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REVIEW

Enhancing bioaugmentation in wastewater treatment: the emerging role of aggregating bacteria as mediators in cell immobilization—review

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Abstract Bioaugmentation is a promising strategy to enhance biological wastewater treatment by introducing functional microbial strains that improve pollutant degradation and nutrient removal. However, the practical success of bioaugmentation is often limited by the washout of introduced bacteria, low colonization efficiency, and competition with native microbial communities. A key challenge is the lack of natural aggregation or biofilm-forming ability in many functional strains, making them vulnerable to operational stresses and system perturbations. Although conventional immobilization techniques have been applied to improve microbial retention, these approaches can be costly and may reduce microbial activity. Aerobic granules, highly structured microbial aggregates known for their strong settling properties, dense architecture, and intrinsic stability, have recently emerged as a valuable source of naturally aggregating and biofilm-forming bacteria. These granule-derived microorganisms exhibit functional traits that support biological immobilization, enhancing the persistence and performance of introduced strains. Acting as bridging microorganisms, they promote coaggregation and physical integration with functional bacteria, facilitating biofilm formation and supporting community stability. Although several case studies highlight the potential of these bacteria in improving bioaugmentation outcomes, a comprehensive exploration of their functional traits, ecological interactions, and engineering applications remains limited. This review systematically examines recent advances in bioaugmentation strategies using aggregating bacteria, particularly those derived from aerobic granules, elucidating their mechanisms of action and role in supporting microbial persistence and synergy. By focusing on their capacity to promote microbial immobilization and integration in engineered systems, this work highlights a promising direction for improving bioaugmentation performance. The review identifies key research gaps and provides a framework for designing more resilient and effective microbial strategies for wastewater treatment.

Keywords Bioaugmentation · Aggregating bacteria · Immobilization · Wastewater treatment · Biofilm · Aerobic granules

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1 Introduction

Wastewater treatment is a vital component of global environmental protection, aiming to reduce the adverse impacts of effluents on natural ecosystems. However, the growing complexity and volume of wastewater, driven by urbanization, industrialization, and population growth, are challenging the efficiency of conventional treatment technologies. To address this challenge, biological wastewater treatment processes are increasingly favoured due to their eco-friendly and cost-effective nature (Zhang et al. 2017). In principle, these processes rely on the metabolic activity of microorganisms to degrade pollutants, with their overall effectiveness depending heavily on the presence and activity of specific functional microbial populations. However, biological performance is often limited by the slow growth rates of certain functional microorganisms, such as nitrifiers and phosphorus-accumulating organisms, which struggle to persist in systems operating under short sludge retention times (SRT) (Gong et al. 2024; Huang & Cui 2025). These constraints highlight the need for targeted strategies that can reinforce the presence and activity of slow-growing functional microorganisms, thereby improving the overall stability and performance of biological treatment systems.

To overcome these limitations, bioaugmentation has emerged as a targeted biological strategy. It involves the deliberate introduction of specialized microbial strains or consortia with specific degradation capabilities to strengthen native microbial communities and improve treatment performance (Chen et al. 2015; Ma et al. 2022; Raper et al. 2018). In practice, specialized microbial strains or consortia with specific functional capabilities are intentionally introduced into existing treatment systems, where the functional bacteria perform specific, beneficial roles in ecosystems, soil, water, and industrial processes. Rather than being defined by their shape or taxonomy, functional bacteria are categorized by *function*, such as nutrient cycling, symbiosis with hosts, fermentation, bioremediation, and genetic engineering. By boosting the abundance and activity of functional bacteria, bioaugmentation offers a practical and targeted solution to enhance biological treatment efficiency where natural microbial growth alone is insufficient, particularly in systems with short SRTs.

