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BIOFILMS IN BIOENERGETICS AND ECOBIOTECHNOLOGY: RESEARCH HISTORY AND APPLICATION PERSPECTIVES

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The historical paradigm shift from studying planktonic bacteria to understanding the protective extracellular polymeric substance (EPS) matrix has fundamentally transformed environmental biotechnology. Once considered operational impediments, biofilms are now recognized as highly organized microbial consortia and essential tools for advanced wastewater treatment, bioremediation, and sustainable bioenergy production.

Aim. The study aimed to analyze the evolution of knowledge regarding biofilms - from early observations to their contemporary role in bioelectrochemical systems and nitrification - thereby outlining future perspectives for the application of microbial communities.

Materials and Methods. A comprehensive theoretical synthesis of contemporary literature was conducted, focusing on EPS matrix dynamics, spatial stratification in granular and membrane bioreactors, and direct interspecies electron transfer (DIET). The analysis encompasses structural data on methanogenic archaea, exoelectrogens (e.g., *Geobacter*), and rhizosphere biofilms within constructed wetland-microbial fuel cells (CW-MFCs).

Results. Biofilm engineering exploited spatial substrate gradients to facilitate syntrophic metabolism, significantly improving nitrogen removal via ANAMMOX processes and maximizing methane yields. The EPS matrix functions both as a chemical buffer against toxic stress and a conductive scaffold. In bioelectrochemical systems, *c*-type cytochromes and flavins facilitate efficient long-range electron transfer. Furthermore, biofilm-mediated horizontal gene transfer and quorum-sensing manipulation enhance the biodegradation of recalcitrant xenobiotics, heavy metal biomineralization, and plastic depolymerization.

Conclusions. Transitioning to the targeted engineering of biofilm architecture using synthetic ecology principles significantly intensified environmental resource recovery. Managing the EPS matrix transforms conventional biological treatment into robust, self-regulating biotechnological systems capable of continuous, sustainable energy generation under fluctuating environmental conditions.

Key words: biofilms, biotechnology, microorganisms, bioremediation, bioenergy, quorum sensing, wastewater treatment.

Biofilms are dynamic microbial communities comprising associations of microorganisms (specifically bacteria, archaea, microalgae, and fungi) attached to a surface and embedded within a self-produced matrix of extracellular polymeric substances (EPS). Processes within biofilms are of profound interest from the perspectives of evolutionary biology, microbiology, medicine, and biotechnology. From an evolutionary standpoint regarding the development of biological entities, biofilms played a critical role by providing early microorganisms with protection against the hostile conditions of the primordial Earth, thereby establishing ecological niches that facilitated the emergence of complex multicellular forms. This evolutionary trajectory is supported by

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biofilm formation in archaea, particularly methanogens, which represent more ancient microbial lineages. Within these microbial consortia, key evolutionary innovations occurred — such as intercellular communication (*quorum sensing*), signaling molecule networks, and horizontal gene transfer — which significantly accelerated bacterial adaptation to environmental stress factors [1].

From a medical perspective, biofilms are a primary driver of resistance to antimicrobial therapy, as their structured extracellular matrix establishes a physical barrier that substantially restricts the penetration of therapeutic agents into target pathogenic cells. Chronic infections associated with biofilms necessitate the development of novel diagnostic and therapeutic strategies that focus not only on inhibiting bacterial proliferation but also on disrupting the structural integrity of this protective microbial architecture [2].

In natural ecosystems, biofilms represent a fundamental mode of microbial existence, ensuring the stability of biogeochemical cycles by effectively retaining metabolically active communities. Due to the unique architecture of the extracellular matrix, these microbial structures enable organisms to survive and function under persistent environmental stresses, such as fluctuations in moisture, nutrient availability, or temperature. Consequently, the study of natural biofilms is key to understanding global biotransformation processes that underpin the self-purification of water bodies and the restoration of soil fertility; thus, these mechanisms can be implemented in bioenergetics and environmental biotechnology, specifically in wastewater treatment and bioremediation [3].

Biofilms also form on various natural and anthropogenic objects; in this context, microbiologically induced corrosion is a critical industrial challenge, driven by biofilms' ability to generate localized electrochemical zones with pH and oxygen concentration gradients on metallic surfaces [4, 5].

The historical aspect of biofilm research is critically important, as it facilitates a paradigm shift from viewing microorganisms as isolated entities to understanding them as integrated, highly efficient engineering systems. Analyzing the historical trajectory from the foundational discoveries of S. Winogradsky to contemporary metagenomics provides a methodological framework for optimizing bioconversion processes, thereby helping to circumvent the inefficient approaches of the planktonic paradigm. Examining past successes and shortcomings in scaling up biofilm reactors enables biotechnologists to implement cutting-edge solutions more rapidly, minimizing temporal and economic costs during the stabilization of industrial systems. Furthermore, understanding the natural evolution of these communities establishes a foundation for designing consortia capable of self-regulation and adaptation under stressful industrial workloads, specifically fluctuating wastewater compositions, methanogen adaptation, and the formation of pollutant-resistant biofilms for remediation. Consequently, the history of research serves as a foundation for transitioning toward the sustainable development of environmental biotechnology, in which biofilms become an indispensable component of circular-economy technologies.

In contemporary biotechnology, biofilms are considered among the most efficient and adaptive modes of microbial organization. For most of the 20th century, microbiological research primarily focused on free-living (planktonic) forms of microorganisms, leading to the development of foundational theories that, however, proved limited when scaled up to industrial conditions. In recent decades, the scientific paradigm has undergone a substantial shift: biofilms are no longer perceived exclusively as a source of biofouling or pathogenic threats. Instead, they have been transformed into a powerful toolkit for environmental biotechnology and bioenergetics.

The relevance of this topic is driven by the imperative transition to a circular economy, in which waste is converted into energy resources. The use of microbial consortia immobilized within an extracellular polymeric matrix ensures the resilience of biotechnological processes to environmental fluctuations, toxic substances, and variations in pH or temperature.

This review aims to analyze the evolution of knowledge regarding biofilms — from early observations to their contemporary role in bioelectrochemical systems and nitrification thereby outlining future perspectives for the application of microbial communities.

Historical overview: from "animalcules" to fundamental laws

The history of biofilm research mirrors the broader development of microbiology. The earliest records of biofilms date back

to 1676, when Antonie van Leeuwenhoek (Fig. 1), utilizing a self-constructed microscope, first documented the presence of living microorganisms within human dental plaque, which he designated as *animalcules* (microscopic animals). Although Leeuwenhoek lacked the terminological framework to define the concept of a biofilm, he effectively became the first to visualize microbial adhesion to biological tissue surfaces and the subsequent formation of complex architectural structures. His detailed descriptions of these "animalcules" and their capacity for surface adherence laid the empirical foundation for future microbiology, even though the scientific paradigm of that era was not yet prepared to recognize the social nature of the microbial world. Leeuwenhoek's observations constituted the first historical scientific evidence that bacteria are capable of forming structured plaques, which are recognized today as biofilms [6].

However, the prolonged dominance of Robert Koch's postulates, which aimed to isolate pure cultures, established a methodological barrier that long impeded understanding of complex polymicrobial communities. Contemporary research demonstrates that the high resilience of biofilms to external factors is driven by phenotypic adaptation and the architecture of the extracellular matrix, rather than by genetic mutations alone. Recognizing this distinction became a pivotal step, enabling biotechnology to transition from combating biofilms to actively utilizing them as functional engineering systems [7].

The Ukrainian scientist Sergei Winogradsky made a substantial contribution to the understanding of biological processes at the end of the 19th century, specifically through his groundbreaking insights into nitrification processes (1890–1891). Winogradsky did not merely demonstrate that the conversion of ammonia into nitrates is a biological rather than a chemical process; he discovered the phenomenon of chemolithoautotrophy. Winogradsky's research demonstrated that microorganisms (*Nitrosomonas* and *Nitrobacter*) can derive energy from the oxidation of inorganic compounds and establish stable communities. This discovery laid the foundation for understanding metabolic synergism. In nature, these bacteria never function in isolation but instead form spatial consortia in which the metabolites of one species serve as substrates for another. It is precisely this spatial organization, initially described by Winogradsky, that has become the conceptual prototype for modern biofilm reactors [8].

