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REVIEW ARTICLE

Biofilm Dynamics in Fluoride-Based Wastewater Systems: A Mini-Review on *Pseudomonas* spp. and *Bacillus* spp. Biofilms in Semiconductor Manufacturing WWTP

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Abstract

Biofilm formation and growth is a significant concern for water treatment professionals, as it can lead to the contamination of water systems and pose a threat to public health. Biofilms are complex communities of microorganisms that adhere to surfaces and are embedded in an extracellular matrix of polysaccharides and proteins. They are notoriously difficult to control, as they provide a protective environment for bacteria, viruses, and other harmful organisms to grow and proliferate. This review article highlights some of the factors that favor biofilm growth, as well as various strategies for controlling biofilm in water systems. Adopting the best available technologies, such as wellhead protection programs, proper distribution system maintenance, and filtration and disinfection, can prevent the formation and growth of biofilms in water systems. A comprehensive and multi-faceted approach to biofilm control can reduce the occurrence of biofilms and ensure the delivery of high-quality water to consumers.

Keywords Bacteria interactions · Biofilm attachment · Wastewater treatment plant (WWTP) · Control strategy · Biofilm removal

Introduction

The management of industrial wastewater, particularly in the semiconductor sector, is critically challenged by the disposal of fluoride-based (F-based) waste. This byproduct from essential manufacturing processes such as etching and cleaning introduces fluoride ions into wastewater systems, inhibiting the growth and adhesion of microorganisms that are crucial for bioremediation [1]. This section explores how biofilms—complex communities of these microorganisms—overcome the harsh conditions imposed by fluoride ions. The remarkable adaptability of biofilms in fluoride-rich wastewater environments highlights the evolutionary resilience of certain microbial species. Notably, species such as *Pseudomonas* spp. and *Bacillus* spp. have evolved mechanisms to resist high fluoride concentrations [2]. Not to be overlooked, *Archaeal* species, renowned for their tolerance to extremities, are also essential in constructing these resilient biofilms. Biofilms' structural and compositional adjustments—such as the fortification of extracellular polymeric substances (EPS)—play a critical role in counteracting the deleterious effects of fluoride ions by trapping and neutralizing them. Certain adjustment extends to metabolic

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alterations that enable microorganisms within the biofilms to degrade, assimilate, or otherwise neutralize fluoride compounds, significantly reducing their toxicity [3].

The influence of fluoride on the architecture of biofilms is profound. Fluoride ions fundamentally alter their physical structure, making them denser and less porous. This adaptation significantly enhances the biofilms' resilience, crucial for surviving and functioning efficiently in high-fluoride environments. By fundamentally transforming biofilm structures, fluoride stress compels biofilms to adopt strategies that enhance their defensive and operational capabilities. Biofilms also play an indispensable role in the treatment of fluoride-rich wastewater. They act as natural biofilters, reducing fluoride concentrations through dual mechanisms: adsorption onto their denser matrix and direct incorporation into microbial cells. This effective reduction of fluoride levels is vital for maintaining the operational integrity of advanced wastewater treatment systems. In these systems, biofilms form the backbone of specially designed bioreactors. These bioreactors are tailored to foster and sustain populations of fluoride-resistant microbes, leveraging biofilms' innate adaptability. By enhancing the biofilm's structural and compositional traits, these bioreactors significantly improve the efficiency and sustainability of wastewater treatment processes. This strategic use of biofilm resilience not only optimizes natural capabilities but also offers an advanced method to manage and treat fluoride-laden wastewater, demonstrating the critical role of biofilms in environmental management and industrial applications [4]. Advancements in genetic and proteomic analysis of these resistant biofilms have started to reveal the intricate inner workings of these microbial consortia, delineating the cooperative strategies that enable their survival under fluoride stress. This burgeoning field of research is key to refining biofilm-based reactor designs for more efficient fluoride mitigation, representing a leap forward in the technology of wastewater treatment [5, 6]. The well-established resilience of *Pseudomonas* spp. and *Bacillus* spp. in fluoride-rich environments merely scratches the surface of a vast microbial world with the potential for fluoride resistance and biofilm formation. It is vital to consider the contribution of a broader range of microbial species to obtain a holistic picture of biofilm dynamics in semiconductor wastewater treatment plant (WWTPs) [7]. Although the roles of specific microbial consortia—including *algae, fungi*, and *extremophiles*—in confronting fluoride-rich conditions are becoming increasingly recognized, it is crucial to understand that these organisms represent just a small segment of the vast microbial diversity present in industrial effluents. *Algae* utilize their fluoride-binding cell walls, *fungi* leverage their versatile metabolisms to transform fluoride compounds, and *extremophiles* thrive under severe conditions, each contributing uniquely to the enhancement of biofilm resilience against fluoride stress.

This review endeavors to expand the scope of investigation to include a wide variety of microbial species and consortia, elucidating their pivotal roles in formulating more effective biofilm-based strategies for wastewater treatment. Given the intricate interactions between microbial biofilms and fluoride within the specific context of semiconductor manufacturing processes, substantial attention is devoted to the adaptive strategies of species such as *Pseudomonas* spp. and *Bacillus* spp. These species have demonstrated exceptional resilience to fluoride exposure, underscoring their importance in industrial biofilm applications. Moreover, a broader ecological spectrum that includes a diverse array of fluoride-resistant microorganisms deserves further exploration for its potential to enhance biofilm robustness and contribute to more efficient wastewater bioremediation efforts [8]. An interdisciplinary approach that integrates microbial ecology with advanced environmental engineering is essential. This approach will drive the development of innovative and sustainable wastewater treatment methodologies that are specifically tailored to meet the challenges of the semiconductor industry. As we confront the dynamic landscape of semiconductor fabrication, it is imperative that our environmental management strategies adapt accordingly. These strategies should not only aim to mitigate pollution but also align with ecological principles, ensuring that technological advancements and environmental stewardship proceed hand in hand [9]. By broadening the narrative to encompass a more extensive range of microbial interactions, this review seeks to spur further research into holistic and sustainable solutions uniquely suited to address the environmental challenges faced by the semiconductor industry. Through such comprehensive research efforts, we aim to develop a deeper understanding of the ecological dynamics at play, ultimately fostering more effective and ecologically sound wastewater treatment paradigms [10].