While bioaugmentation has demonstrated benefits in terms of process stability, nutrient removal, and system resilience (Wang et al. 2024), its broader application is often hindered by challenges such as washout of the introduced strains, limited colonization, and competition with indigenous microbes (Herrero & Stuckey 2015). This is largely associated with the lack of natural aggregation or biofilm-forming ability in many bioaugmented strains, making them vulnerable to environmental stress and operational disruptions such as shock loads. These sudden fluctuations in wastewater composition caused by industrial discharges, pH shifts, or toxic compounds can destabilize microbial communities and reduce treatment efficiency. To mitigate these issues, conventional carrier-based immobilization techniques, including physical and chemical entrapment, have been used to enhance microbial retention and protect introduced strains (Li et al. 2013). However, these synthetic carrier-based immobilized systems often involve high costs, complexity, or reduced microbial activity.

As an alternative to conventional immobilization methods, growing attention has focused on microbial aggregates that naturally develop within engineered wastewater systems. Among these, aerobic granules have emerged as an important ecological niche enriched with microorganisms that display strong aggregation and attachment capabilities (Pincam et al. 2024). Their compact structure, steep microbial stratification, and resilience under fluctuating operational conditions (Zheng et al. 2024) create an environment that selects for bacteria with inherent abilities to adhere, cooperate, and withstand hydraulic stress. These characteristics make aerobic granules a promising reservoir of aggregating bacteria that can potentially support bioaugmentation by improving the retention and stability of introduced strains (Cydzik-Kwiatkowska et al. 2022). Rather than functioning merely as passive biomass, bacteria originating from granules often participate actively in interspecies interactions, aiding the establishment of functional populations and strengthening community-level robustness (Zhao et al. 2021). Recognizing this untapped potential, recent research has begun exploring how granule-associated organisms can be leveraged to facilitate microbial integration and enhance the long-term success of bioaugmentation strategies (J. Liu et al. 2025a, b; Zhang et al. 2025). These findings suggest that granule-associated organisms play a key role in supporting sustained bioaugmentation success.

This review aims to critically examine the emerging role of aggregating bacteria as mediators of cell immobilization in bioaugmentation for wastewater treatment. While previous studies have examined microbial immobilization (Najim et al. 2024; Ogundolie et al. 2024) and bioaugmentation (Chettri et al. 2024; Herrero & Stuckey 2015; Raper et al. 2018) separately, they rarely address the functional role of naturally aggregating bacteria in enhancing the persistence, colonization, and synergistic performance of introduced strains. The review first provides an overview of the bioaugmentation process to identify limitations in microbial persistence and gaps in conventional strategies. Key factors influencing bioaugmentation performance, including microbial interactions, aggregation traits, and environmental conditions, are then examined, as these aspects are rarely addressed systematically in the literature. Following this, the functional role of aggregating bacteria in mediating cell immobilization and facilitating community integration is discussed. Finally, by synthesizing microbial trait-based insights with engineering considerations, we propose a novel framework to guide the design of more resilient and effective bioaugmentation strategies in wastewater treatment systems.

2 Overview of bioaugmentation

Bioaugmentation, also known as seeding, is an *in-situ* biological approach to contaminant remediation, where supplementary microorganisms with specific biodegradation capacities are introduced to enhance the microbial community's diversity and improve the degradation performance in a polluted environment (Chettri et al. 2024; Ma et al. 2022; Raper et al. 2018). Numerous studies have focused on evaluating bioaugmentation strategies for wastewater treatment, aiming to enhance the effectiveness of nutrient removal and improve overall system performance (Chen et al. 2015; Hong et al. 2024; Jia et al. 2019; Liu et al. 2018; Wang et al. 2023; Yang et al. 2022). Collectively, they have demonstrated that bioaugmentation has become an important tool for strengthening biological treatment processes, underscoring the need to understand how different implementation strategies influence its overall success.