Despite these advancements, the dominant planktonic paradigm maintained its stronghold until the 1970s. A turning

Fig. 1. **Timeline of the biofilm-based technologies: a historical perspective**

point for biotechnology came with the work of J. William Costerton (1978), published in the journal *Scientific American* under the title "How Bacteria Stick" (which introduced the broader concept published as "The bacterial biofilm: A common form of persistence in nature"). Costerton substantiated that a biofilm is not an accidental colonization but rather a highly organized structure and a microbial survival strategy. He introduced the concept of the EPS matrix as a community exoskeleton that protects against adverse environmental factors, including antibiotics and toxicants. He validated the concept of the biofilm as a functional mode of microbial existence in nature. This shift in focus from pure culture to microbial consortium became the departure point for modern biotechnology. It enabled a transformation in the perception of biofilms: from a problematic fouling to a controllable biotechnology that underpins contemporary energy and environmental engineering solutions [9].

It became evident that studying pure cultures *in vitro* does not reflect the actual physiology of microbes in nature. This provided the impetus for developing novel engineering solutions where the biofilm began to be considered an active biocatalyst. Understanding the mechanisms of *quorum sensing* — intercellular communication mediated by chemical signals — definitively legitimized the status of the biofilm as a distinct, highly organized structure. The development of the *quorum-sensing* concept itself began in the 1970s with research on the luminescence of the marine bacterium *Vibrio fischeri*, in which a density-dependent mechanism of gene expression regulation via the secretion of signaling molecules was discovered. This fundamental discovery demonstrated that bacteria exhibit collective behavior and coordinated actions, such as biofilm formation, marking a pivotal moment in understanding microorganisms as socially organized systems [10–12].

Today, we are witnessing a new stage in the evolution of biofilm research — a transition from the study of natural biofilms to their targeted engineering driven by synthetic biology. Integrating Winogradsky's concepts of metabolic cooperation with Costerton's principles regarding the protective role of the matrix allows contemporary scientists to design bioreactors in which the biofilm functions not as a static entity, but as a dynamic tool. This integration opens avenues for developing systems capable of simultaneously executing advanced wastewater treatment to remove nitrogen compounds and generating electrical energy, which constitutes the core of modern environmental biotechnology.

Wastewater treatment. Nitrification and metabolic synergism: from foundational discoveries to engineering implementation

Wastewater treatment biotechnology has undergone a sequence of historical shifts (Table 1). At the beginning of the 20th century, in 1914, Arden and Lockett published a foundational paper describing the activated sludge process. They demonstrated that the aeration of wastewater in the presence of microbial flocs provides intensive degradation of organic matter [13].

Edward Frankland established the conceptual foundation for the use of trickling biofilters through a series of intermittent soil filtration experiments, which demonstrated that biological oxidation, rather than mere mechanical straining, is the key to purification. His work became a cornerstone document for the development of trickling biofilters [14, 15].

The research conducted by Rittmann and McCarty (1980) laid the foundation for modeling biofilms as engineered entities. They integrated diffusion processes and Monod kinetics to describe the steady-state conditions of a biofilm [16].

Bruce E. Logan synthesized the transition from conventional treatment practices to microbial fuel cells, which served as the departure point for contemporary engineering of electrogenic biofilms [17].

Furthermore, Rusyn and Gómora-Hernández [18] demonstrated comprehensive approaches toward integrating biofilm systems into constructed wetlands for simultaneous purification and energy production.

Nitrification, which consists of a two-stage biological oxidation of ammonia to nitrite and subsequently to nitrate, constitutes a key link in the global nitrogen (N) cycle. In contemporary industrial environmental biotechnologies, nitrification in biofilm systems is one of the primary processes for treating wastewater with high concentrations of ammonium compounds. The efficiency of this process is predicated on metabolic synergism and the complex spatial and functional organization of the biofilm, which enables sequential transformations unattainable in planktonic cultures [19–21].

Table 1. **Historical evolution of biofilm-based wastewater treatment technologies**

Stage	Key technology	Primary source
Empirical	Irrigation fields / Biofilters	[15]
Industrial	Activated sludge	[13]
Engineering	Mathematical modeling of biofilms	[16]
Energy-focused	Bioelectrochemical systems (MFCs)	[17]

The history of the discovery of nitrification is a striking example of the evolution of scientific thought — from the assumption of a purely chemical nature of the phenomenon to its recognition as a microbiological process. At the end of the 19^{th} century, the debate surrounding the mechanism of nitrification became the foundation for the development of an entire field of environmental microbiology.

A fundamental understanding of nitrification began with the pioneering work of Théophile Schloesing and Achille Müntz, who demonstrated that ammonia oxidation occurs in soil and can be halted by antiseptics or high temperatures, providing indisputable evidence of the involvement of living organisms. The subsequent stage was marked by the research of Percy Frankland and Grace Frankland, who substantiated the two-stage nature of the process; utilizing serial dilution methods, they isolated pure cultures of nitrifiers and demonstrated that distinct functional groups of bacteria carry out the conversion of ammonia to nitrite and nitrite to nitrate.

Sergei Winogradsky resolved this debate by discovering chemolithoautotrophy and identifying the specific microorganisms that drive nitrogen transformations in nature. Subsequently, William Costerton and his successors integrated these discoveries into the biofilm paradigm, demonstrating that the high efficiency of nitrification is driven by the metabolic synergism of microbial consortia within the protective matrix. Contemporary studies confirm that it is precisely the architectural organization of the biofilm, grounded in the natural principles laid out by Winogradsky, that enables nitrifiers to achieve stability and resilience to external factors within industrial systems [22, 23].

The modern understanding of nitrification is predicated on the conceptual foundation established by Winogradsky and augmented by knowledge of the social architecture of bacteria. Within a biofilm, nitrification is carried out via metabolic synergism, in which spatial organization effectively replaces complex technological reactor configurations. Several key aspects dictate biofilm functionality.

First, spatial stratification. Distinct oxygen and substrate gradients develop throughout the biofilm depth. Ammonia-oxidizing bacteria (AOB, e.g., *Nitrosomonas*) typically concentrate in the outer layers where oxygen accessibility is maximized. Conversely, nitrite-oxidizing bacteria (NOB, e.g., *Nitrobacter*), which are more sensitive to elevated oxygen concentrations and exhibit lower growth rates, occupy deeper, microaerophilic niches. This sequential substrate consumption minimizes the accumulation of toxic nitrite [24–28].

Second, the role of the EPS. The matrix functions as a chemical buffer, shielding nitrifiers — which are highly sensitive to external stress — from elevated concentrations of toxic substances or abrupt pH fluctuations. This biofilm structure provides a level of stability unattainable in activated sludge reactors, where cells are continuously subjected to hydrodynamic stress [29].

Third, the microbial consortium. As Winogradsky noted, nature does not operate with pure cultures in isolation. Contemporary metagenomic research confirms the presence of heterotrophic companion bacteria in nitrifying biofilms. These heterotrophs consume the metabolic byproducts of nitrifiers and stabilize the matrix's physical structure, thereby acting as the architects of the community [30].

Engineering control of the biofilm by regulating biofilm thickness, carrier surface area, and hydraulic flow rate opens avenues for constructing reactors that operate on the principles of natural design. Applying the principles of metabolic synergism enables the implementation of advanced technologies such as ANAMMOX (anaerobic ammonium oxidation), where nitrification and denitrification are coupled within a single consortium, ensuring exceptional energy efficiency in wastewater treatment.

The ANAMMOX process in biofilm systems ensures high nitrogen removal efficiency due to the unique capacity of specialized bacteria to function under low-oxygen conditions [31–33].

