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Bacterial biofilms: Structure, composition and significant role in aquaculture

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Abstract

Bacterial biofilms are complex communities of microorganisms that adhere to biotic and abiotic surfaces and are embedded in a self-produced extracellular polymeric matrix. The formation of a biofilm matrix is a multi-step process, and the compositions of its matrix depends on the bacterial species. These constituents include extracellular DNA, proteins, lipids, enzymes, adhesion molecules, and exopolysaccharides. Biofilms can facilitate the transfer of genes conferring resistance, enhance survival, increase resistance to antimicrobial agents, and prevent desiccation. Bacterial biofilms play a significant role in nutrient recycling, bioremediation, improved water quality, and the abundance of microbial communities in biofilm-based intensive aquaculture systems. Biofilms are rich in single-cell proteins, which can be very easily harvested and digested by aquaculture species as a natural feed source. However, biofilm-forming pathogenic bacteria, such as *Vibrio* spp., exhibit increased antibiotic resistance posing a threat to shrimp aquaculture. This chapter highlights the concept of biofilms, their development, structure and composition, properties, significant beneficial role, adverse effects, management and control strategies, and the potential future economic importance of bacterial biofilms.

Keywords: Biofilm, Exopolysaccharide, Virulence gene, antimicrobial resistance, Probiotics, Shrimp aquaculture

Highlights

- Matrixome refers to the complete set of biomolecules (polysaccharides, proteins, nucleic acids, lipids, lipoproteins) that drive biofilm structure, function, and virulence.
- The biochemical and structural traits of the matrixome determine key biofilm properties such as adhesion, spatial/chemical heterogeneity, microbial interactions, antimicrobial resistance, and virulence.
- Biofilms contain high levels of single-cell proteins, making them a natural nutrient source for aquatic organisms.

Introduction

The rapidly growing shrimp aquaculture industry faces an increased risk of pathogenic bacterial infections associated with biofilm in shrimp hatcheries and grow-out farms. Pathogenic bacteria can form a biofilm matrix, protecting them from antimicrobial compounds, developing antimicrobial resistance, or spreading AMR-related diseases. Strain variants, and environmental stress factors influence the biofilm development. In general, biofilms form on the interfaces such as filter systems, tanks, ponds and coarse surfaces, and their presence is associated with recurring disease outbreaks in shrimp culture systems (Karunasagar et al., 1996; Arunkumar et al., 2020; Balducci et al., 2023).

The concept of Biofilm

A biofilm is formed when microbial cells attach to either living (biotic) or non-living (abiotic) surfaces and are surrounded by a matrix of extracellular polymeric substances (EPS), also referred to as a slime layer. This matrix is composed of various substances, such as polysaccharides, proteins, nucleic acids, lipids, and enzymes, which provide structural support and facilitate the adhesion of biofilm to surfaces. Factors like bacterial species, surface properties, and environmental conditions, including pH, nutrient levels, and temperature, influence the development of biofilms. Additionally, the structure of biofilm communities and their EPS matrix can shield bacteria from antimicrobial agents, enzyme degradation, physical forces, and the host's immune system (Karygianni et al., 2020).

Mechanism of biofilm formation

Biofilm formation is a common mode of bacterial growth that proceeds through five distinct stages (Figure1): 1) Initial attachment, 2) Monolayer/Matrix production, 3) Microcolony formation, 4) Maturation, 5) Dispersion (Karygianni et al., 2020).

1) Initial attachment: In the first step, the planktonic bacterial cell move toward the surface for attachment.
2) Monolayer / matrix production: In the second stage, the bacteria forms a monolayer and produce an extracellular matrix or "slime" providing protection.
3) Microcolony formation: In the third stage, bacteria multiply and form microcolonies, which exhibit significant growth and cell-cell communication, such as quorum sensing. The biofilm grows three-dimensionally, and the attachment becomes irreversible.
4) Maturation: In the fourth stage, biofilm starts to mature; it develops into an organised structure which can be flat or mushroom-shaped, depending on the nutrient availability.
5) Dispersion: Finally, during the fifth and lasssst stage, biofilm cells detach from the colony and transitorily return to a planktonic form, spreading out and initiate a new cycle of biofilm formation.