Biofilm Developmental Stages and F-Based Semiconductor Wastewater

Biofilm development within semiconductor wastewater systems is a continuous, multi-stage process intricately impacted by fluoride at each transition (Table 1). The journey begins with initial adherence, where fluoride ions challenge bacterial colonization by modifying surface protein structure and impeding signaling pathways, setting a precedent for the biofilm's eventual stability and complexity [11]. This early interference has cascading effects on the entire lifecycle of the biofilm. As resilient bacteria establish initial adherence, they progress to form microcolonies, with quorum sensing as a pivotal player in coordinating subsequent growth [12]. Fluoride's persistent influence alters EPS production, impacting not only the biofilm's protective

Table 1 Overview of fluoride's impact at each biofilm development stage and its implications for wastewater treatment

Stage	Effect of fluoride on biofilm	Impact on wastewater treatment
Initial adherence	Alters protein structure on cell surface Disrupts signaling for biofilm initiation	Leads to less structured biofilm formation, impacting treatment efficiency
Microcolony formation	Interferes with quorum sensing Alters EPS production and composition	Affects biofilm density and protective capabilities
Mature biofilm	Promotes compact and less porous structure	Impacts nutrient and waste transport, potentially reducing bioremediation capacity
Dispersion	Causes premature dispersion or inhibit normal cell release	Disrupts biofilm stability and regeneration, possibly leading to overgrowth or decreased treatment efficacy

capabilities but also its structural formation. This disruption manifests in altered biofilm density and porosity, which are strategic adaptations to the persistent presence of fluoride. The maturation stage sees the biofilm developing a more compact architecture in response to fluoride, a defensive adjustment that nevertheless may affect the biofilm's functional efficacy in treating wastewater. The balance between fluoride resistance and treatment efficiency becomes critical at this stage [13]. Finally, the dispersion of cells, a natural phase of biofilm renewal and propagation, can be expedited or inhibited by fluoride. Premature dispersion may lead to a less effective biofilm for wastewater treatment, while inhibited dispersion could result in overgrowth and system inefficiencies [14].

Biofilm Development and Fluoride Interference

Biofilm formation within semiconductor wastewater systems is a complex process initiated by the adhesion of bacteria to surfaces. This critical first step, foundational to subsequent biofilm maturation, faces significant challenges due to the presence of fluoride ions. In species such as *Pseudomonas* spp., which are adept at forming biofilms, fluoride ions present a unique hurdle [15]. They bind to functional groups on surface proteins and adhesins—essential for the bacteria's initial adherence and stable anchorage on substrates. This interaction not only alters protein conformation but also modifies the characteristics of the bacterial cell surface, thereby hindering microbial attachment. Such biochemical and physical interference by fluoride ions plays a pivotal role in dictating the early stages impeding the robustness of biofilm formation (Fig. 1) [16, 17].

Fluoride ions significantly impact biofilm dynamics not only by hindering physical attachment but also by disrupting the intricate signaling networks essential for biofilm regulation. In *Pseudomonas* spp., this disruption can manifest as a delayed or structurally weakened biofilm, underlining the multifaceted nature of fluoride interference. These biochemical challenges have profound consequences that permeate the entire biofilm lifecycle. As bacteria begin to overcome the initial fluoride challenges and adhere to surfaces, they initiate microcolony formation. During this phase, interbacterial communication becomes crucial. In species like *Bacillus*, quorum sensing mechanisms play a pivotal role in orchestrating collective behaviors necessary for coordinated growth and extracellular polymeric substance (EPS) production. Fluoride ions can disrupt these communication pathways, adversely affecting EPS composition and quantity, which in turn compromises the biofilm's structural integrity and defensive capabilities [18]. Furthermore, interkingdom interactions, involving communication between different microbial domains such as bacteria and fungi or bacteria and microalgae, are also susceptible to fluoride's disruptive effects. These interactions often enhance biofilm resilience by diversifying the metabolic capabilities and environmental tolerances of the community. The interference of fluoride with these cross-kingdom dialogues could lead to less cohesive and functionally impaired biofilms, thus challenging the stability and efficacy of biofilms in environments with high fluoride concentrations [19].

Microcolony Formation in Fluoride-Rich Environments

As biofilms approach maturity, their inherently complex and three-dimensional structures undergo significant adaptations to cope with high fluoride conditions. For instance, *Pseudomonas* spp. biofilms tend to become denser and less porous. This structural compactness serves as a defensive measure to minimize fluoride penetration, yet it can simultaneously hinder the biofilm's metabolic efficiency. This is due to the restricted transport of nutrients and waste, which is critical in wastewater treatment applications where biofilm activity directly influences treatment efficacy [6, 15]. During this crucial stage of biofilm maturation, bacterial communities rely heavily on intricate communication and coordination mechanisms, predominantly facilitated by quorum sensing. This sophisticated bacterial communication system is essential for regulating gene expression relative to cell population density, orchestrating key aspects of biofilm development, including the production of extracellular polymeric substances (EPS). In industrial settings,

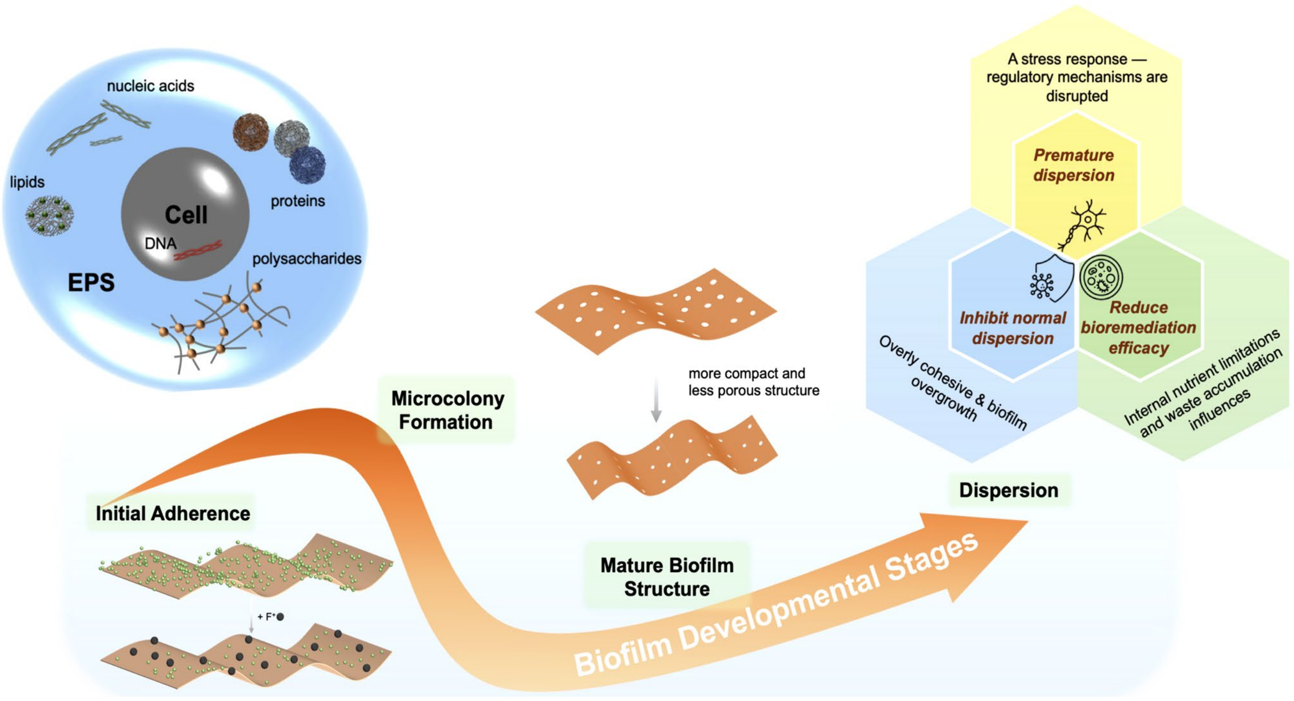

Fig. 1 Schematic diagram of developmental stages of biofilms

particularly within wastewater treatment systems handling fluoride-rich effluents, the disruption of quorum sensing by fluoride ions poses significant challenges. Such disruption can result in changes to the composition and quantity of EPS [20]. Altered EPS production due to fluoride interference can lead to biofilms that are mechanically weaker and less capable of withstanding the harsh conditions typical of industrial wastewater systems. These changes not only affect the biofilm's physical properties but also its performance in bioremediation processes, as the altered biofilm may not effectively degrade or treat wastewater contaminants as efficiently as required. Understanding these dynamics is crucial for optimizing biofilm-based wastewater treatment technologies, particularly for industries dealing with high fluoride concentrations [3, 10].