In modern biofilm reactors, the formation of dense granules results from a controlled engineering process in which substrate gradients drive a unique syntrophic organization: ammonia-oxidizing bacteria (AOB) occupy the outer aerobic layer, providing a continuous flux of nitrite to the inner anaerobic layers inhabited by ANAMMOX bacteria. This spatial architecture transforms the granule into an autonomous bioreactor, wherein the syntrophic proximity minimizes the concentration of toxic intermediates and maximizes nitrogen conversion efficiency.

In membrane bioreactors (MBRs), biofilms forming directly on membrane surfaces act as highly efficient biocatalytic layers that intensify nitrification even at high hydraulic flow rates. By establishing oxygen gradients throughout the biofilm depth, the membrane system enables simultaneous nitrification in the outer layers and denitrification within the inner anaerobic zones. This integrated architecture minimizes the need for an external carbon source by enabling bacteria to utilize metabolic products within a single consortium efficiently. Furthermore, the integration of membranes into the nitrification-denitrification process prevents the washout of slow-growing nitrifiers, thereby ensuring high stability of nitrogen compound removal in wastewater treatment under industrial workloads [34, 35].

Despite the widespread application of MBRs in advanced wastewater treatment, their long-term viability is persistently challenged by the detrimental accumulation of unwanted microbial layers.

While biofilms are intentionally cultivated on carriers in fixed-bed systems or electrodes in bioelectrochemical cells, their uncontrolled accumulation on filtration membranes, commonly known as membrane biofouling, remains a primary operational bottleneck in MBRs. Biofouling is driven by initial microbial colonization of the membrane surface, followed by cell proliferation and extensive EPS secretion. This self-producing matrix creates a dense hydrodynamic resistance layer that progressively increases transmembrane pressure, drastically reduces permeate flux, and shortens the operational lifespan of expensive hollow-fiber or flat-sheet modules. Furthermore, standard physical backwashing and chemical cleaning-in-place protocols often fail to eradicate mature, multi-layered biofilms entrenched in membrane pores completely.

To mitigate this critical limitation and maintain stable flux performance, modern MBR engineering incorporates several advanced anti-fouling strategies. These include:

1) continuous or intermittent air sparging (two-phase cross-flow filtration) to induce high shear stress and scour away loose biofilm structures;

2) surface modification of polymer membranes via grafting hydrophilic nanoparticles, zwitterionic polymers, or antimicrobial peptides to lower initial cell attachment;

3) *quorum quenching* biotechnology, which utilizes immobilized or free enzymes to degrade N-acyl-homoserine lactones and disrupt inter-bacterial communication required for biofilm maturation;

4) the integration of conductive carbon-based or metal-organic framework membranes capable of electro-rejection and localized electrochemical oxidation of microbial cells upon applying a minor electrical potential.

The efficiency of the metabolic activity of microbial consortia within biofilm systems is substantially limited by the diffusion barriers of the extracellular matrix (Fig. 2). As early as the classic works of Rittmann and McCarty (1980), it was theoretically substantiated that the rate of substrate transformation is a function of both the biochemical activity of the cells and the diffusional capacity of the EPS matrix. This relationship is critical for understanding the functionality of anaerobic granules and electrogenic biofilms, in which the inner layers of the consortium may experience substrate starvation despite high substrate concentrations in the bulk liquid flow. Consequently, contemporary biofilm engineering strategies aimed at optimizing cytochrome density (e.g., OmcZ) or stimulating methanogenesis must account for the kinetic limitations imposed by the steady-state biofilm model [36, 37].

Bioenergetics and microbial fuel cells: biofilms as drivers of sustainable energy

The application of biofilms in bioenergetics encompasses a range of technologies wherein a complex consortium of microorganisms converts organic waste into energy carriers, specifically biogas, electricity, bioethanol, and biohydrogen.

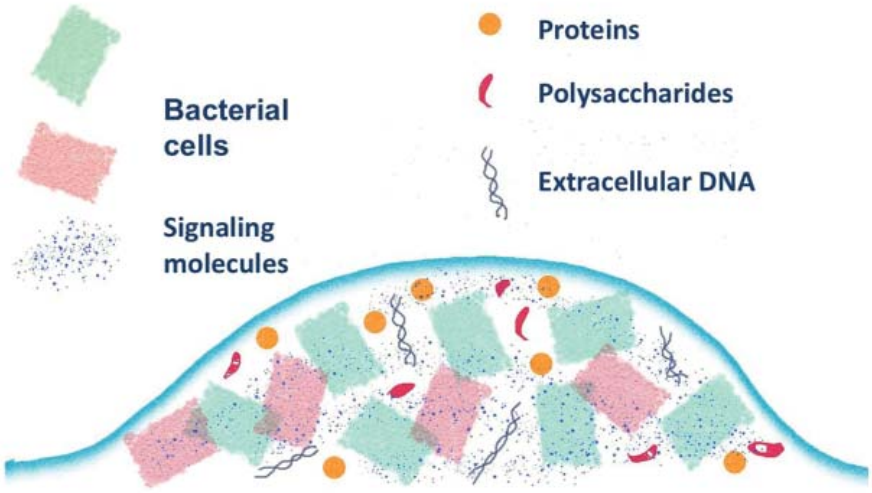

Fig. 2. **Schematic representation of mature biofilm architecture and EPS matrix composition**

The history of bioelectrochemistry dates back to 1911, when the British botanist Michael Potter, while investigating the metabolic activity of yeast (*Saccharomyces cerevisiae*), demonstrated that microorganisms can generate an electric current during metabolism. Although his discovery did not have immediate engineering applications at the time, Potter laid the foundation for the concept of microbial fuel cells (MFCs), demonstrating that cells can transfer electrons to an electrode [38, 39].

While the underlying principles trace back to Galvani's 18th-century discovery of animal electricity and Grove's 1839 chemical fuel cell, the technological evolution of microbial systems was gradual. Following Potter's breakthrough, Cohen demonstrated in 1931 that connecting MFCs in series could generate over 35 Volts, which later sparked interest during the 1960s space program as a potential dual-purpose waste-disposal and power-generation system. The development of analytical MFCs using synthetic mediators by Bennetto in the 1980s and 1990s was another critical milestone. Together, these historical advancements catalyzed the transition to the modern era of bioelectrochemistry, shifting the focus toward mediator-less electroactive biofilms and the expansion of MFCs into a broader class of bioelectrochemical systems for product synthesis and water desalination [40, 41].

Today, MFCs have evolved into highly efficient systems in which the biofilm forming on the anode acts as a biocatalyst, transforming organic substrates and transferring electrons to the anode. A biofilm enriched with exoelectrogens constitutes a highly ordered semiconductor structure, in which the electron-transfer rate depends directly on the density and metabolic activity of the cells [42, 43].

The EPS within the established biofilm is of paramount importance. Research on EPS, highlighted in Flemming (2016), became defining in the transition from describing biofilms as mere slime formations to understanding them as functionally organized biopolymer systems. A significant step in this direction was marked by the foundational works of J. William Costerton, whose concept of the biofilm as a microbial city in the 1980s shifted the paradigm of microbiology by proving the key role of the matrix in cell protection. Another milestone was the development of confocal laser scanning microscopy (CLSM), which enabled scientists such as Hans-Curt Flemming to visualize EPS architecture in three dimensions. These developments enabled the identification of complex interactions among polysaccharides, proteins, and extracellular DNA (eDNA), which today form the basis for engineered control of biofilms in industrial biotechnology. Contemporary advancements in this field confirm that the matrix is not merely a metabolic byproduct, but an active external organ that ensures the adaptation of microorganisms to extreme environmental conditions [44].

Within exoelectrogenic biofilms, the EPS matrix serves as a critical conductive scaffold that hosts specific *c-type* cytochromes, such as OmcZ, OmcS, and OmcE, which directly mediate electron transfer to the anode surface.

