followed by lengthy sun exposure, and applying chlorinated alkaline detergents to dislocate embedded cells, stand instrumental in mitigating biofilm prevalence (Shukla et al., 2019; Niveditha & Babu, 2016). Nevertheless, supplementary preventative measures warrant exploration, chiefly in light of surging concerns revolving around infectious diseases and antibiotic resistance. One compelling avenue gaining momentum entails crafting surfaces with topographies specifically configured to suppress biofilm initiation, representing a pioneering stride forward in tackling biofilm interference (Thavasi et al., 2014). Novel manufacturing techniques spawning nanostructured materials inherently repelling biofilm settlement manifest great promise (Sukenik et al., 2018). Such innovations integrate seamlessly with existing disinfection protocols, propelling the collective quest for streamlined biofilm governance in aquatic spheres. Recent breakthroughs shed light on exploiting quorum quenching - the disruption of bacterial communication channels - to neutralize biofilm viability (Brackman et al., 2011). Enzymes such as aceSA, lactonase, and oxidoreductase cleave signaling molecules integral to bacterial cooperation, effectively annihilating their social hierarchy and averting biofilm consolidation (Gruber et al., 2014). Similarly, phage therapy introduces viruses specialized in destroying specific bacterial targets, complementing conventional antibiotics without eliciting resistance (Lu & Collins, 2007). Combining multiple strategies promises synergistic action, maximizing therapeutic potential vis-à-vis singular modalities. Expounding on the significance of photodynamic therapies, visible light illuminates porphyrins deposited on bacterial membranes, prompting the release of destructive singlet oxygen radicals (Qiu et al., 2016). Alternatively, metal-based nanoparticles furnishing silver, zinc, titanium dioxide, gold, or copper exact lethal damage on biofilms, capitalizing on catalytic redox reactions generating copious reactive oxygen species (ROS) (Hasan et al., 2019). Both approaches portray minimal risks for humans, livestock, and crops, positioning themselves as auspicious alternatives to traditionally employed chemotherapeutic agents. All things considered, bridging the divide between empirical biofilm removal techniques and avant-garde surface modifications holds tremendous sway in spearheading improved biofilm administration within aquatic environments (Song et al., 2018). Given the plethora of ground breaking discoveries unfurled herein, anticipation runs rife regarding forthcoming progress charting new territories in biofilm regulation.

## PROSPECTS

As we look ahead, the prospect of unlocking the full potential of biofilms in the aquatic environment appears incredibly promising. While biofilm formation is indeed a survival tactic utilized by microorganisms in response to fluctuating environmental conditions, it is crucial to recognize their broader implications and possibilities. Although acknowledged merely as a hassle in certain aquaculture scenarios, biofilms possess untapped value and applicability in various fields.

Future endeavours ought to focus on unravelling the intricate microenvironment within natural aquatic biofilms communities. Expanded comprehension of their ecology, physiology, and genetic makeup will lay the foundation for leveraging biofilms across a spectrum of industries. Several key areas deserve special emphasis moving forward:

**Genetic heterogeneity:** Uncovering the vast array of microorganisms dwelling within biofilms necessitates exhaustive identification and classification. Genomic sequencing and metagenomics analyses will aid in pinpointing novel species, unravelling symbiotic relations, and exposing unique genetic traits.

**Environmental monitoring:** Developing sensitive and selective biosensor platforms utilizing biofilms could revolutionize aquatic surveillance programs. Monitoring pollution levels, tracking invasive species, and detecting trace chemicals would greatly benefit from such innovations.

**Bio-control agents:** Exploration of biofilms as natural barriers against pathogens bears enormous potential. Identification of defensive compounds and underlying mechanisms can inspire novel therapeutic strategies to manage aquatic diseases.

**Resource recovery:** Harvesting valuable commodities from biofilms, such as pigments, enzymes, and pharmaceuticals, exemplifies the latent economic worth of these microbial clusters. Optimization of culturing conditions and downstream processing methods is essential to drive commercialization.

**Climate change mitigation:** Capitalizing on biofilm capacity to assimilate carbon and cycle nutrients opens doors for developing climate-resilient aquatic systems. Manipulating microbial compositions and habitat configurations can amplify these benefits, fostering greener and more sustainable practices.