Conventional bioaugmentation strategies primarily involve the addition of free-floating microbial cells, either as pre-adapted pure strains, microbial consortia, genetically engineered bacteria, or vectors carrying specific biodegradation genes, into biological treatment systems to enhance pollutant degradation (El Fantroussi & Agathos 2005). Although isolated pure strains have been widely applied in bioaugmentation for wastewater treatment (Nancharaiah et al. 2008; Plangklang & Reungsang 2009; Zhu et al. 2015), the use of enriched mixed microbial cultures is increasingly preferred as mixed consortia often exhibit greater metabolic diversity, ecological stability, and resilience under fluctuating environmental conditions, thereby enhancing the overall effectiveness and robustness of bioaugmentation strategies (Bhatt et al. 2021; Feng et al. 2021; Mrozik & Piotrowska-Seget 2010). This shows that the success of conventional bioaugmentation depends not only on the type of inoculum used but also on the conditions that govern its survival, stability, and activity within the treatment system.

2.1 Factors influencing the stability and efficiency of bioaugmentation

The success of any bioaugmentation application fundamentally depends on the ability of the introduced bacteria to persist and remain active within the treatment environment, a process governed by multiple interconnected factors (Fig. 1).

2.1.1 Selection of bioaugmentation strain and its characteristics

The most critical step in achieving successful bioaugmentation is the selection of appropriate microorganisms with desirable functional traits. These microorganisms should exhibit a high degradative potential toward specific pollutant(s) and possess key physiological and ecological characteristics such as biofilm-forming ability, aggregation capacity, and production of extracellular polymeric substances (EPS). Additional traits that enhance their effectiveness include bio-flocculating activity, motility, and the synthesis of biosurfactants and autoinducers. To ensure persistence within the