These protein components are integrated into the extracellular matrix, where they interact with the functional groups of polysaccharides and flavins (specifically riboflavin) that serve as soluble electron shuttles to facilitate long-range electron transfer. The EPS matrix, rich in proteins, humic acids, and eDNA, ensures a stable spatial organization of these cytochromes, preventing their degradation and maintaining a high density of electrical contact. Research confirms that the expression level of the OmcZ cytochrome directly correlates with the efficiency of the current generation in MFCs, as it forms the densest contacts between the cell and the anode. The utilization of nanostructured materials enables the effective immobilization of these biomolecules, transforming the biofilm into a highly efficient bioelectrochemical system. Consequently, the coordinated action of cytochromes, flavins, and the biopolymer EPS matrix constitutes a defining factor for achieving maximum power outputs in contemporary bioenergetic technologies [45–47].

Biofilm formation is equally critical in methanogenesis research. The process of methane production has evolved from simple observations of gas evolution in wetlands to the understanding of methane as an energy resource managed by biofilm consortia.

A fundamental scientific breakthrough was the realization that methanogenic archaea have an inherent propensity to colonize and form dense granular biofilms that serve as strategic structures for survival and efficient metabolism. Granular systems offer a distinct advantage over planktonic counterparts, as the spatial architecture of the biofilm precisely provides the syntrophic balance between hydrolytic bacteria and methanogenic archaea necessary for methanogenesis. Within this structure, EPS functions not only as a mechanical scaffold but also as a key factor for retaining microorganisms within the reactor under high hydraulic loads.

Contemporary data indicate that metabolic activity within the biofilm is regulated by a complex network of signaling molecules within the *quorum-sensing* system, enabling the consortium to adapt in a coordinated manner to fluctuations in environmental composition. Specifically, the use of CLSM has enabled the identification of distinct spatial gradients in oxygen, pH, and substrate concentrations within the granules, thereby ensuring the energetic efficiency of converting organic compounds into methane.

Modern engineering solutions focus on the controlled design of the EPS matrix, in which researchers use quorum-quenching methods to regulate biofilm thickness, thereby preventing membrane biofouling.

In addition to structural optimization, contemporary bioreactors exploit the high genetic plasticity of biofilms; owing to active horizontal gene transfer, microbial communities can rapidly acquire the capacity to degrade novel types of xenobiotics and recalcitrant industrial pollutants.

The integration of methanogenic biofilms into next-generation membrane reactors has emerged as a technological breakthrough, allowing for the coupling of advanced wastewater treatment processes with continuous energy recovery. Consequently, the transition from empirical observation of microbial processes to synthetic ecology methods has transformed biogas plants into highly efficient biotechnological systems capable of stable operation under modern environmental challenges. Without a biofilm-based organization, methanogenesis would be infeasible, as archaea exhibit an exceptionally slow growth rate and are prone to being washed out of the reactor if not immobilized within the granular structure [48–50].

Metagenomic analysis of archaeal biofilms demonstrates their unique capacity for adaptation to extreme environments through specific regulatory pathways that ensure survival under high temperatures and salinity [51].

Unlike the bacterial extracellular polymeric substance (EPS), the archaeal EPS exhibits a distinct biochemical profile enriched in highly glycosylated proteins, characteristic ether-linked isoprenoid lipids, and pseudomurein rather than peptidoglycan. This unique chemical composition imparts extraordinary thermostability and chemical resilience to the archaeal biofilm. Furthermore, archaeal adhesion and matrix structuration are driven by the archaellum — an ATP-powered rotary motor whose complex, multi-archaellin filament architecture provides exceptional structural flexibility for robust surface colonization [52, 53].

Archaea within biofilms play a crucial role in the global nitrogen and carbon cycles, particularly in anaerobic niches, where their syntrophic organization enables complex energetic transformations unattainable to individual species [51, 54–56].

Archaea, which, according to phylogenetic analysis, belong to the most ancient lineages

of life on Earth, have historically adapted to the extreme conditions of the early biosphere, shaping their unique biochemical resistance. As noted by Orell et al. [54], the capacity of these organisms to form biofilms was a survival strategy in primordial environments characterized by high temperatures, extreme pH, and elevated metal concentrations, thereby allowing them to maintain microbial community stability under persistent environmental stress [57].

A crucial milestone in understanding biofilm functionality for subsequent biotechnological applications was the discovery of syntrophic associations, in which distinct microbial groups that cannot independently degrade organic matter form dense, granular biofilms for collective metabolism. The spatial architecture of the biofilm enables methanogenic archaea to maintain the extremely low hydrogen concentrations required for efficient methane production. Contemporary research confirms that the EPS matrix within methanogenic biofilms functions not only as a structural glue but also as an active diffusion barrier that shields sensitive methanogenic enzymes from toxic compounds. Consequently, the transition from investigating planktonic processes to designing granular methanogenic systems has significantly enhanced biogas yields in industrial bioreactors. This evolutionary leap in biofilm understanding has transformed wastewater treatment from simple organic removal into a process of sustainable energy production [58, 59].

According to recent data, optimizing the spatial architecture of the EPS matrix significantly intensifies direct interspecies electron transfer (DIET), which is critical for enhancing methane yields and current densities in MFCs. DIET becomes feasible due to the dense spatial organization of the biofilm on the anode surface, where direct contact between exoelectrogenic bacteria (e.g., *Geobacter* spp.) and methanogens or other consortium partners is mediated by conductive protein structures (nanowires [60].

The use of biofilm systems for bioethanol production substantially enhances the resilience of producing strains to fermentation end-products, particularly ethyl alcohol, a critical factor for continuous biotechnological processes. The immobilization of yeasts and bacteria within the EPS matrix establishes a protective environment that minimizes ethanol's inhibitory effect on cell viability, thereby enabling fermentation at higher target product concentrations.

Integrating this approach with the use of lignocellulosic feedstocks, such as wheat straw and legume crop residues, ensures highly efficient sugar conversion through the synergistic action of enzymes retained directly within the biofilm structure [61–62].

Such systems demonstrate superior performance compared to conventional suspension fermentation methods, as they ensure rapid recovery of the microbial population following stress workloads. Implementing biofilm technologies in the production cycle also optimizes the utilization of substrates and byproducts, making the process a more economically viable model for the deep processing of biomass. Furthermore, the higher ethanol yield reduces the energy consumption required for subsequent distillation [63–65].

However, scaling such high-efficiency bioprocesses and maintaining long-term operational stability ultimately hinges on mastering microbial adhesion at solid-liquid interfaces. Transitioning from macroscopic reactor performance to targeted surface engineering requires a deeper insight into the physical rules governing initial cell attachment.

Integrating modern methods for analyzing surface interactions provides a deeper understanding of microbial adhesion dynamics, which is critical for designing novel biotechnological materials. Specifically, investigating the impact of physical factors, such as a static magnetic field, on the interaction between microorganisms and silicon surfaces opens new perspectives for the controlled formation of biofilms. The application of fractal analysis in such experiments enables quantitative assessment of biofilm architectural complexity and spatial organization under varying environmental conditions, including solvent composition. These data confirm that the physicochemical characteristics of the substrate, combined with external physical influences, can be utilized as tools for biofilm design — either stimulating its formation in biosensors or, conversely, preventing biofouling on sensitive components [66].

Contemporary biofilm engineering actively employs hybrid abiotic-biotic electrodes to increase power output in MFCs, in which the matrix acts as a conductive medium for electron transfer [67].

In the field of plant microbial fuel cells (Plant-MFCs), rhizosphere biofilms effectively convert root exudates into electrical current [68]. These exudates serve as a rich, continuous metabolic substrate for electroactive microorganisms, primarily consisting of major carbohydrates (such as glucose, fructose, and sucrose), specific amino acids (including glutamate and aspartate), and organic acids. This biochemical diversity demonstrates the high adaptability of microbial consortia to natural plant-derived nutrient sources, directly influencing both the structural composition of the biofilm and the resulting electricity outputs [69, 70].