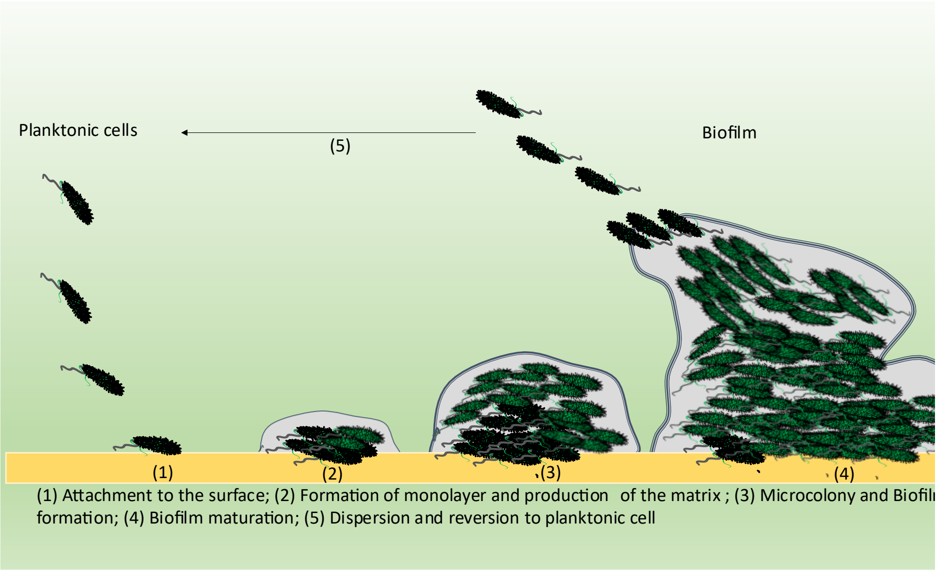

Fig 1. Illustration of the mechanism of bacterial biofilm development stages

Biofilm structure and composition

Biofilms primarily comprises of microbial cells (10%) and EPS (90%). Many bacterial EPS contain backbone structures with 1,3- or 1,4-β-linked hexose residues. The EPS of gram-negative bacteria has polyanionic properties due to uronic acids (such as D-glucuronic, D-galacturonic, and mannuronic acids) or ketal-linked pyruvates. On the other hand, in Gram-positive bacteria, the chemical composition of EPS may be quite different and cationic. EPS may be associated with metal ions, divalent cations, and other macromolecules (such as proteins, DNA, lipids, and even humic substances). The production of EPS can be influenced by the nutrient status of the growth medium; particularly an excess of available carbon with limitation of nitrogen, potassium, or phosphate can promote EPS synthesis (Karygianni et al., 2020) (Figure 2 and Table 1).

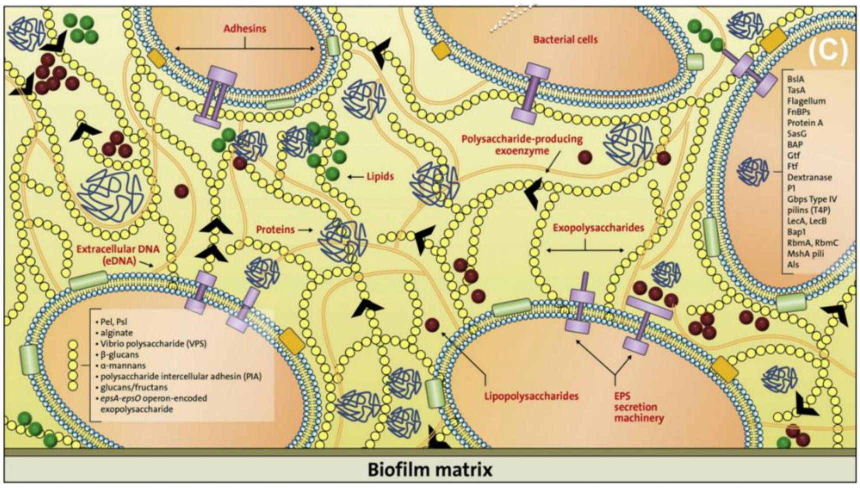

Fig 2. Composition biofilm matrix in structured microbial communities (Source: Karygianni et al., 2020)