Mature Biofilm Structure

As biofilms mature within fluoride-rich environments like those found in semiconductor wastewater systems, they undergo notable structural changes. Biofilms formed by species such as Pseudomonas spp., which are renowned for their resilience, exhibit significant morphological adjustments in response to fluoride toxicity. These biofilms tend to develop a denser and less permeable structure, a direct response aimed at minimizing the adverse effects of fluoride [20]. This densification helps shield the biofilm's inner layers from fluoride ions, enhancing protection for the microbial community. Furthermore, the reduced porosity restricts fluoride penetration, effectively lessening its toxic impact on the core of the biofilm. However, these structural changes, while protective against fluoride toxicity, can adversely affect the biofilm's functional capacity, especially within wastewater treatment systems [12, 21]. The increased compactness and decreased permeability alter the diffusion dynamics within the biofilm matrix. Such alterations can impede the transport of nutrients and the expulsion of waste products from the microbial cells. In the context of wastewater treatment, these changes may compromise the biofilm's bioremediation effectiveness. Nutrient scarcity and waste build-up could lead to a decline in metabolic activity and reduce the efficiency of the microbial community involved in treatment processes [22].

Dispersion

Finally, fluoride's influence extends to the dispersion phase. While high fluoride levels can induce premature cell release, potentially undermining biofilm stability and bioremediation efforts, they can also inhibit dispersion, leading to overgrowth and operational inefficiencies within wastewater systems. This stage, which involves the controlled release of cells from the mature biofilm, is essential for colonizing new areas and maintaining the biofilm lifecycle. However, fluoride can disrupt this finely tuned process in several ways [23]. Firstly, high fluoride levels can lead to premature dispersion of cells from the biofilm. This premature release is often a stress response, where the biofilm's regulatory mechanisms are disrupted, leading to an early detachment of cells. While this might initially seem beneficial for spreading the biofilm, it can have detrimental effects on the biofilm's functionality in wastewater treatment. A sustained and stable microbial community is essential for continuous and effective bioremediation. Premature dispersion disrupts this stability, leading to a reduction in the biofilm's capacity to treat wastewater effectively. The prematurely dispersed cells might also be less equipped to form new, effective biofilms elsewhere, further reducing the overall efficiency of the bioremediation process. Conversely, fluoride can also inhibit normal dispersion processes. In this scenario, the biofilm remains overly cohesive, and cells do not disperse at the necessary rate or in the appropriate numbers. This inhibition can lead to biofilm overgrowth, where the biofilm becomes too dense and thick, potentially leading to system inefficiencies [12]. Overgrown biofilms can obstruct flow in wastewater treatment systems, leading to blockages and reduced treatment efficiency. Additionally, an overgrown biofilm might suffer from internal nutrient limitations and waste accumulation, further reducing its bioremediation efficacy.

Throughout the biofilm lifecycle, fluoride presents a continuous obstacle, shaping each developmental phase and demanding adaptive responses from microbial communities. From the initial hindrance to adherence, through the disruption of intra-biofilm communication and structural maturation, to the challenges in dispersion, fluoride's presence is a constant driver of biofilm evolution [17]. Addressing these interconnected stages holistically is essential for devising effective treatment strategies, ensuring that semiconductor wastewater systems remain efficient and sustainable. Understanding the nuances of each stage's response to fluoride can inform the optimization of biofilm-based treatments, ultimately contributing to a more robust and reliable approach to managing the intricate challenges of semiconductor wastewater.

Chemical Interactions with F-Based Wastewater

Stability and Reactivity

The interaction between fluoride ions and biofilm-forming bacteria at the onset of biofilm development is crucial. It dictates not just the immediate attachment and structural development, but also the long-term stability and degradative capacity of the biofilm. Fluoride ions, by virtue of their electronegativity and small radius, can penetrate the biofilm matrix, influencing both the physical structure and the metabolic pathways within. The resulting impact on biofilm establishment can be profound, with fluoride disrupting essential enzymatic functions that biofilms rely on for their sustenance and pollutant breakdown [12]. In *Pseudomonas* spp., fluoride's presence has been shown to substantially affect the biofilm's stability and its enzymatic reactivity. Studies such as those have already underscored the challenges faced by biofilms against fluorinated compounds, primarily due to the resilience of the C-F bond which resists enzymatic cleavage. Extending these insights, observed that specific enzymes responsible for aromatic compound degradation in *Pseudomonas* spp. were markedly less effective when confronted with fluorinated analogs. This led to a significant reduction, up to 60%, in the enzymatic breakdown of these stable compounds. Similarly, for *Bacillus* spp., which are known for their robust biodegradation capabilities, the enzymatic assault on fluorinated phenols is notably impeded. The oxidative enzymes that this species deploys for degrading phenolic compounds encounter formidable resistance when these molecules are fluorinated. Illustrated that the degradation efficiency of these biofilms plummeted by 50–65% for mono-fluorinated phenols when compared to non-fluorinated varieties, suggesting that the presence of fluorine atoms critically hampers the enzymatic degradation process.

The compendium of these studies highlights a significant obstacle: the chemical tenacity of fluorine compounds, which thwart the biofilm's natural degradation pathways. As these compounds remain recalcitrant within the biofilm matrix, their persistence in wastewater systems becomes a pivotal concern. It suggests that biofilm-based treatment strategies might need to be revisited and optimized to address the unique challenges posed by fluorinated pollutants. The implications are twofold: there is a pressing need to enhance the biofilm's capacity to handle fluorine's inhibitory effects, and to understand the adaptive responses of biofilm communities at the very initial stages of formation in the presence of fluoride. This dual approach could lead to more effective strategies for managing fluorine's impact on biofilm functionality and, ultimately, the sustainability of wastewater treatment practices in the semiconductor industry.

EPS Modification

The study on *Pseudomonas* spp. biofilms is a significant contribution to this field. Their research went beyond noting a 40% increase in certain polysaccharide components of the EPS. They also identified specific types of polysaccharides that were upregulated, including alginate, a known component in *Pseudomonas* spp. biofilms that contributes to their robustness and resistance to environmental stressors [17]. This upregulation was correlated with an increased resistance to fluoride toxicity, suggesting a direct adaptive response of the biofilm to the presence of fluoride.