Within constructed wetland-microbial fuel cell (CW-MFC) systems, biofilms forming on the surfaces of the filler material (plant root systems and gravel substrates) function as powerful bioelectrochemical reactors that simultaneously provide wastewater treatment and green energy generation. These complex consortia utilize rhizospheric oxygen and organic plant exudates to establish gradients that stimulate both aerobic pollutant degradation processes and anaerobic methanogenesis or exoelectrogenesis. According to recent data, the synergy between the root biofilm and anode materials intensifies electron transfer, transforming "passive" wetland systems into active resource-recovery installations. Research highlights that it is precisely the biofilm architecture and its capacity to adapt under fluctuating hydrodynamic workloads that dictate the stability of power output and the efficiency of nitrogen and phosphorus removal. Consequently, the modern engineering approach lies in the targeted design of the root environment and substrate materials to maximize the metabolic capacity of microbial communities within CW-MFCs [18].

The integration of an MFC and a biogas plant significantly enhances biohydrogen production efficiency by optimizing microbial metabolic processes. This combination of technologies ensures the utilization of bioelectrochemical mechanisms to stimulate hydrogen synthesis under anaerobic conditions. Co-operating these systems facilitates deeper conversion of organic substrates, substantially improving the energy yield of the final product. Implementing such integrated solutions represents a promising avenue for advancing modern biotechnology and the sustainable production of renewable energy [71].

One of the latest directions in biofilm research regarding biogas production is biogas biofiltration aimed at hydrogen sulfide removal. This process is based on the biological oxidation of sulfides mediated by sulfur-oxidizing bacteria of the genus *Thiobacillus*. In bioscrubbers, these bacteria form dense biofilms on packing materials, where hydrogen sulfide is absorbed from the biogas into the aqueous phase and immediately oxidized to elemental sulfur or sulfates. This allows the replacement of expensive chemical reagents with an efficient, stable biotechnological process that maintains microbial community activity under continuous gas flow [72, 73].

A distinct and promising trajectory in the development of bioelectrochemical systems involves the utilization of photosynthetic microalgae and cyanobacteria as biological agents for biocathodes. Traditional MFCs often require expensive platinum catalysts or toxic chemical oxidants (e.g., hexacyanoferrates) for oxygen reduction at the cathode. The application of microalgae in the cathodic chamber addresses this challenge; through photosynthesis, they directly generate oxygen, which serves as the terminal electron acceptor. Beyond electrical energy generation, such consortia can efficiently remove nitrogen and phosphorus compounds from wastewater, sequester carbon dioxide, and accumulate valuable biomass rich in lipids and carotenoids (e.g., lutein, astaxanthin) [74].

Experimental studies confirm the viability of integrating microalgae into MFC cathodic processes. Specifically, in two-chamber H-type systems with a salt bridge — where fermented sludge after methanogenesis served as the anolyte and cultures of *Chlorella vulgaris*, *Desmodesmus armatus*, and *Parachlorella kessleri* were used as cathodic inoculum — stable and sustained electrical energy generation was demonstrated. An important technological advantage of such systems is their adaptability; in the presence of a pre-established stable anodic biofilm, current and voltage parameters remain relatively stable even under fluctuating light sources. This paves the way for utilizing cost-free natural sunlight instead of energy-intensive artificial lighting [75].

Analysis of potential fields for the practical implementation of biofuel cells indicates that their utility extends well beyond classical bioenergy. In addition to scaling for municipal and industrial wastewater treatment, critical areas of development include the design of biosensors (particularly for the online monitoring of biochemical oxygen demand, BOD) and the creation of autonomous power

sources for remote environmental stations and wireless sensor networks. In such systems, the architecture and metabolic activity of the microbial biofilm determine the device's durability, sensitivity, and operational stability under varying environmental conditions [76].

Bioremediation and future perspectives of biofilm application in environmental biotechnology and bioenergetics

The roots of modern bioremediation date back to the early 20th century, when primary methods for biological wastewater treatment were first implemented. For a long time, purification was believed to be exclusively the result of free-swimming bacterial activity; however, a fundamental paradigm shift initiated by the works of microbiologists in the 1970s and 1980s, most notably J. William Costerton, demonstrated that the slime on stones or bioreactor pipes is not merely metabolic waste, but a highly organized microbial consortium whose metabolism is directed toward resource processing.

Today, biofilms are recognized as the most powerful tool for bioremediation. Their purification capacity is predicated on a combination of metabolic diversity and the physicochemical properties of the EPS matrix.

Microorganisms within the biofilm can remove pollutants, such as heavy metals, because the EPS matrix acts as a highly efficient ion-exchange resin. Due to the abundant presence of negatively charged functional groups, specifically hydroxyl, carboxyl, phosphate, and sulfate groups, the EPS effectively binds positively charged metal ions. Quantitative assessments confirm the high efficacy of this biotechnology: for instance, biofilms formed by *Bacillus* sp. achieved maximum removal efficiencies for As(V), Cd(II), and Cr(VI) of 73.65%, 57.37%, and 61.62%, respectively, in individual metal solutions. Even in complex multi-metal solutions, the biofilm maintained significant sequestration capacities, achieving removal rates of 48.92%, 28.7%, and 35.46% for the respective metals [77].

Beyond passive sorption, certain bacteria within the biofilm can alter the oxidation state of metals, converting them into less toxic or insoluble forms that precipitate and are subsequently removed from the system. Specifically, *Bacillus subtilis*, *Bacillus licheniformis*, and *Sporosarcina pasteurii* precipitate metals as carbonates, while *Desulfovibrio desulfuricans* releases sulfur compounds that bind metals into insoluble sulfides [78–80].

Petroleum Products and Xenobiotics: Biofilms establish ideal conditions for the degradation of recalcitrant organic pollutants. Within the dense matrix structure, oxygen gradients are formed, enabling the execution of complex metabolic pathways — ranging from the aerobic oxidation of light petroleum fractions to the anaerobic transformation of halogenated hydrocarbons — within a single biofilm layer. Furthermore, microorganisms within the biofilm share enzymes via horizontal gene transfer, allowing the community to rapidly adapt to novel xenobiotics entering wastewater [81, 82].

Emerging technologies in biofiltration use apparatuses in which the biofilm is immobilized on an inert carrier (ceramics, plastics, or charcoal). In modern biofilters, managing microbial metabolism — specifically through the use of molecules that stimulate *quorum sensing* — accelerates the formation of the active biofilm layer on the carrier. In contrast, quorum *quenching* methods help control biofilm thickness, thereby preventing filter clogging. This ensures the stable removal of toxicants such as phenols, pesticides, and pharmaceutical residues, which are often resistant to conventional treatment methods.

This builds upon the legacy of George Robinson, a pioneer of microbial ecology whose mid-20th-century research helped elucidate the role of microbial communities in the cycling of matter. His findings formed the basis for the concept of aquatic self-purification, which engineers subsequently transformed into contemporary biofilm-based biofilters [83].

The plant root system establishes a unique ecological niche hosting specialized biofilms that play a crucial role in the bioremediation of contaminated soils.

Root exudates, dynamically adjusted by the plant in response to environmental stress and specifically enriched in dominant carbohydrates (e.g., glucose, sucrose) and amino acids (e.g., glutamate, glycine), along with various organic acids, serve as a versatile energy source for microbial communities. These specific chemical ingredients not only nourish the biofilm but also act as chemoattractants that stimulate microbial metabolic activity, thereby promoting the synergistic enzymatic degradation of complex organic pollutants (such as polycyclic aromatic hydrocarbons) and the effective chelation and immobilization of heavy metals [84, 85].

This synergy, known as rhizoremediation, ensures a higher degree of purification than in uninoculated soils, as the biofilm matrix shields microorganisms from toxic stress and supports their stable functioning. Studies underscore that the architecture of these rhizosphere biofilms enables the effective management of pollutant fluxes, transforming the root zone into an active barrier for groundwater protection. In addition to biodegradation, root-associated biofilms facilitate the biomineralization of toxic ions, fixing them as safe mineral compounds directly within the root contact zone. Implementing this technology in industrial land reclamation cycles enables the transformation of degraded territories into productive ecosystems with minimal energy consumption [86, 87].