Table 1. Composition and Functions of Extracellular Polymeric Substances (EPS) in Biofilms

Major EPS Class	Microorganism	Composition	Location	Functions
Polysaccharides	*Bacillus subtilis*	epsA-epsO operon-encoded exopolysaccharide,	Extracellular	Adhesion, scaffolding, stability, sorption, nutrient
		γ-PGA (poly-γ-glutamate)	Extracellular	Adhesion, scaffolding, sorption, nutrient
	Vibrio sp.	Vibrio polysaccharide (VPS)	Extracellular/cell-associated	Adhesion, cohesion, scaffolding, stability
		β-glucans, α-mannans	Extracellular Cell wall	Forming mannan-glucan complex (MGCx), scaffolding, protection, antifungal resistance, bacterial–fungal interaction
	Pseudomonas sp.	Psl	Extracellular/cell-associated	Adhesion, scaffolding, stability, protection against immune response, cell-to-cell binding
		Pel	Extracellular/cell-	Adhesion,

			associated	scaffolding, stability, cell-to-cell binding, protection against antibiotics
		Alginate	Extracellular	Adhesion, scaffolding, water/nutrient retention, protection against harsh environments/immune response/antimicrobials, stability
	Enterobacteria	Polysaccharide intercellular adhesin (PIA) or poly-β (1-6)-N-acetylglucosamine (PNAG)	Extracellular	Adhesion, cohesion, scaffolding, stability, protection against antibiotics
Protein	*Bacillus subtilis*	Biofilm surface layer protein (BslA)	Extracellular	Surface hydrophobicity, protection
		Translocation-dependent antimicrobial spore component (TasA)/TasA anchoring and	Extracellular Cell wall	Scaffolding, cell-to-cell binding

		assembly protein (TapA)		
		Flagellum	Cell-associated	Adhesion, motility, mechanosensing
	Vibrio sp.	Biofilm-associated protein (Bap1)	Cell-associated/ extracellular	Adhesion, scaffolding, hydrophobicity, stability, protection
		Rugosity and biofilm modulators (RbmA/RbmC)	Cell-associated/ extracellular	RbmA: cell-to-cell binding RbmC: scaffolding, stability
		Mannose-sensitive hemagglutinin (MSHA) pili	Cell-associated	Adhesion, motility, mechanosensing
		Flagellum	Cell-associated	Adhesion, motility, mechanosensing
	Pseudomonas sp.	Type IV pilins (T4P)	Cell-associated	Adhesion, scaffolding, twitching motility, mechanosensing
		Lectins (LecA/LecB)	Cell-associated/ extracellular	Adhesion, cell-to-cell binding, stability, cytotoxin
Nucleic acids	Wide distribution in bacteria, archaea, and fungi	eDNA	Extracellular	Scaffolding, adhesion, cohesion, nutrient source, DNA damage repair,

				gene transfer, interaction with other matrix components
Lipids	Bacillus	Teichoic and lipoteichoic acids	Cell-associated/extracellular	Adhesion, cohesion, protection, immune evasion
Lipopolysaccharides	Wide distribution in Gram-negative bacteria	LPS (endotoxin)	Cell-associated/ extracellular	Adhesion, colonization and host invasion, activation of immune response

The properties of biofilm

Biofilm exhibit several properties including surface adhesion, spatial and chemical heterogeneity, synergistic/competitive polymicrobial interactions, increased tolerance to antimicrobials, and virulence attributes (Karygianni, & Thurnheer, 2020)

Structural Properties

- Extracellular Polymeric Substance (EPS) Matrix: The EPS matrix, composed of polysaccharides, proteins, lipids, and extracellular DNA, provides structural stability and protection to the biofilm. It helps the cells adhere to surfaces and each other.
- Heterogeneous Structure: Biofilms have a heterogeneous structure with water channels that allow nutrient and waste transport. The architecture can vary depending on the microbial composition and environmental conditions.
- Surface Adherence: Biofilms can form on various surfaces, including metals, plastics, natural tissues, and environmental surfaces like rocks and aquatic sediments.