Furthermore, the study observed changes in the protein profiles within the EPS, noting an increase in stress response proteins, including those associated with efflux pumps and oxidative stress management. This indicates that the biofilm is not only altering its physical makeup but also its functional protein expression in response to fluoride exposure. In the case of *Bacillus* spp. biofilms, the research provided detailed insights into the physicochemical changes of the EPS. They reported not only an increase in viscosity but also a significant alteration in the EPS's ionic composition. The study found an increased concentration of calcium and magnesium ions within the EPS, which are believed to play a role in stabilizing the matrix in the presence of fluoride. This stabilization could be a mechanism to prevent the penetration of fluoride ions into the deeper layers of the biofilm, thereby protecting the microbial community. Additionally, the decrease in porosity observed in Bacillus spp. biofilms was quantified using advanced imaging techniques [12]. The study reported a reduction in pore size by approximately 30–35%, which significantly impacts the biofilm's permeability and its interactions with the surrounding environment. These studies collectively enhance our understanding of how biofilms adapt to fluoride stress at a molecular level. The modifications in the EPS composition and structure are clear indicators of a defensive response, aimed at preserving the integrity and functionality of the biofilm in hostile environments [18].

Physical Interactions with F-Based Wastewater

Biofilm Morphology and Initial Formation

The interplay between fluoride ions and biofilm formation begins at the earliest stages, dictating the future morphology and functionality of microbial communities. Documented an increase in biofilm thickness of 20–25% under high fluoride conditions, a response that likely serves as a defense mechanism against fluoride intrusion. Yet, this adaptive response may not be entirely beneficial; the augmented thickness can impede oxygen and nutrient flow within the biofilm, potentially dampening its metabolic vigor and, by extension, its wastewater treatment capabilities. Furthermore, the study highlights how fluoride can compact the biofilm matrix, as evidenced by a 30% reduction in pore size. Such structural condensation may impact the diffusion of essential substances and retard the biofilm's efficiency in processing contaminants. This diminished porosity is a critical consideration for wastewater treatment, as it influences the biofilm's capacity for contaminant absorption and bioremediation.

For Bacillus spp. biofilms, fluoride's influence extends to surface adherence characteristics. Research revealed a 15–20% reduction in the biofilms' adherence strength, potentially due to modifications in the EPS matrix, an integral factor in biofilm stability. Such a reduction in adherence could have significant operational consequences in wastewater treatment systems, where biofilm stability on substrates is paramount. The initial reactions to fluoride—thicker but less permeable biofilms for *Pseudomonas* spp., and weaker adhesion for *Bacillus* spp.—set the stage for a series of downstream effects. These alterations in biofilm morphology underscore the necessity for a nuanced understanding of the initial fluoride interaction. It is this early exposure that shapes the biofilm's structural evolution and functional capacity, emphasizing the need for targeted management strategies in the treatment of semiconductor wastewater. Through this lens, the early stage encounter with fluoride is not merely a hurdle but a determinant of the biofilm's ultimate resilience and efficacy in environmental processing [19].

Absorption and Accumulation

In *Pseudomonas* spp. biofilms, the EPS matrix has been shown to play a significant role in the absorption of fluoride compounds. A study revealed that the polysaccharide components of the EPS in *Pseudomonas* spp. biofilms have a high affinity for fluoride ions. This affinity leads to the adsorption and accumulation of fluoride within the biofilm matrix. The study quantified that *Pseudomonas* spp. biofilms could absorb up to 30% more fluoride than their planktonic counterparts, indicating the effectiveness of the EPS in sequestering these compounds. The absorption rate was also found to be influenced by the maturity of the biofilm, with more mature biofilms exhibiting higher absorption capacities. This is likely due to the increased density and complexity of the EPS in older biofilms.

For *Bacillus* spp. biofilms, the mechanism of fluoride absorption appears to be somewhat different. Research showed that the protein components of the EPS in *Bacillus* spp. biofilms play a more significant role in fluoride binding. The study demonstrated that certain proteins within the EPS could bind fluoride ions, leading to their accumulation within the biofilm (Fig. 2). The rate of absorption was observed to vary with the EPS composition, which is influenced by factors such as the biofilm's age and the environmental conditions. Older *Bacillus* spp. biofilms with a more developed protein matrix showed a higher capacity for fluoride accumulation. Such findings underscore the complexity of the interactions between fluoride compounds and biofilms. The ability of biofilms to adsorb and accumulate fluoride has significant implications for wastewater

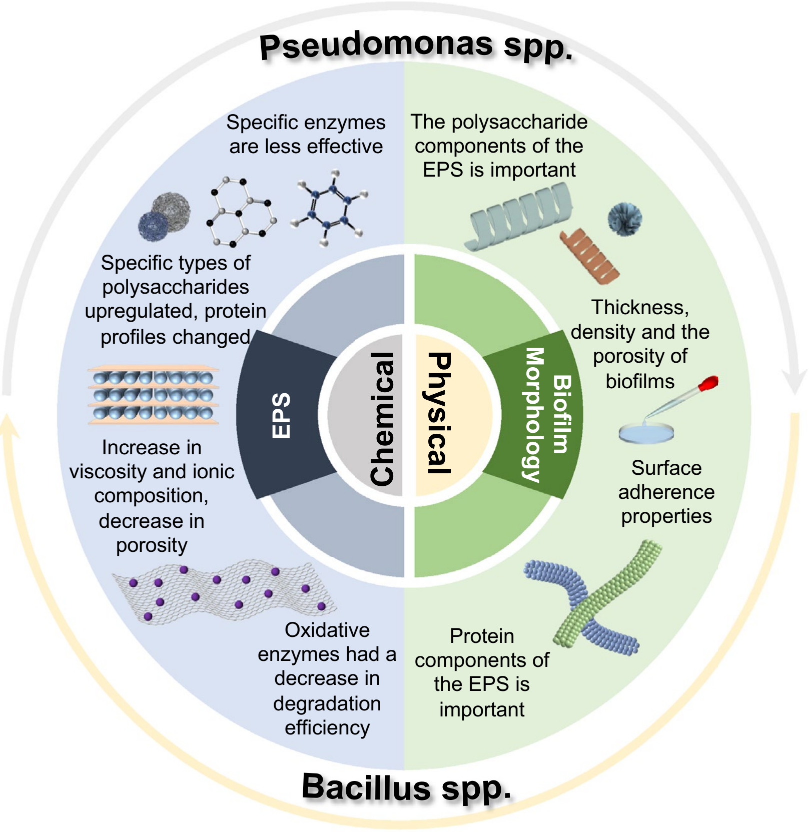

Fig. 2 Summary of physical and chemical interactions with F-based wastewater

treatment, particularly in the semiconductor industry, where fluoride-rich effluents are common [16, 17].