The research of Ananda Mohan Chakrabarty significantly influenced modern bioremediation in the 1970s. He engineered the first genetically modified organism (a strain of *Pseudomonas putida*) capable of degrading complex petroleum hydrocarbons. Although his work focused primarily on genetics, it demonstrated how strains behave within biofilms -specifically, that in natural environments, bacteria inherently seek protection within a matrix. Chakrabarty's findings prompted scientists to investigate the biofilm mode of life for these degradative strains [88].

Subsequently, in the 1990s, J. William Costerton demonstrated that bacteria within biofilms are many times more resilient to toxicants and disinfectants than their planktonic counterparts. This breakthrough discovery explained why bioremediation under real-world conditions (such as the treatment of contaminated soils) requires fundamentally different approaches than those used in laboratory flasks [89].

EPS components play a critical role in biofilm functionality: neutral polysaccharides and amyloids form the structural framework that ensures community stability and its resilience to external influences. Hydrophobic polysaccharides and biosurfactants promote adhesion, water channel formation, efficient metal sorption, and the solubility of hydrophobic organic pollutants. Nucleic acids mediate the transfer of genes responsible for xenobiotic degradation, while enzymes (such as oxidoreductases and hydrolases) directly catalyze the breakdown of compounds, including surfactants. Furthermore, monomers and polymers in the matrix serve as nutrient substrates during resource deficits, supporting the initial acclimatization of microorganisms exposed to toxic pollutants [90].

As contemporary research highlights, active horizontal gene transfer (HGT) occurs within the EPS matrix of biofilms in biotechnological systems, ensuring not only adaptation to toxic stressors but also the formation of metabolic syntrophy. Understanding HGT mechanisms enables a transition from passive microbial cultivation to the active modeling of consortia capable of stable, industrial-scale degradation of complex anthropogenic pollutants [91].

One of the most advanced frontiers is the utilization of biofilms for plastic depolymerization, particularly polyethylene terephthalate (PET). Specialized consortia, including strains of *Ideonella sakaiensis*, form biofilms on plastic surfaces and secrete specific enzymes (PETases) that cleave polymer chains into monomers. This process, governed by the principles of diffusional limitations described in Rittmann's works, allows for the conversion of persistent waste into biodegradable substrates [92, 93].

To systematically organize the diverse mechanisms and microbial agents involved in these remediation processes, Table 2 summarizes the primary pollutant categories, along with their corresponding biofilm-mediated transformation pathways and efficiencies.

Engineering parameters and control of biofilm systems

The efficiency of biofilm functioning in wastewater treatment technologies, bioenergy, and bioremediation depends on several engineering parameters, particularly biofilm thickness, the ratio of living to dead microorganisms, bioreactor hydrodynamic regimes, carrier materials, and *quorum-sensing* signaling molecules within the biofilm. The efficiency of biofilm-based biotechnologies is determined by the complex interplay between the physicochemical parameters governing mass transfer and the intrapopulational biological regulation of microorganisms. The primary factor limiting the rate of metabolic processes in a biofilm is its thickness, which directly regulates the diffusive fluxes of substrates and metabolites in accordance with the laws of diffusion.

For instance, a study on the effect of biofilm thickness (30–1000 μm) in moving

Table 2. **Biofilm-mediated bioremediation processes, key microbial agents, and mechanisms**

Pollutant category	Key microorganisms	Primary mechanism	Efficiency/Result	References
Heavy Metals (As(V), Cd(II), Cr(VI))	*Bacillus* sp., *Pseudomonas*	Biosorption via EPS functional groups (hydroxyl, carboxyl, phosphate)	Removal efficiency up to 73.65% (individual) or 48.92% (multi-metal)	[77]
Ions (Carbonates and Sulfides)	*Bacillus subtilis, B. licheniformis, Sporosarcina pasteurii, Desulfovibrio desulfuricans*	Biomineralization, precipitation (as carbonates or insoluble sulfides)	Conversion into insoluble, non-toxic precipitates	[78–80]
Petroleum Products & Xenobiotics	*Consortium bacteria* (enhanced via HGT)	Aerobic oxidation, anaerobic transformation of halogenated compounds	Stratified degradation within oxygen/ anoxic gradients	[81, 82]
Soil Pollutants & Hydrocarbons (Rhizoremediation)	Rhizosphere microbial consortia	Synergistic enzymatic degradation, chelation driven by plant root exudates	Enhanced detoxification and groundwater protection	[86–87]
Plastics (Polyethylene terephthalate)	*Ideonella sakaiensis* and specialized consortia	Enzymatic depolymerization via secreted PETases	Conversion of persistent polymer waste into biodegradable monomers	[92, 93]

bed biofilm reactors (MBBR) revealed that the specific rate of phosphorus release and uptake increases with an increase in thickness up to 110 μm. However, it significantly decreases within the range of 550–1000 μm due to the diffusion limitations of substrate mass transfer [94].

Regarding biofilms in bioelectrochemical systems, monitoring anodic biofilms using optical coherence tomography revealed that the maximum current density (up to 3.7 A/m^2) is generated at a minimal film thickness in the range of 10–30 μm. Mathematical modeling based on Fick's laws established that the threshold thickness of the anodic consortium, without acetate diffusion limitation, is 55 μm (at a potential of −0.2 V). This confirms the critical need for engineering control of this parameter to prevent intra-biofilm mass transfer resistance, as excessive thickening of the exoelectrogenic biofilm reduces the efficiency of electron transfer to the electrode [95].

The biochemical composition of the biofilm is also crucial; for example, a high protein content in the EPS of exoelectrogenic biofilms protects microorganisms from Cr(VI) toxicity and stimulates extracellular electron transfer via aromatic proteins [96].

Regulation of the *Tetradesmus obliquus* microalgal biofilm on a polyurethane carrier using the phytohormone indole-3-acetic acid (IAA, 1–5 mg/L) significantly accelerates its startup, providing biomass accumulation up to 48.2 mg/g and decreasing the absolute value of the zeta potential to –2.49 mV, which stimulates primary cell adhesion. This process is accompanied by structural remodeling of the extracellular matrix, characterized by an increase in the protein-to-polysaccharide ratio to 0.68 and an enrichment of tryptophan-like proteins by 16%. This ensures high system stability against shock loads and increases the efficiency of total nitrogen removal up to 87% [97].

It has been established that dynamic fluctuations in shear stress and turbulent flow structures have a more pronounced effect on biofilm thickness, density, and structure than the absolute magnitude of shear stress. This is driven by a combination of intensified mass transfer and mechanotransduction (specifically, the activation of c-di-GMP synthesis and extracellular matrix production). In this context, spatial heterogeneity and microcolony size distribution follow a log-normal pattern, the asymmetry of which

reflects a shift in the kinetic balance between cell deposition and their accelerated growth under the influence of non-stationary hydrodynamics. These results support the feasibility of using passive vortex induction as a low-energy engineering approach for targeted regulation of biofilm architecture in biofiltration systems [98].

The application of real-time CLSM and numerical modeling of *Shewanella oneidensis* consortia under low laminar flow conditions (Reynolds numbers Re = 6.7–54) confirmed that detachment is a purely physical process driven by shear forces, during which the basal cell layer maintains adhesion to the substrate even under maximum loads. Local morphological heterogeneities (finger-like protrusions and roughness) narrow the fluid flow channels, causing a multi-fold microscale increase in shear stress (from 31 to 299 mPa). This leads to the selective washout of rough zones and, consequently, the gradual hydrodynamic smoothing of the biofilm surface [99].

On the other hand, investigations of turbulent fluid motion revealed that increasing the pulsation intensity to an optimal value of q = 2,50 cm/s accelerates convective-diffusive transport due to the emergence of a pronounced percolation velocity gradient. This directly modulates the biofilm's porosity and thickness, ensuring a pollutant removal efficiency of 96.93% [100].