Ultimately, realizing the future prospects of biofilms demands interdisciplinary collaborations blending fields such as microbiology, ecology, genomics, analytical chemistry, and environmental engineering. Through concerted efforts, we can translate basic scientific discoveries into tangible societal gains, brightening the horizon for biofilm research and its applications.

## CHALLENGES

Biofilms, omnipresent in aquatic environments, exhibit both beneficial and detrimental influences, giving rise to a series of challenges. Serving as reservoirs of microbial diversity, they participate in fundamental ecological processes, such as biogeochemical cycles, pollutant degradation, and resource capture (Azevedo et al., 2008). Despite these advantages, biofilms pose substantial difficulties, mainly attributed to their role in mediating the transmission of pathogens and perpetuating chronic infections (Parsek & Singh, 2003). Controlling biofilm dissemination and minimizing associated hazards require dedicated research initiatives. Key challenges confronting biofilm investigations encompass.

**Complexity:** Deciphering biofilm architecture, organization, and functioning calls for sophisticated experimental models and computational simulations (Stewart, 2003). Capturing the essence of these complex systems remains an open pursuit for researchers.

**Variability:** Spatiotemporal fluctuations in biofilm composition and behaviour add layers of complexity, demanding elaborate sampling schemes and statistical frameworks (Xavier et al., 2005). Standardizing quantitative assessments of biofilm characteristics continues to vex investigators.

**Resistance:** Robust resistance profiles distinguish biofilms from their planktonic counterparts, limiting the efficacy of conventional antimicrobials (Hall & Mah, 2017). Designing novel therapeutic strategies to overcome this recalcitrance ranks among pressing priorities.

**Quorum sensing:** Coordinated behaviours driven by chemical communications within biofilms shape their responses to external cues (Atkinson & Williams, 2009). Counteracting such coordination necessitates detailed understanding of the regulatory circuits governing signal transduction.

**Dispersal:** Timely detection and prediction of biofilm dispersion events hold significant ramifications for public health and environmental safety (Harper & Camper, 2016). Devising reliable sensors responsive to subtle changes preceding detachment can inform timely intervention measures.

Collectively, these challenges call for integrated and cross-disciplinary approaches combining classical microbiology, molecular biology, biochemistry, physics, mathematics, and engineering to fully grasp biofilm phenomena and realize practical solutions. Addressing these obstacles stands to deliver wide-ranging benefits, translating theoretical insight into applied solutions, and fostering healthier, safer, and more sustainable aquatic systems.

## CONCLUSION

Biofilms occupy a fascinating yet intricate niche within aquatic environments, representing the outcome of microorganisms' physiological adaptations to changing environmental conditions. Historically, biofilms remained overlooked, overshadowed by their planktonic counterparts. Yet, recent explorations have begun unearthing their profound significance and vast potential applications. Despite these revelations, much uncertainty clouds the complex microenvironment nested within natural aquatic biofilms communities. Further examination of their ecology, physiology, and genetic diversity remains an absolute necessity. Delving deeper into these facets of biofilm biology will undoubtedly open unprecedented

opportunities for broadening their reach and utility in various fields. However, biofilms have simultaneously demonstrated the potential to wreak havoc, particularly in aquaculture settings, mandating diligent monitoring and vigilant management. Mitigating the negative consequences of biofilm growth, whilst preserving their positive contributions, necessitates careful navigation of competing interests. Looking ahead, the successful resolution of lingering challenges associated with biofilm research will likely rely on interdisciplinary collaboration, innovation, and commitment. Embracing convergence across seemingly disparate disciplines, such as microbiology, ecology, genomics, and engineering, promises to accelerate discovery and hasten translation from benchside to bedside. Ultimately, mastering biofilm complexities will allow society to tap previously untenable resources, improve aquatic environments, secure healthy food supplies, and strengthen global health security. Armed with a clear vision of the tantalizing rewards waiting just beyond today's horizons, let us press forward boldly and confidently, united in our mission to demystify and harness the power contained within these incredible microbial marvels called biofilms.

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