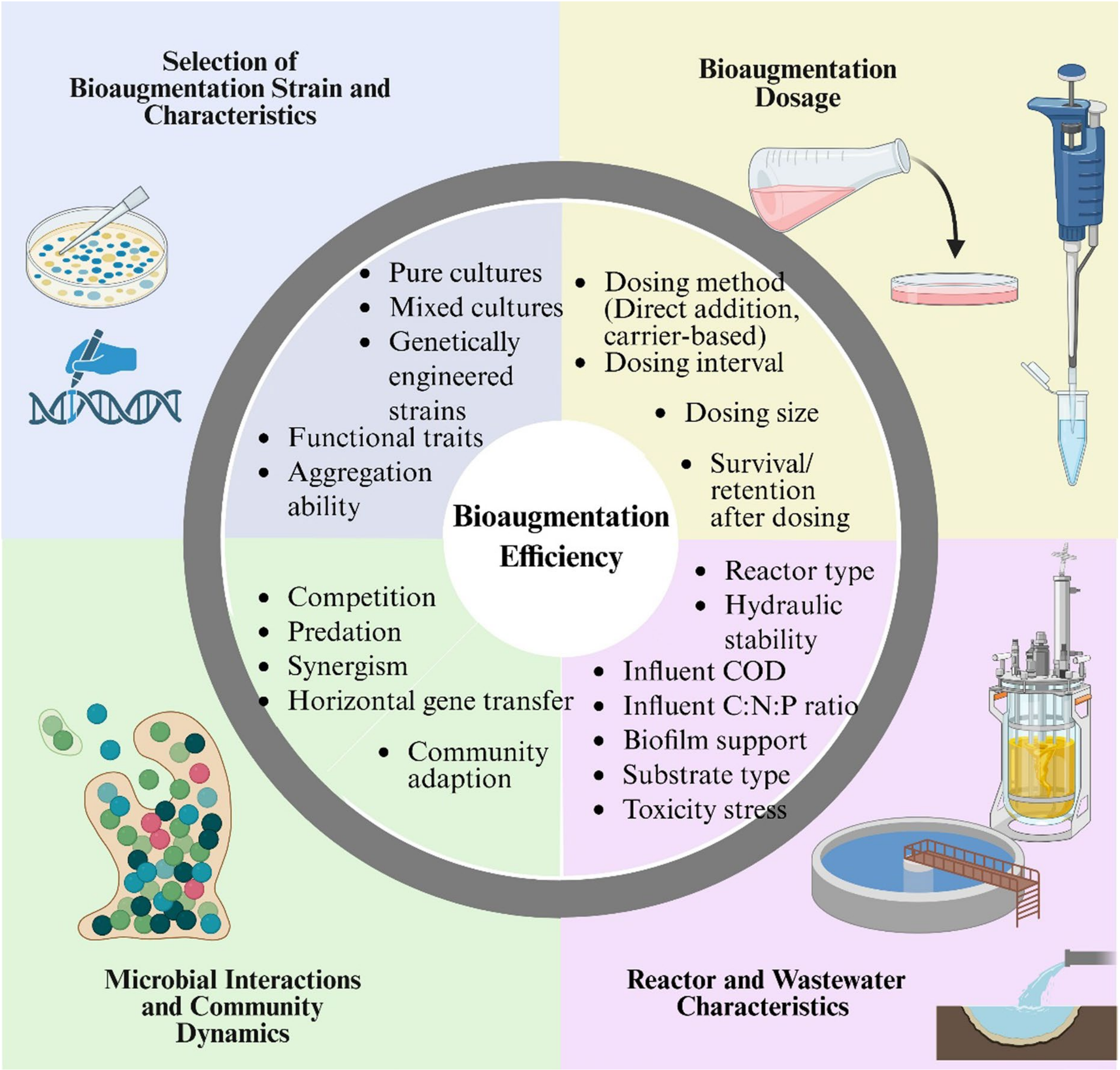

Fig. 1 Interconnected roles of strain selection, dosing strategy, reactor configuration, wastewater characteristics, and microbial community dynamics in determining bioaugmentation efficiency

treatment system, the selected strains must be able to survive post-inoculation, integrate into existing sludge flocs, or contribute to the formation of microbial granules. Improper selection of microbial strains for bioaugmentation in industrial wastewater treatment can lead to unsatisfactory or inconsistent results. To enhance compatibility and performance, studies have focused on isolating inoculum from the native microbial communities of the target environment (Aguilar-Romero et al. 2025; Carlos et al. 2016; Dong et al. 2025; Fan et al. 2025; Feng et al. 2021; Shan et al. 2023, 2025; Wang et al. 2022). This approach offers a significant advantage, as the selected strains are already adapted to the specific physicochemical conditions and pollutant profiles of the system, increasing their chances of survival and activity post-inoculation (Chettri et al. 2024).Therefore, the use of environmentally adapted strains represents one of the key strategies for achieving efficient bioaugmentation.

These selected strains could be either pure cultures or microbial consortia. In many bioaugmentation approaches, the use of multiple strains in consortia has been beneficial in improving pollutant treatment (Raper et al. 2018). For instance, the bacterial consortium HM-4, composed of four isolates (*Bacillus cereus*, *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Stenotrophomonas acidaminiphila*), was tested for its ability to decolorize six azo dyes. While individual isolates could fully decolorize only two of the dyes (AR-119 and AB-113), the consortium successfully decolorized all six dyes at 20 mg/L and did so at a significantly faster rate than any single isolate (Khehra et al. 2005). This shows that well-designed microbial consortia can achieve faster and more comprehensive pollutant degradation than single strains alone in bioaugmentation.