Microbial Community Properties

Biofilms typically consist of multiple microbial species, including bacteria, fungi, algae, and protozoa, creating a complex and dynamic community. Cells within a biofilm exhibit phenotypic diversity, meaning they can express different genes and exhibit different behaviours compared to their planktonic counterparts. This diversity enhances adaptability and resilience in fluctuating environmental conditions (Ortiz-Estrada et al., 2019).

Physiological Properties

- Enhanced Resistance: Biofilm-forming bacteria are significantly more resistant to antibiotics, disinfectants, and environmental stresses compared to planktonic bacteria. The EPS matrix limits the penetration of antimicrobial agents and protects the cells.
- Metabolic Cooperation: Microorganisms within biofilms engage in metabolic cooperation, where different species or cells utilise metabolic byproducts of others, enhancing overall survival and efficiency.
- Quorum Sensing: Bacteria in biofilm communicate through quorum sensing, a process where they release and detect signalling molecules to coordinate gene expression and behaviour in response to population density.

Functional Properties

- Nutrient Acquisition: Biofilms can efficiently acquire and utilise nutrients from their environment. The EPS matrix trap nutrients, while the heterogeneous structure creates nutrient gradients, optimising the metabolic activities of different cells.
- Waste Removal: The water channels within biofilms facilitate the removal of waste products, preventing toxic build up and maintaining a conducive environment for microbial growth.
- Environmental Adaptation: Biofilms can adapt to a wide range of environmental conditions, including extreme pH, temperature, and salinity. This adaptability is partly due to the protective EPS matrix and the genetic diversity within the biofilm.

Ecological Properties

- Habitat Creation: Biofilms create microhabitats that support diverse microbial communities, contributing to biodiversity. These microhabitats can protect sensitive species from harsh environmental conditions.
- Ecological Impact: Biofilms play crucial roles in natural ecosystems, including nutrient cycling, bioremediation, and forming the basis of food webs in aquatic environments. They can also influence the corrosion of metals and the material degradation in man-made environments.

The significant role in aquaculture

In aquaculture, biofilms play a crucial role in nutrient cycling, breaking down waste products, and reducing the need for water replacement, contributing to lower operational costs and improved productivity.

A. Beneficial Effects

Nutrient cycling

Biofilms are crucial in the nitrogen cycle, particularly in converting toxic ammonia excreted by shrimp into nitrite and subsequently into less harmful nitrate. This process is vital for maintaining water quality and preventing ammonia toxicity, which can be lethal to shrimp (Thompson et al., 2002).

Biofilter system

Biofilms are integral to the operation of biofilters in recirculating aquaculture systems (RAS). These biofilters harbor nitrifying bacteria that convert ammonia to nitrite and then to nitrate, crucial for maintaining safe water conditions (Pandey & Kumar, 2022).

Biodiversity support

Biofilms provide habitat and food for a variety of microorganisms and small invertebrates, supporting microbial and ecological biodiversity. This contributes to the resilience and productivity of aquaculture systems (Ortiz-Estrada et al., 2019).

Bioremediation

Biofilms help to decompose organic matter, including uneaten feed and shrimp waste, reducing the build-up of harmful substances in the water thereby maintaining a cleaner and more stable aquatic environment. These bioremediation efforts to clean up pollutants in aquaculture systems, including heavy metals and other contaminants helping to maintain a healthier culture environment (Pandey & Kumar, 2022).

Probiotic system

Probiotic bacteria have emerged as a valuable tool in sustainable aquaculture. Lactic acid bacteria (LAB) are Gram-positive, catalase-negative, non-spore-forming rods or cocci microorganisms that produce lactic acid as a major metabolic end-product of carbohydrate fermentation.

The principal mechanisms of probiotic action include 1) inhibiting enteric pathogens by producing lactic acid, hydrogen peroxide, and bacteriocins 2) competitive exclusion of enteric pathogens by blocking adhesion sites, 3) competition for nutrients, and 4) modulation of the host immune system. The genera Lactobacillus and Bifidobacterium have emerged as LAB's most commonly used probiotic strains.