Biological Responses to F-Based Wastewater

Impact on Microbial Community

In fluoride-rich wastewater environments, selective pressures favor the emergence of fluoride-resistant microbial species. Research on mixed-species biofilms, which included species such as *Pseudomonas* spp. and *Bacillus* spp., highlighted a notable shift in community composition under high fluoride conditions. Their findings revealed an increased dominance of certain fluoride-resistant *Pseudomonas* spp., while fluoride-sensitive species significantly declined. This ecological shift resulted in reduced biodiversity within the biofilm [7]. The diminished diversity within the biofilm has significant consequences for its metabolic functionality. Each microbial species plays a unique role within the biofilm's metabolic network; thus, the loss of specific species diminishes the community's overall metabolic flexibility. Documented that biofilms under fluoride stress demonstrated decreased capabilities in critical functions like nitrogen fixation and organic matter degradation, with reductions in these processes estimated at 20–30% [4]. Furthermore, the prevalence of fluoride-resistant species alters the biofilm's metabolic dynamics. Research observed that fluoride-resistant *Pseudomonas* spp. exhibited enhanced mechanisms for coping with stress, including increased activity of efflux pumps and antioxidant systems. While these physiological changes aid survival in harsh environments, they may redirect energy from other essential metabolic activities, thus potentially diminishing the biofilm's overall efficacy in wastewater treatment operations [14].

Metabolic Alterations

A study demonstrated that certain strains of *Pseudomonas* spp. upregulate specific metabolic pathways in response to fluoride. These pathways include enhanced glycolysis and increased production of stress-related metabolites like polyhydroxyalkanoates (PHAs), which are thought to play a role in protecting cells from fluoride toxicity. The study also noted an increase in energy production pathways, suggesting that these biofilms expend more energy to cope with the stress imposed by fluoride. For *Bacillus* spp. biofilms, the response to fluoride exposure appears to involve alterations in nutrient metabolism. A 2021 study by Kumar and Sharma found that *Bacillus* spp. in fluoride-rich environments showed a marked shift in nitrogen and sulfur metabolism. This shift was characterized by an increased uptake of ammonium and sulfate ions, possibly as a mechanism to counteract fluoride toxicity. The study also observed changes in the lipid profile of the biofilms, indicating alterations in membrane composition, which could be a response to maintain cellular integrity against fluoride stress. Such survival mechanisms may also result in changes in the biological activity of the biofilms. For instance, the altered metabolic pathways can impact the biofilm's ability to degrade organic pollutants or participate in nutrient cycling. This was evident in a study, which showed that fluoride-stressed *Pseudomonas* spp. biofilms had a reduced capacity for degrading aromatic compounds, a key function in many wastewater treatment processes [17].

Synergy and Competition in Mixed-Species Biofilms

The interplay between *Pseudomonas* spp. and *Bacillus* spp. within mixed-species biofilms offers intriguing prospects for optimizing fluoride removal from wastewater. In these communities, *Pseudomonas* spp. can form robust biofilms that serve as a scaffold for *Bacillus* spp., fostering synergistic interactions that enhance the stability and resilience of the composite biofilm. Such synergy may be critical in environments with high fluoride concentrations, as it can improve the collective ability of the biofilm to sequester and degrade fluoride compounds. However, these interactions are not always cooperative. Antagonistic behaviors, such as the production of inhibitory compounds by one species that affect the growth of the other, can lead to a decrease in microbial diversity and, as a result, a less robust biofilm [12]. For instance, *Pseudomonas* spp. are known to produce a range of secondary metabolites that could suppress *Bacillus* spp. growth, potentially compromising the biofilm's capacity for fluoride removal. Understanding these complex interactions is essential, as the delicate balance between cooperation and competition within mixed-species biofilms determines their efficiency in fluoride bioremediation.

Mechanisms of Fluoride Removal by Biofilms

Biofilms exhibit a multifaceted capability for removing fluoride from environments, utilizing both physical sequestration and biochemical transformation. At the physical level, fluoride ions are primarily immobilized within the biofilm matrix. This occurs through adsorption onto extracellular polymeric substances (EPS) and direct uptake by microbial cells, where fluoride binds to specific proteins or accumulates in intracellular compartments. Biochemically, biofilms leverage enzymatic actions to transform fluorinated compounds, thereby reducing their toxicity and enhancing their removability. Enzymes such as dehalogenases, although less prevalent and less studied than those acting on chloride bonds, play crucial roles in breaking down carbon–fluorine bonds. Additionally, oxidoreductases found in species like *Pseudomonas* spp. can oxidize fluoride compounds, facilitating their breakdown into less harmful forms [19]. The metabolic pathways involved in fluoride detoxification are intricate. They often include the upregulation of stress response pathways, which provide the necessary energy and reducing power to combat fluoride-induced oxidative stress. Some pathways lead to the production of polyhydroxyalkanoates (PHAs) and other compounds that can sequester fluoride ions, removing them from the biofilm's active metabolic zones. For instance, in Bacillus spp., there is an adaptive shift towards metabolic pathways that generate reducing agents or the expression of efflux systems that actively pump fluoride ions out of the cells [9]. These diverse mechanisms underline the biofilm's adaptive responses to fluoride stress, demonstrating its remarkable capacity for the bioremediation of fluoride-contaminated environments. By elucidating these complex interactions, the full potential of biofilms in environmental management and wastewater treatment can be realized, offering a robust solution for mitigating fluoride pollution.

Treatment Efficacy and Environmental Implications

Removal Efficiency

The efficiency of biofilms in treating F-based wastewater in the semiconductor industry is significantly influenced by the absorption capacity of the biofilm, particularly its extracellular polymeric substances (EPS) matrix. The EPS, composed mainly of polysaccharides and proteins, plays a pivotal role in the biofilm's interaction with fluoride ions. Recent studies have demonstrated that biofilms of *Pseudomonas* spp. and *Bacillus* spp. are capable of adsorbing and accumulating substantial amounts of fluoride. This process primarily occurs through the binding of fluoride ions to the polysaccharide and protein components of the EPS. For instance, research specifically addressed this phenomenon in the context of semiconductor wastewater. The study found that *Pseudomonas* spp. biofilms exhibited a high fluoride absorption capacity, suggesting that these biofilms can effectively reduce fluoride concentrations in wastewater [12, 20].

However, the ability of these biofilms to biologically degrade or chemically transform fluoride compounds presents a more complex challenge. The same study by Chen et al. (2022) indicated that while *Pseudomonas* spp. biofilms were proficient in fluoride absorption, their capacity to chemically alter or degrade these compounds was limited. This finding is significant as it highlights a crucial gap in the biofilm-based treatment of F-based wastewater. The fluoride absorbed by the biofilms is not necessarily rendered harmless or removed from the wastewater system but is merely sequestered temporarily [18]. This limitation underscores the need for additional treatment steps or the engineering of biofilms with enhanced capabilities. The development of biofilms that can not only absorb but also transform fluoride compounds into less harmful forms would be a significant advancement in the treatment of semiconductor wastewater. Such advancements could involve genetic or metabolic engineering of the biofilm-forming bacteria to enhance their fluoride-degrading capabilities or the integration of biofilm treatment with other wastewater treatment processes to ensure comprehensive fluoride removal.

Influence Factors on Biofilm Efficacy

The performance of biofilms in treating F-based wastewater in semiconductor settings is significantly affected by environmental conditions such as pH, temperature, nutrient availability, and the concentration of fluoride compounds. These factors can alter the biofilm's physical and chemical properties, impacting its ability to absorb and process fluoride (Fig. 3).