Genetically modified organisms could be used to overexpress pollutant degradation genes or increase survivability (Raper et al. 2018). Under extreme wastewater conditions such as high salinity, the presence of recalcitrant organics, and potentially toxic elements, isolating naturally occurring strains with the capacity to tolerate multiple stresses and perform targeted functions is time-consuming and inconsistent (Al-Ansari et al. 2021). Generally, in industrial wastewater, complex mixtures of organic compounds, metals, and other contaminants co-exist. Research shows that co-contaminants often exhibit synergistic toxic effects, leading to greater overall toxicity than individual pollutants alone (Wen et al. 2018). Microbial consortia or engineered strains with enhanced resistance to multiple contaminants, along with versatile metabolic pathways, may offer more robust and effective bioaugmentation solutions under these complex conditions. However, the deployment of engineered microbes for environmental biotechnology is tightly governed by biosafety and regulatory frameworks that vary across regions (Lea-Smith et al. 2025). Frameworks in the UK, EU, and USA impose rigorous assessments due to concerns over unintended ecological effects, including disruption of native microbial communities and horizontal gene transfer. Public acceptance of engineered strains is often constrained by information gaps and limited awareness of biosafety issues, underscoring the need for transparent communication and education when deploying genetically engineered microbes in environmental systems (Ampadu-Ameyaw et al. 2021). Further, the widespread release of genetically engineered bacteria into natural environments remains controversial due to potential impacts on human health, issues closely linked to modern discussions on ecological risk, regulatory oversight, and public perception (Rafeeq et al. 2023). Therefore, although engineered strains offer promising solutions for complex wastewater environments, overcoming these biosafety and regulatory challenges remains essential before their widespread adoption can be realised.

2.1.2 *Bioaugmentation dosage*

The techniques of dosing or applying microorganisms to a treatment system affects the efficiency of bioaugmentation. The dosage and dosing interval, whether through a single or repeated application are two critical factors in the bacterial dosing strategy for bioaugmentation. Selecting an appropriate dosing method can significantly enhance an introduced strain's competitiveness against indigenous microorganisms and improve its adaptability to the treatment environment (Ma et al. 2022). Lee et al. (2022) studied the effect of bioaugmentation dosage interval with *Methanosarcina thermophila* using two dosing intervals as single and repeated where 10% v/v of the microbes grown on biochar (1 g/L) were added at setup of the reactors in single dosing, and same dose was added over 10 feeding cycles in repeated dosing. The best repeated reactor showed 37% more yield, while the best single reactor presented 32%. This repeated dosing, or frequent dosing method, is useful in bioaugmentation to avoid wash away due to the temporary stability of the newly introduced strains (Herrero & Stuckey 2015). However, the process can be labour-intensive and costly, depending on the microbial strain and the bioaugmentation technique employed.

When considering the characteristics of bioaugmentation dosing, the initial inoculum size or dosing size is a critical factor, as it must be sufficiently large to overcome initial predation and competition pressures, yet not so excessive that it disrupts the existing microbial community or ecosystem balance in the reactor system (Raper et al. 2018). Evidence across environmental matrices supports this; Gupta et al. (2022) observed that inoculum concentrations in the range of 10^7-10^9 CFU/mL consistently enhanced diesel and biodiesel degradation, indicating that a threshold abundance is often necessary to establish functional activity. However, dosage optimisation is shaped not only by biological requirements but also by operational constraints. Ahmed and Baitharu (2023) emphasised that factors such as the availability and quality of seeding biomass, transfer logistics, and cost–benefit considerations collectively restrict the practical upper limits of bioaugmentation dosing. Thus, while higher doses may improve short-term performance, sustainable applications require dosing strategies that balance ecological effectiveness with operational feasibility.

However, some studies (Lyon & Vogel 2013) indicate that enhanced microbial abundance does not always equate to improved degradation efficiency, reinforcing the need for strategic dosing that integrates inoculum amount, timing, and frequency. The work of Lyon and Vogel (2013) illustrates this interplay vividly; when *Pseudomonas cepacia* was introduced at very low densities (230–360 cells/mL) into non-sterile lake water, the inoculum collapsed under protozoan grazing and nutrient limitations, preventing p-nitrophenol mineralisation. Increasing the dose to $\geq 10^4$ cells/mL, however, enabled survival and successful degradation, and interventions such as protozoan suppression or nutrient supplementation restored activity even at lower starting densities. Collectively, these findings demonstrate that adequate initial inoculum size is essential to overcome early ecological losses in bioaugmentation, particularly in complex systems like wastewater, where competition and grazing can rapidly diminish introduced populations.