Probiotic bacteria such as *Lactobacillus* spp. (*Lactobacillus fermentum*, *L. rhamnosus*, *L. plantarum*, *L. delbrueckii*, *L. reuteri*, *L. acidophilus)*, *Lactiplantibacillus plantarum* and *Bacillus subtilis* can form biofilms, which enhance colonisation and longer permanence in the host mucosa, preventing pathogenic bacteria from colonising. The EPS of these bacteria containing proteins and lipoteichoic acid, are involved in cell adhesion on host epithelial surfaces promoting biofilm formation. This adherence provide an antagonistic effect against intestinal pathogens by producing antimicrobial substances such as bacteriocins, biosurfactants, and H_2O_2. Mature probiotic biofilms exhibit excellent antibacterial activity and higher tolerance to gastric pH than a newly formed biofilm. For example, *B. subtilis* is a non-pathogenic bacterium that forms spores and exhibits efficient probiotic properties. It can help maintain a beneficial microflora balance in the gastrointestinal tract (GIT). Moreover, *B. subtilis* generates an extracellular matrix that shields it from harsh environments. Recent studies have demonstrated that super-intensive indoor shrimp culture system utilize heterotrophic and probiotic-based biofilms to regulate nitrogen metabolism including dissimilatory nitrate reduction, assimilatory nitrate reduction, denitrification, nitrification, nitrogen fixation, and anaerobic ammonia oxidation (anammox). Administering inactivated bacterial biofilm (*V. harveyi*) orally can serve as a novel immunostimulant to enhance shrimp's growth perfomance, survival, and health. Similarly, adding inactivated *V. alginolyticus* biofilms on chitin flakes as dietary supplementation demonstrated antimicrobial effects, increased phenoloxidase activity, and elevated total haemocyte count, boosting immunity in shrimp (Vinay et al., 2019; Pandey & Kumar, 2022).

B. Detrimental effects:

Pathogen virulence/disease transmission

Biofilms associated with many pathogenic bacteria are a significant contributor to virulence mechanisms in aquaculture systems. Overproduction of the matrix polysaccharide, such as, alginate leads to the formation of a mucoid biofilm. The matrix components including surface proteins, amyloid curli, eDNA, O-antigen, and extracellular enzymes, such as proteases lipase, esterase, DNAse, RNAse, and fibrinolysin, are recognised as potential virulence factors. Biofilm can restrict penetration of antimicrobials, disinfectants, and immune effectors, resist phagocytosis, and facilitate infection in the host niches. Biofilm-forming pathogens colonising in host tissues are can be 100-1000 times more resistant to antibiotics and disinfectants than their planktonic counterpart increasing the development of antimicrobial resistance to antimicrobial agents reducing its efficiency.

In aquaculture, studies show that pathogens like *Vibrio anguillarum, V. parahaemolyticus, V. alginolyticus, V. harveyi, V. campbellii, V. fischeri, Aeromonas hydrophila, A. salmonicida, Yersinia ruckeri, Flavobacterium columnare, F. psychrophilum, Piscirickettsia salmonis, Edwardsiella tarda, E. ictaluri, E. piscicida, Streptococcus parauberis,* and *S. iniae* can survive in the environment by transforming their planktonic form to biofilm form. Pathogenic *Vibrio* and *Pseudomonas* can form biofilms in shrimp culture systems, enabling their survival in the gastrointestinal tract and hepatopancreas causing persistent infections in shrimp (Karunasagar et al., 1996; Otta et al., 2001; Balducci et al., 2023).

Biofouling

Excessive biofilm growth can lead to biofouling, where biofilms accumulate on surfaces such as tank walls, piping, and filtration systems. This accumulation can impede water flow, reduce aeration efficiency, and clog filters, leading to increased maintenance costs and operational challenges.

Variable in biofilm composition

The composition of biofilms can vary widely, leading to unpredictable effects on shrimp health and water quality. Changing environmental conditions, such as temperature, salinity, and nutrient levels, can alter biofilm communities, sometimes shifting the balance from beneficial to harmful effects.