1. **Temperature:** Research highlighted the temperature-dependent nature of fluoride absorption in *Bacillus* spp. biofilms. Their study found that as the temperature increased, the biofilms showed enhanced capacity to absorb fluoride [11]. However, this increased absorption was accompanied by a compromise in the structural integrity of the biofilms. At higher temperatures, the stability of the extracellular polymeric substances (EPS) matrix, which is crucial for maintaining the biofilm's structure, was adversely affected. This finding is particularly relevant for semiconductor wastewater treatment, where temperature variations are common [6].
2. **pH:** The pH of the wastewater is another critical factor influencing biofilm efficacy. Variations in pH can change the ionization state of fluoride ions, thereby affecting their interaction with the biofilm EPS. For instance, in an acidic environment, the increased protonation may enhance the binding of fluoride ions to the EPS, while in alkaline conditions, the decreased protonation might reduce this interaction [5]. This aspect was explored in a study focusing on *Pseudomonas* spp. biofilms, where the fluoride binding efficiency varied significantly with pH changes [12].
3. **Nutrient availability:** The presence and concentration of nutrients in the wastewater also play a vital role. Nutrients are essential for the growth and maintenance of biofilms. A nutrient-rich environment can promote robust biofilm development, enhancing its capacity to interact with and absorb fluoride. Conversely, nutrient limitations can lead to weaker biofilms with reduced fluoride treatment efficiency [21].
4. **Fluoride concentration:** The concentration of fluoride in the wastewater directly impacts the biofilm's response. High concentrations of fluoride can be inhibitory to biofilm formation and function, as fluoride ions can interfere with essential biological processes within the microorganisms. On the other hand, biofilms might adapt to moderate fluoride concentrations by altering their EPS composition or metabolic pathways to cope with the stress.

In summary, managing these environmental parameters is critical for optimizing the performance of biofilm-based systems in semiconductor wastewater treatment. By

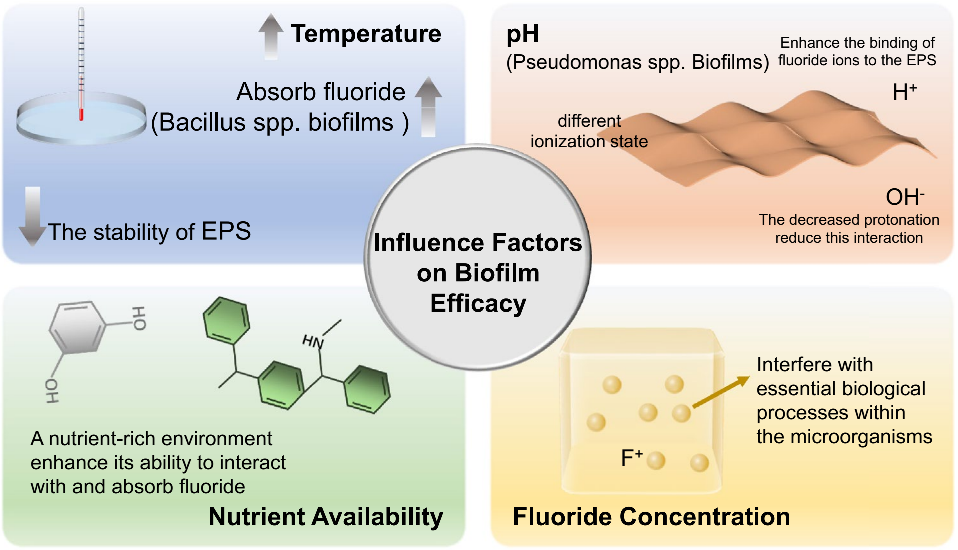

Fig. 3 Schematic diagram of factors affecting biofilm efficacy

understanding and controlling the interplay between temperature, pH, nutrients, and fluoride concentrations, it is possible to enhance the resilience and efficiency of biofilms, ensuring more effective and sustainable fluoride removal processes.

Environmental Release

In addressing the environmental release of fluoride from biofilms used in treating semiconductor wastewater, it is critical to evaluate the implications for environmental protection regulations and the potential for secondary contamination. Study highlights the impermanent nature of fluoride ion sequestration by biofilms. The stability of this process is influenced by environmental conditions, such as pH levels and ionic strength, which can induce the desorption of fluoride. An increase in pH, for instance, can diminish the fluoride ions' binding affinity to the extracellular polymeric substances (EPS) within the biofilm, leading to their re-entry into the water. The natural decay or disruption of biofilms presents another route for fluoride release. Over time, biofilms may degrade due to physical or chemical stressors, leading to a breakdown and subsequent release of absorbed pollutants, including fluoride, into the environment. This release could occur gradually or abruptly, contingent on the disruption's nature and the biofilm's stability. This potential release poses substantial concerns regarding environmental regulations, especially for the semiconductor industry, which relies on biofilms for wastewater treatment [22]. It necessitates stringent monitoring and management of the treatment process to prevent secondary environmental contamination. This includes consistent evaluation of biofilm integrity and environmental conditions.

To mitigate the risk of fluoride release, several strategies can be implemented. Regular biofilm replacement or rejuvenation, precise control of environmental factors, and pairing biofilm treatment with complementary wastewater treatment methods are viable strategies. There is also a clear opportunity for research into the development of biofilms that maintain a higher fluoride affinity, even under variable conditions, which could significantly contribute to more sustainable and environmentally responsible wastewater management. Furthermore, the discussion of greenhouse gases, particularly fluoride-based gases, is pertinent. These gases, commonly used in industrial applications, have high global warming potential. The release of fluorides from industrial processes into the atmosphere can exacerbate the greenhouse effect. Thus, effective fluoride sequestration and control in wastewater treatment not only prevent water pollution but also potentially reduce the emissions of fluoride-based greenhouse gases by curtailing their cycle through environmental systems. The semiconductor industry, in this context, could significantly contribute to environmental sustainability efforts by innovating and implementing robust fluoride management practices in their wastewater treatment processes [15].

Scaling Up Biofilm-Based Treatments for Industrial Applications

Scaling biofilm-based treatments from laboratory to industrial scales is fraught with challenges that stem from both technical and operational complexities. One of the primary concerns is maintaining the structural integrity and biological activity of the biofilms amidst the fluctuating conditions typical of industrial wastewater streams. Biofilms that are effective in controlled, small-scale systems may struggle to cope with the variable flow rates, pollutant loads, and chemical compositions encountered in full-scale operations. Technical challenges include designing bioreactors that can support biofilm growth without compromising system efficiency. This involves ensuring adequate nutrient supply, appropriate shear forces, and effective distribution of the wastewater across the biofilm. Furthermore, operational challenges encompass maintaining consistent biofilm thickness to prevent clogging, managing biofilm detachment due to shear stress, and avoiding the buildup of inert biomass, which can reduce treatment efficiency. Other critical issues include controlling the presence of predators or invasive species that can destabilize the biofilm ecology, as well as the need for regular biofilm regeneration to maintain peak metabolic activity. Additionally, the integration of biofilm systems into existing wastewater treatment infrastructures requires careful planning to ensure compatibility and effectiveness.