The physicochemical properties of carrier materials determine the kinetics of primary cell adhesion and the longevity of the system. While hydrophilic polymeric materials with a high specific surface area are applied in conventional biological treatment, electrically conductive carbonaceous materials, such as felt, cloth, or brushes, are utilized for MFC anodes. The surface of these conductors is further optimized through acid treatment or amination to create a positive surface charge, facilitating electrostatic interaction with negatively charged bacterial cell envelopes.

A distinct trajectory involves modifying biofilm carriers. For instance, modifying plastic carriers with granular activated carbon in MBBRs provides a well-developed macroporous structure, which significantly intensifies biomass accumulation and improves microorganism retention on the surface. This promotes targeted enrichment of the consortium with key functional genera (*Zoogloea*, *Thauera*, *Nitrospira*, and *Nitrosomonas*), increasing the efficiency of carbon (up to 81.8%) and nitrogen (up to 74.9%) removal under high organic loading rates, while ensuring high dynamic stability of the biofilm [101].

Furthermore, the efficiency of biofilm processes, particularly diffusion, is affected by the molecular size of the particles consumed by the biofilm microorganisms. An analysis of diffusion kinetics in aerobic granular sludge (AGS) revealed that the heterogeneous porous structure of the granules does not restrict the transport of compounds with molecular weights up to 4 kDa. In contrast, the penetration of larger macromolecules (from 10 kDa) is hindered by the dense zones of the cellular matrix. Given that over 60% of the soluble chemical oxygen demand (sCOD) in actual wastewater is represented specifically by the low-molecular-weight fraction (no more than 1 kDa), most of the organic substrate can freely diffuse deep into the granular biofilm. This indicates that intra-porous diffusion is not a limiting factor for the biodegradation of most soluble pollutants when using AGS technology, thereby confirming the high engineering efficiency of such systems [102].

Alongside this, the architecture, mechanical stability, and metabolic cooperation within the biofilm at all stages of its life cycle are coordinated by quorum-sensing chemical signals. These signaling molecules, specifically acyl-homoserine lactones in Gram-negative bacteria, oligopeptides in Gram-positive bacteria, and the universal autoinducer-2 for interspecies communication, regulate the expression of genes responsible for the synthesis of EPS matrix components. The accumulation of a critical concentration of quorum-sensing molecules in the localized microenvironment of the biofilm not only triggers maturation processes or synchronized cell detachment to colonize new surfaces but also directly regulates electrochemical activity in MFCs. Notably, *quorum-sensing* signaling molecules regulate the biosynthesis of endogenous electron shuttles, such as flavins and phenazines, thereby ensuring molecular synergy between the reactor's engineering parameters and the biological productivity of the microbial community [103, 104].

Biofilm formation is influenced by temperature, pH, the microorganisms within the consortium (specifically their competition and coexistence), nutrient availability, the presence or absence of mixing, process duration, and other technological parameters [105].

Thus, achieving maximum stability and productivity in biofilm systems requires comprehensive optimization. This includes

maintaining the film thickness within the substrate diffusion limit (tailored to each reactor type), ensuring hydrodynamic flow fluctuations to stimulate EPS synthesis, and using porous or chemically modified carriers to accumulate target microbiota selectively. Additionally, it involves selecting appropriate temperatures and pH levels, optimizing nutrient composition, and supplementing limiting substances during treatment processes, depending on the consortium microorganisms and substrates used in the technology.

Despite the advantages of utilizing biofilm technologies, there is an inherent risk of HGT within biofilms, particularly concerning the potential spread of antibiotic resistance in ecological and industrial systems. The high cell density, restricted motility of microorganisms, and the protective role of the EPS matrix make biofilms hotspots for the transfer of mobile genetic elements, such as plasmids; notably, the conjugation frequency in such consortia can exceed that of planktonic cultures by two to three orders of magnitude. Furthermore, the high concentration of eDNA within the matrix facilitates natural transformation processes, which are further intensified in thick biofilms (over 500 μm) due to the emergence of pronounced oxygen and substrate gradients. This leads to anaerobic cell lysis in the basal layers and the massive release of genomic material. The presence of antibiotics or heavy metals in wastewater or anaerobic digestion waste serves as an additional trigger for the bacterial stress response, thereby stimulating horizontal gene transfer.

To mitigate these biological risks and prevent the environmental dissemination of antibiotic resistance genes, several targeted engineering and operational solutions must be implemented. These include:

1) periodic backwashing or carrier scouring to control biofilm thickness strictly below the critical diffusion threshold (typically under 100–150 μm), thereby preventing deep anaerobic zones and massive cell lysis;

2) applying targeted *quorum quenching* enzymes or signal inhibitors to suppress high-density cell signaling that triggers stress responses;

3) integrating advanced oxidation processes or enzymatic treatment before discharge;

4) strictly regulating influent selective pressures by minimizing concentrations of antibiotics and heavy metals in industrial wastewater streams.

This necessitates the implementation of engineering solutions to minimize these biorisks. Such solutions may include optimizing hydrodynamic parameters for the controlled detachment of excess biomass and employing *quorum quenching* techniques to reduce matrix density.

The economic factors hindering the implementation of bioelectrochemical systems include, in particular, the high capital costs associated with selective proton-exchange membranes (such as Nafion, which can account for 30–50% of total reactor material costs) and specialized carbon-based electrode materials. This expenditure significantly impedes their commercial competitiveness compared to conventional wastewater treatment and energy generation technologies. Specifically, capital expenses for pilot-scale bioelectrochemical reactors often exceed those for standard activated sludge setups severalfold, while operating costs remain vulnerable due to frequent maintenance needs. Beyond economic burdens, the main technical barriers to scaling up these systems remain the limited extracellular electron transfer rate (yielding power densities typically restricted to fractions of a watt per square meter in unoptimized systems), rapid membrane and electrode biofouling, and difficulties in maintaining stable reactor performance over long-term continuous operation exceeding several months. To overcome these technological and economic limitations, further research focused on developing low-cost alternative membranes, scaling up reactor architecture, and engineering novel biocompatible, highly conductive materials is critically required [106].

Future perspectives of biofilm application in bioenergetics and environmental biotechnology

The transition from understanding biofilms as mere survival mechanisms to deploying them as active biocatalysts has driven a wide array of modern biotechnological applications. As summarized in Table 3, engineered biofilm processes now form the backbone of both environmental remediation and renewable energy generation. The inherent advantages of the extracellular polymeric substance (EPS) matrix, such as protection against toxic shocks and the establishment of precise metabolic microenvironments, enable specific microbial consortia to perform highly specialized tasks. For instance, in wastewater

Table 3. **Comparative characteristics of biofilm-based biotechnological processes**

Technology	Main objective	Key microorganisms	Role of biofilm	Product / Result	References
Wastewater Treatment	Removal of organics and nitrogen	*Nitrosomonas*, *Pseudomonas*	Community protection, nitrification	Treated water, biomass	[24–28]
Soil Bioremediation	Removal of toxicants / metals	*Bacillus*, *Pseudomonas*	Biosorption, stress protection	Soil detoxification	[78–80]
Methane Production	Waste processing (biogas)	*Methanosaeta*, *Syntrophobacter*	Syntrophy, consortium stability	Biomethane CH_4	[48–51]
Bioethanol Production	Fermentation of sugars / cellulose	*Saccharomyces*, *Zymomonas*	Enhanced ethanol tolerance	Bioethanol (fuel)	[63–65]
Electricity Production (MFC)	Water treatment + energy	*Geobacter*, *Shewanella*	Direct Interspecies Electron Transfer (DIET)	Electric current	[60]
Biogas Desulfurization	Removal of H_2S from biogas	*Thiobacillus*	Oxidation of hydrogen sulfide	Purified methane, fertilizer, sulfur	[72–73]

treatment and soil bioremediation, genera such as *Nitrosomonas*, *Pseudomonas*, and *Bacillus* leverage the protective biofilm architecture to efficiently remove organic loads, nitrogen compounds, and heavy metals.