2.1.3 *Microbial interactions and community dynamics*

The interaction between introduced microbial strains, indigenous microorganisms, and pollutants plays a critical role in the success of bioaugmentation by influencing the structure, function, and stability of microbial communities. These interactions can result in either synergistic cooperation or competitive exclusion, ultimately affecting the persistence and activity of the bioaugmented strains within the treatment system.

One important mechanism facilitating community adaptation is horizontal gene transfer (HGT), where introduced bacteria can share catabolic genes, often carried on plasmids with native microbes. This gene exchange enhances the overall degradation capacity of the community by spreading pollutant-degrading abilities beyond the inoculated strains. For example, Nancharaiah et al. (2008) demonstrated plasmid transfer of a green fluorescent protein-tagged *Pseudomonas putida* KT2442 strain to indigenous aerobic granule microbes, facilitated by biofilm formation and EPS. EPS secretion not only promotes microbial aggregation and stable biofilm development but also supports genetic exchange and metabolic cooperation.

Bioaugmentation often causes shifts in the microbial community structure. While overall diversity may temporarily decline, the abundance of introduced strains or indigenous bacteria capable of degrading target pollutants generally increases. For instance, Feng et al. (2021) reported that bioaugmentation with a synthetic consortium of *Pseudomonas putida* and *Rhodococcus ruber* to degrade benzene, toluene, and styrene (BTS), achieved above 90% removal efficiency in both sludge and sewage while *Acinetobacter* emerged as the most dominant genus in sludge samples after bioaugmentation, accounting for 27.8% of total sequences, followed by *Pseudoxanthomonas* (13.2%), *Comamonas* (4.7%), and *Flavobacterium* (4.4%). The increase in abundance of *Acinetobacter* and *Pseudoxanthomonas* compared to the non-augmented system suggests that the introduced strains not only persisted but also altered the indigenous community's composition and promoted the growth of other functional degraders to enhance the overall catabolic potential of the microbial community. This dominance may result from selective advantages such as high degradation efficiency for hydrocarbons (e.g., BTEX, alkanes, PAHs), effective biofilm formation, and horizontal gene transfer. For instance, *Pseudomonas* and *Rhodococcus* are known to secrete EPS, which enhances biofilm development and cell-surface hydrophobicity. Such biofilms facilitate microbial aggregation and enable lateral gene transfer of catabolic plasmids within the community, potentially enhancing the metabolic capabilities of both the inoculated and native strains.

Similarly, Wang et al. (2024) found that introducing the auto-aggregating *Pseudomonas* strain XL-2 enriched beneficial genera such as *Flavobacterium* and *Paracoccus* which contribute to EPS production and denitrification, thereby improving granulation and nitrogen removal. This has shown the potential of bioaugmentation to mitigate the impact of pollutants and help to preserve and maintain various bacterial species within the ecosystem (Shi et al. 2015). Thus, the use of auto-aggregating bacteria offers a promising approach for simultaneously improving contaminant removal and maintaining a balanced and resilient microbial community.

Environmental factors such as nutrient availability, temperature, pH, salinity, and dissolved oxygen (DO) also influence microbial aggregation, community composition, and bioaugmentation outcomes. Microenvironmental conditions within microbial aggregates and biofilms, such as oxygen gradients, oxidation–reduction potential (ORP), and substrate diffusion, affect microbial activity and interactions. Microelectrode studies by Shao et al. (2022) revealed that oxygen penetration into activated sludge flocs varies with particle size and external DO concentration, underscoring the importance of monitoring and optimizing these parameters for bioaugmented systems.

Recent advances in molecular tools, including fluorescence labelling, omics, and high-throughput sequencing, have improved the understanding of microbial interactions and community dynamics in bioaugmentation (Ma et al. 2022). These methods enable detailed profiling of microbial populations, gene transfer events, and functional shifts, providing insights that can guide the selection and management of inoculum to enhance treatment performance. A thorough understanding of microbial interactions and community dynamics is essential to optimize bioaugmentation strategies and achieve efficient pollutant removal.