Environmental Sustainability of Biofilm-Based Fluoride Removal in the Semiconductor Industry

Biofilm-based systems for fluoride removal offer significant environmental benefits beyond pollutant reduction, primarily by minimizing the need for chemical treatments that can generate secondary pollutants. These systems harness the natural degradative processes of biofilms, which are composed of fluoride-resistant bacteria such as *Pseudomonas* spp. and *Bacillus* spp. These bacteria are adept at forming dense, protective biofilms that reduce fluoride penetration effectively, particularly important in the semiconductor industry where wastewater treatment standards are stringent [23]. A critical consideration in sustainable biofilm operations is the fate of fluoride ions after treatment. Life Cycle Assessment (LCA) provides a quantitative framework to evaluate the environmental impacts of these systems, measuring how different fluoride concentrations affect biofilm efficiency. This assessment includes an analysis of energy consumption, chemical use, and the longevity of biofilm systems under varying conditions of fluoride exposure—from trace levels of 1 ppm to concentrations exceeding 15 ppm [24]. LCA also examines the implications of post-treatment fluoride management. If not properly handled, fluoride can leach back into the environment, contaminating soil and water sources, which poses a threat to ecological health. Sustainable practices include the recovery and reuse of fluoride from biofilms, especially in industries like glass or ceramics where fluoride is a valuable input. Alternatively, fluoride can be transformed into non-leachable forms suitable for safe disposal, such as stable mineral forms or incorporation into construction materials, preventing secondary contamination [19, 22]. Moreover, the LCA explores the trade-offs between reducing fluoride levels in wastewater and the potential increase in resource use required to maintain optimal biofilm functionality. This helps semiconductor manufacturers not only comply with environmental regulations but also optimize their wastewater treatment processes for maximum sustainability and cost efficiency. Such assessments are crucial for developing strategies that reduce the environmental footprint while ensuring the effectiveness of fluoride removal.

Future Directions

Towards Innovative Biofilm Systems and Policy Influence

Deploying biofilms for fluoride-based (F-based) wastewater treatment brings environmental challenges into concert with technological innovations, directly supporting the Sustainable Development Goals (SDGs) on Clean Water and Sanitation (SDG 6) and Industry, Innovation, and Infrastructure (SDG 9). Confronting the resistance of fluoride compounds to biodegradation represents a significant hurdle for biofilm applications in treating industrial wastewater, especially from semiconductor manufacturing [11]. Traditional biofilm methodologies often fall short in reducing fluoride concentrations to acceptable levels due to the chemical tenacity of fluoride compounds. To address this, "innovatively designed biofilm systems" refer to advanced bioreactors that incorporate not just natural biofilm-forming organisms but also those that are genetically modified or selected for their heightened resistance and degradation abilities against fluoride. Such systems could include novel configurations that maximize contact between biofilms and wastewater, utilize specialized growth media to enhance microbial activity, or apply innovative process controls to optimize degradation pathways.

The complex contaminant spectrum in semiconductor wastewater, including various pollutants along with fluoride, challenges the specificity and efficiency of biofilm targeting. This complexity necessitates biofilm systems that are both selective for fluoride and robust against a multitude of interfering substances. Scaling up these systems to industrial levels introduces additional challenges, including the need for consistent performance and resilience under variable wastewater conditions. However, these challenges also offer rich opportunities to expand our understanding of microbial survival in extreme environments [25, 26]. Delving into these survival strategies may reveal new avenues for bioremediation. The potential of custom-designed biofilm systems, potentially utilizing genetically tailored microbes, represents a transformative advancement in wastewater treatment technology. Embracing these opportunities requires collaborative efforts across disciplines—microbiology, environmental engineering, and the semiconductor industry itself—to engineer breakthrough solutions that can tackle the multifaceted demands of industrial wastewater treatment.

Furthermore, the successful implementation of these innovative biofilm systems can have a profound impact on environmental policy and regulation. By demonstrating that advanced, effective treatment of F-based wastewater is achievable, regulators might be influenced to establish more stringent environmental standards [27]. This, in turn, would push the semiconductor industry and others toward adopting these sustainable practices, contributing to global efforts in environmental protection. The ripple effect of such a technological leap could set new benchmarks for industrial wastewater management, fostering a culture of sustainability that aligns with the SDGs and enhances the industry's license to operate within a greener future.

Targeted Bioremediation Strategies for Fluoride Mitigation

The advancement of bioremediation technologies, particularly for fluoride-based (F-based) wastewater, stands at the forefront of combining environmental sustainability with industrial innovation, resonating with the objectives of Sustainable Development Goal 12 (SDG 12) on responsible consumption and production. A central challenge in fluoride remediation is the precise targeting of fluoride compounds due to their chemical stability and widespread presence amidst a variety of pollutants. Traditional biofilms exhibit broad-spectrum bioremediation capabilities, yet they often lack the specificity required for efficient fluoride mitigation [28]. To address this, efforts in genetic and metabolic engineering are pivotal, focusing on the creation of biofilms that specifically target and degrade fluoride compounds.

The pursuit involves identifying and enhancing genes in biofilm-forming microbes that confer fluoride resistance and sequestration abilities. The strategic manipulation of such genetic pathways can yield biofilms tailored for high-fluoride environments, preserving their functional integrity and enhancing their treatment efficacy. Metabolic engineering further complements this by refining biodegradation pathways, particularly by augmenting the fluoride affinity and catalytic activity of pertinent enzymes. Through such targeted modifications, the biofilms can achieve a higher degradation rate, offering a more directed and efficacious approach to fluoride remediation. The implications of these targeted bioremediation strategies are manifold. By focusing on the specific degradation of fluoride compounds, these engineered biofilms minimize the environmental impact of industrial processes, contributing to the conservation of aquatic ecosystems and aligning with broader environmental protection initiatives. This specificity is crucial as it translates into more efficient resource utilization, reduced reliance on chemical treatments, and an overall decrease in the environmental footprint of industrial activities, particularly in the semiconductor sector [29].

In an era of heightened environmental scrutiny, the semiconductor industry's shift towards adopting such specific bioremediation techniques is not merely an environmental necessity but also a strategic business decision. These innovative technologies, which epitomize the spirit of SDG 12, have the potential to redefine the industry's approach to wastewater management. By advancing these genetically and metabolically engineered biofilms, we are setting the stage for industrial operations where environmental responsibility is deeply embedded and where sustainability goes hand in hand with industrial progress. The future success of these biofilm technologies in managing F-based wastewater could serve as a model for policy development, urging regulatory bodies to advocate for high precision bioremediation methods. Demonstrating the effectiveness of such targeted approaches could catalyze the adoption of stringent environmental standards, driving the semiconductor industry towards sustainable evolution and inspiring similar shifts across diverse industrial landscapes.