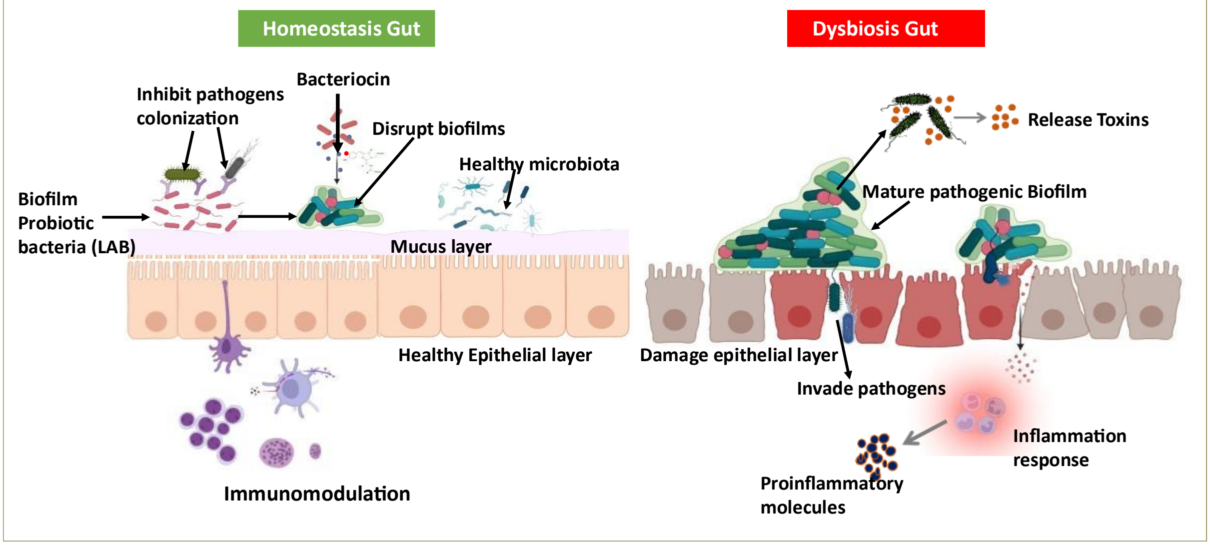

Fig 3. The action of microbial biofilm on the gut epithelial barrier (designed in BioRender.com).

Management Strategies

Bacterial biofilms play a significant role in aquaculture, influencing both production efficiency and disease management. Biofilms can enhance fish growth and health by serving as a protein source and habitat, while also aiding in waste nutrient conversion (Pandey & Kumar, 2024). However, they can also harbor antibiotic-resistant bacteria, posing risks to both aquaculture and food safety (Haque et al., 2024). The management of biofilms through innovative techniques, such as quorum-sensing inhibition, has emerged as a promising strategy to mitigate these risks (Monzón-Atienza et al., 2024).

Anti-biofilm strategies can be broadly categorized into physical, chemical and biological treatments. : A) Physical treatment- Regular removal of biofilms from surfaces can help prevent biofouling and the buildup of harmful bacteria. Example: Electrical, Ultrasound, Radiation treatment. B) Chemical treatment- Using disinfectants and biocides selectively can help control biofilm formation. Example: Surface coating, Disinfectant, locking solution, Chelating agent, etc., C) Biological treatment -Introducing specific strains of beneficial bacteria can help establish biofilms that support shrimp health and suppress growth of pathogens. Example: probiotics, phage therapy, bioenzymes (Karygianni et al., 2020; Zhao et al., 2023).

Two major approaches to biofilm management (Figure 4):

I. Prevent biofilm formation at early stages: This approach targets the initial stages of biofilm formation by, inhibition of c-di-GMP / c-di-AMP signalling (which regulates biofilm initiation), blocking bacterial adhesion to the surface; inhibition of exopolysaccharide-producing exoenzymes (which reduce matrix formation); use of small molecules like L-arginine, alkali (to interfere with early attachment and signalling).

II. Disruption of established biofilms at the mature stage: This approach focuses on, Targeting the biofilm matrix, including polysaccharides, proteins, and extracellular DNA (eDNA); Enzymatic degradation using protease, polysaccharide hydrolases, DNases and application of nanoparticles (NPs) to penetrate and destabilise the matrix; physical disruption by applications of photodynamic/ photothermal, ultrasound or acoustic shockwaves, and high velocity sprays and jet irrigation.