Beyond environmental cleanup, the spatial organization of biofilms is fundamental to modern bioenergetics. Complex syntrophic interactions within granular or anodic biofilms facilitate sustainable energy recovery, underpinning processes like methane generation (*Methanosaeta*, *Syntrophobacter*), bioethanol fermentation (*Saccharomyces*, *Zymomonas*), and direct electricity production in microbial fuel cells (*Geobacter*, *Shewanella*). Furthermore, specialized ecological niches, such as those exploited for biogas desulfurization using *Thiobacillus*, highlight the immense versatility of these structured microbial communities. Consequently, Table 3 provides a comprehensive overview of how targeted biofilm engineering, tailored to specific primary objectives and key microorganisms, results in distinct, valuable end products, serving as a conceptual roadmap for the detailed processes discussed in this review.

The future of biofilms lies within the domain of synthetic ecology. Using 3D bioprinting, it is possible to engineer specific architectures for biofilm formation, in which bacteria are positioned in precise spatial sequences to ensure the staged purification of water or soil from complex pollutant mixtures, or the conversion of organic waste into energy carriers.

Integrating genetically modified strains into the biofilm structure — such as those that luminesce via *lux* genes or alter their electrical potential upon encountering a specific toxicant (e.g., exoelectrogens like *Geobacter* or *Shewanella*) — will transform future treatment facilities into systems embedded with intelligent biosensors or biocomputers. Driven by preprogrammed automation, these systems will be capable of responding autonomously to fluctuations in environmental composition.

Another promising frontier is the recovery of valuable resources from waste during bioremediation. For instance, employing specific biofilms not only for metal removal but also for their subsequent concentration and recovery shifts the paradigm of wastewater purification from a cost-intensive operation to a profitable model.

Conclusions

In summary, biofilms represent a powerful yet complex frontier in modern environmental biotechnology, effectively bridging

bioenergetics and waste valorization. While harnessing the natural resilience of microbial consortia offers exceptional advantages across wastewater treatment and environmental remediation, the broader transition of these systems from laboratory scales to industrial implementation remains constrained by several fundamental challenges. The inherent dichotomy of the extracellular polymeric substance matrix—acting simultaneously as a protective shield and a formidable diffusion barrier—poses intricate engineering challenges that have yet to be fully overcome at a commercial scale.

Specifically, in bioenergetics, the practical deployment of microbial fuel cells and larger bioelectrochemical systems is severely hindered by notoriously low extracellular electron transfer rates, which fundamentally limit overall power density and energy recovery. This kinetic bottleneck occurs because the dense EPS matrix, while securing the consortium to the electrode, eventually imposes substantial internal ohmic resistance as the biofilm thickens, impeding the efficient flow of electrons via cytochromes and endogenous mediators. Prohibitive structural and economic barriers heavily compound this biological limitation. The reliance on expensive selective proton-exchange membranes, such as Nafion, alongside the need for noble-metal catalysts or specialized carbon-based electrode materials, significantly increases capital expenditure. Consequently, this economic burden undermines the commercial competitiveness of bioelectrochemical reactors when compared to conventional, energy-intensive wastewater treatment technologies. Furthermore, long-term reactor stability is chronically compromised by severe membrane biofouling. The continuous, unregulated overproduction of the EPS matrix leads to rapid clogging of membrane pores and fouling of active electrode sites, which physically obstruct substrate diffusion, sharply reduce metabolic efficiency, and necessitate frequent, costly maintenance interventions.

Beyond these operational and economic barriers, the widespread application of robust biofilm systems introduces significant, yet often overlooked, biological risks. The extraordinarily high cell densities packed within the structural confines of the EPS matrix establish an ideal microenvironment for accelerated horizontal gene transfer. Because these microbial communities are continuously exposed to sub-lethal concentrations of diverse anthropogenic pollutants in municipal and industrial effluents, they endure constant selective pressure. This turns the biofilm into a highly active ecological hotspot for the exchange of mobile genetic elements, raising serious ecological concerns about the environmental dissemination of antibiotic resistance genes and engineered traits via treated effluent discharges.

To bridge the persistent gap between fundamental biofilm ecology and scaled-up reactor engineering, it is essential to resolve these intertwined physical, economic, and biological bottlenecks. Accordingly, upcoming experimental research will pivot toward precision ecological management and targeted materials science. A primary focus of future investigations might be the execution of a comprehensive experimental project dedicated to developing advanced bio-electrode interfaces and low-cost conductive materials. This targeted approach is designed to significantly enhance direct interspecies electron transfer and maximize power outputs, while dynamically mitigating electrode biofouling and drastically reducing the capital costs associated with conventional electrode assemblies.

In parallel with material innovations, biofilm architecture could be optimized and dynamically regulated using quorum-quenching strategies. This dual approach will allow us to maintain biofilm thickness strictly below critical diffusion thresholds, thereby avoiding massive cellular lysis and ultimately minimizing genetic risks of horizontal gene transfer.

A deepening interdisciplinary approach will ultimately bridge the gap between theoretical laboratory-scale breakthroughs and reliable commercial applications, paving the way for highly efficient, sustainable, closed-loop bioenergy and circular bioeconomy frameworks.

Conflict of interest

The authors declare no conflict of interest.

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Author's contributions

D. Koltysheva — conceptualization, literature review, bibliography compilation, analysis and interpretation of results, and drafting of the manuscript; K. Shchurska — critical revision of the manuscript, editing, language polishing, translation editing, and final approval of the manuscript.

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БІОПЛІВКИ В БІОЕНЕРГЕТИЦІ ТА ЕКОБІОТЕХНОЛОГІЇ: ІСТОРІЯ ДОСЛІДЖЕНЬ ТА ПЕРСПЕКТИВИ ЗАСТОСУВАННЯ

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Історична зміна парадигми від вивчення планктонних бактерій до розуміння захисного матриксу позаклітинних полімерних речовин (ППР) докорінно змінила екологічну біотехнологію. Біоплівки, які колись вважалися експлуатаційними перешкодами, сьогодні визнані високоорганізованими мікробними консорціумами та важливими інструментами для сучасного очищення стічних вод, біоремедіації та сталого біоенергетичного виробництва.

Мета. Це дослідження спрямоване на аналіз еволюції знань про біоплівки: від ранніх спостережень до їхньої сучасної ролі в біоелектрохімічних системах та нітрифікації, і, що дозволяє окреслити майбутні перспективи застосування мікробних консорціумів.

Матеріали й методи. Проведено всебічний теоретичний синтез сучасної літератури з акцентом на динаміку матриксу ППР, просторову стратифікацію в гранульованих і мембранних біореакторах та прямий міжвидовий перенос електронів (ПМПЕ). Аналіз охоплює структурні дані про метаногенні археї, екзоелектрогени (наприклад, *Geobacter*) та ризосферні біоплівки у штучних водно-болотних угіддях — мікробних паливних елементах (ШВБУ-МПЕ).

Результати. Інженерія біоплівок використовує просторові градієнти субстрату для полегшення синтрофного метаболізму, що істотно покращує видалення азоту за допомогою процесів ANAMMOX та максимізує вихід метану. Матрикс ППР функціонує як хімічний буфер проти токсичного стресу та як електропровідний каркас. У біоелектрохімічних системах цитохроми типу *c* та флавіни забезпечують ефективний перенос електронів на великі відстані. Крім того, опосередкований біоплівками горизонтальний перенос генів та маніпулювання відчуттям кворуму (*quorum sensing*) покращують біодеградацію стійких ксенобіотиків, біомінералізацію важких металів та деполімеризацію пластику.

Висновки. Перехід до цілеспрямованого конструювання архітектури біоплівок з використанням принципів синтетичної екології суттєво інтенсифікує відновлення ресурсів довкілля. Управління матриксом ППР перетворює традиційну біологічну очистку на надійні, саморегульовані біотехнологічні системи, здатні до безперервного та сталого генерування енергії в мінливих умовах навколишнього середовища.

Ключові слова: біоплівка, біотехнологія, мікроорганізми, біоремедіація, біоенергетика, відчуття кворуму, очищення стічних вод.

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