Leveraging Antagonistic Behaviors in Biofilm Communities for Enhanced Fluoride Removal

Exploring the potential of antagonistic behaviors within biofilm communities presents a promising avenue for advancing the efficacy of biofilm-based fluoride removal systems in semiconductor wastewater treatment. Understanding and harnessing these interactions could lead to innovative strategies that optimize biofilm functionality and resilience. Research into microbial competition within biofilms could reveal how interactions among different microbial species affect their resilience to fluoride and their capacity for fluoride removal [12]. By identifying competitive relationships that promote the dominance of fluoride-resistant microbes, it might be possible to engineer biofilms that are inherently more effective at handling high fluoride loads. This could involve selectively enhancing beneficial microbial strains or suppressing less advantageous ones through targeted microbial management strategies. In addition to competitive interactions, the role of natural inhibitory compounds produced within biofilms offers intriguing possibilities. These compounds, which can suppress non-beneficial bacteria or enhance the structural stability and fluoride resistance of the biofilm, merit detailed investigation. Characterizing these compounds and understanding their mechanisms could provide new methods to manipulate biofilm dynamics, potentially making them more robust against environmental stresses including high fluoride concentrations [13].

Manipulation of quorum sensing mechanisms also represents a significant area of potential research. These signaling pathways, essential for coordinating biofilm development and behavior, could be influenced by external factors such as fluoride exposure. By developing ways to manipulate these pathways—either enhancing or inhibiting specific signals—researchers could directly influence biofilm formation, stability, and functionality. Moreover, the study of biofilm predation—utilizing bacteriophages or predatory bacteria to target specific microbial populations within biofilms—could provide a novel approach to managing biofilm composition. This strategy could help optimize the functional properties of biofilms, making them more effective for wastewater treatment applications. By selectively removing detrimental or inefficient bacterial populations, it might be possible to enhance the overall performance of the biofilm [12, 30].

Finally, an integrative approach that combines insights from microbial ecology, molecular biology, and environmental engineering could lead to the development of holistic strategies. These strategies would not only address the ecological dynamics of biofilm communities but also meet the technical and environmental challenges of semiconductor wastewater treatment processes. By focusing on these areas, future research can significantly advance the application and management of biofilm-based systems for fluoride removal, leading to more sustainable and effective solutions in industrial settings. Such studies will not only address immediate industrial needs but also enhance broader environmental protection efforts by developing systems that are both robust and adaptable to varying treatment conditions [17].

Integrated Treatment Approaches

The complexity of fluoride-based (F-based) wastewater, particularly from industries like semiconductor manufacturing, necessitates a comprehensive approach to treatment. Traditional biofilm-based treatments, while effective, often fall short in addressing the multifaceted nature of this type of wastewater. This is where integrated treatment approaches become crucial. By combining biofilm technology with other physical or chemical methods, a more holistic strategy for wastewater management can be

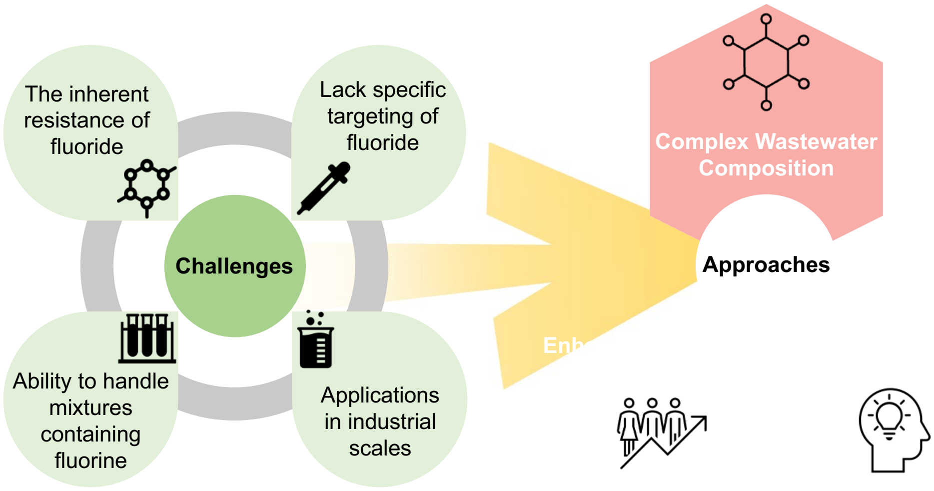

Fig. 4 Challenges and solutions for the future

developed (Fig. 4). This not only enhances the efficiency of the treatment but also aligns with Sustainable Development Goal 11 (SDG 11)—"Sustainable Cities and Communities," aiming to mitigate the environmental impact of industrial processes.

- **Complex wastewater composition:** F-based wastewater is not just about fluoride; it often contains a diverse array of contaminants. This complexity arises from the various chemicals and processes used in industries like semiconductor manufacturing. To effectively tackle this broad spectrum of pollutants, integrating biofilm technology with other treatment methods is essential [30]. This integration ensures a more comprehensive treatment, addressing each contaminant effectively.
- **Enhanced treatment efficiency:** The synergy between biofilm-based treatments and other methods like advanced filtration techniques or chemical precipitation processes can significantly boost the efficiency of wastewater treatment. This combination leads to higher removal rates of fluoride and other contaminants, ensuring a more thorough purification process. The enhanced efficiency is not just about removing more contaminants but also about doing so in a more energy and resource-efficient manner.
- **Resilience and adaptability:** Integrated approaches foster resilience in wastewater management systems. These systems are better equipped to adapt to changing environmental conditions and evolving industrial requirements. The dynamic nature of industrial wastewater, with its fluctuating compositions and concentrations of pollutants, demands a treatment strategy that can be modified and adapted in response to these changes. This adaptability is key to sustainable and long-term wastewater management [31].

By efficiently removing a wider range of contaminants, integrated treatment approaches play a direct role in reducing the environmental impact of industrial activities. This is crucial for minimizing the release of harmful substances into the environment, thereby supporting the goal of creating sustainable cities and communities. The implementation of integrated wastewater treatment methods may also result in significantly improved water quality [4]. This is vital for the health of ecosystems and communities alike. High-quality water resources are a cornerstone of sustainable urban development, and integrated treatment approaches are instrumental in achieving this. As SDG 11 emphasizes the importance of building resilient infrastructure, integrated wastewater management systems are a prime example of this. Their adaptability and effectiveness make them a robust solution for addressing the challenges posed by industrial wastewater. These systems not only manage the immediate concerns of wastewater treatment but also contribute to the overall integrity and resilience of urban environments.

Conclusion

The intersection of biofilm ecology with fluoride-rich wastewater from semiconductor manufacturing offers a compelling case study of environmental adaptation and resilience. This review reveals the profound capacity of microbial communities to withstand industrial pollutants, reflecting the broader themes of sustainability and innovation in environmental management. As the semiconductor industry continues to advance, so too must the strategies for wastewater treatment, ensuring that environmental stewardship remains at the forefront of industrial practice. This review underscores the significance of interdisciplinary approaches to managing industrial wastewater, integrating microbiological insights with environmental engineering solutions to address the challenges posed by fluoride in the semiconductor industry. It highlights the potential of biofilms as a natural resource for mitigating the environmental impact of industrial processes, paving the way for more sustainable manufacturing practices.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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