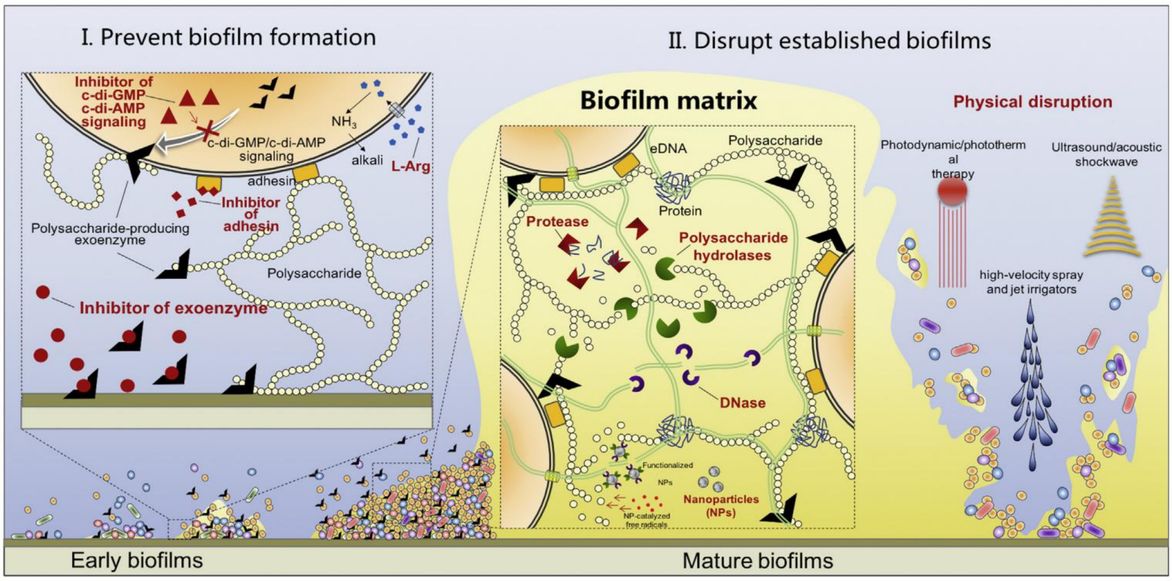

Fig 4. *The management of biofilms through innovative techniques (Source: Karygianni et al., 2020)*

Monitoring and Adjustment

a) Regular Monitoring: Continuous monitoring of water quality and biofilm composition can help early detection of potential issues allowing timely interventions.

b) Environmental Management: Adjusting environmental parameters, such as flow rates and nutrient levels, can effectively manage biofilm development and help maintain a balanced microbial community.

Economic importance of biofilms

The economic importance of bacterial biofilms is multifaceted, affecting various industries positively and negatively.

- Improved water quality
- Bioremediation of wastewater treatment
- Improve soil ecosystem
- Positive role in microbial fermentation process
- Application as a natural biofilm fertiliser
- Act as a biofilm reactor in a bioprocess unit
- Biogas production
- Probiotic biofilms industry
- Applications in bioprocessing and environmental biotechnology
- Biofilter system and membrane-based bioreactors
- Vaccine development

Challenges

Although biofilms offer several benefits, they also have certain challenges. Biofilms can harbour antibiotic-resistant bacteria, complicating treatment options and increasing health risks. Biofilms formed by pathogens can cause persistent infections in the host and contaminate seafood products, leading to spoilage and health threats.

Conclusion

Biofilms are considered the most common form of microbial life. Microbial biofilm can have both beneficial and negative effects on the host. Biofilm is described as the most ubiquitous form of microbial life. Therefore, bacterial biofilms in shrimp aquaculture can significantly enhance system performance and shrimp health through improved water quality and disease control. However, they also pose challenges, such as biofouling and potential pathogen reservoirs. Effective management strategies are essential to harness the benefits while mitigating the risks associated with biofilms in shrimp aquaculture. Future research should focus on the dynamics of biofilm matrix formation, structure organisation, host-biofilm interactions, biofilm detection techniques, and new anti-biofilm strategies. In future, microbial biofilm-based super-intensive systems holds a potential for sustainable and eco-friendly aquaculture operations.

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