2.1.4 Reactor type and wastewater characteristics

The success of bioaugmentation is dependent on compatibility between the microbial inoculum and the operational environment, particularly the type of bioreactor and the characteristics of the influent wastewater (Herrero & Stuckey 2015). Reactor configurations such as sequencing batch reactors (SBRs), continuous stirred tank reactors (CSTRs), membrane bioreactors (MBRs), and biofilm-based reactors differ in hydrodynamics, solids retention time, oxygen transfer, and shear forces, all of which directly influence microbial attachment, survival, and functional expression (Boavida-Dias et al. 2022; Khan et al. 2025; Y. Liu et al. 2025a, b; McAteer et al. 2020). For example, SBRs and aerobic granular sludge systems promote microbial aggregation due to their feast-famine cycles and selective pressure for settleable biomass, favouring the retention of aggregating bacteria. Meanwhile, reactors such as moving bed biofilm reactors (MBBRs) and MBRs provide large surface areas and stable hydraulic environments that support the immobilization and persistence of inoculated strains (Saini et al. 2023). A stable microbial community is more likely to develop in immobilized biomass reactors (Sipma et al. 2010). A study by Zhu et al. (2025) compared the efficacy of SBR and CSTR in enriching complete ammonium-oxidising bacteria (comammox) for nitrogen removal. Their results indicated that the SBR, with fluctuating but generally higher *in-situ* ammonium concentrations (1.0–6.0 g-N/m^3), favoured the proliferation of ammonia-oxidising bacteria (AOB). In contrast, comammox bacteria, which have a highly oligotrophic lifestyle, thrived better in the CSTR under relatively lower ammonium levels ($<$2.0 g-N/m^3). This study exemplifies how reactor configuration and operational conditions influence microbial community dynamics, a factor critical to the success of bioaugmentation.

The composition of influent wastewater, such as its carbon-to-nitrogen (C/N) ratio, nutrient load, presence of inhibitory substances, and biodegradable organic matter, governs the establishment and activity of bioaugmented strains (Chen et al. 2022). For instance, bioaugmentation outcomes differ significantly between municipal and industrial wastewaters due to variations in substrate complexity and toxicity.

In summary, while conventional bioaugmentation methods that rely on freely suspended cells can temporarily enhance the degradation of specific contaminants, their long-term effectiveness is frequently limited. Free cells are vulnerable to operational challenges such as poor retention within the reactor, washout under low sludge retention times (Abeysinghe et al. 2002), competition from established native microbial populations, and predation by protozoa. These factors collectively compromise the stability and sustainability of bioaugmentation in large-scale or continuous systems. Consequently, more robust strategies are required to ensure sustained microbial retention and functional stability, laying the foundation for exploring cell immobilization techniques as a promising alternative to overcome the inherent limitations of free cell bioaugmentation.

3 Towards robust bioaugmentation

3.1 Cell immobilization approaches

Ensuring the persistence of introduced strains is crucial in bioaugmentation, cell immobilization methods have proven to be an efficient way to achieve this goal. Cell immobilization refers to the confinement of viable microbial cells within a specific area to restrict their movement, while still maintaining their biological activity for continuous and repeated use (Martins et al. 2013). In many cases, microorganisms are encouraged to attach to surfaces or natural carriers, which are then introduced into the contaminated medium. These microbes often form biofilms, structured microbial communities that develop on surfaces exposed to water. Biofilms consist of living microorganisms embedded within a self-produced matrix made up of organic polymers, such as polysaccharides or alginate, along with adsorbed nutrients, minerals, and other substances (Nwankwegu & Onwosi 2017). Therefore, this matrix plays a crucial role in maintaining the long-term viability and performance of immobilized cells in bioaugmentation processes.

There are four main methods of cell immobilization: physical adsorption, entrapment, encapsulation, and cross-linking (or covalent bonding). Physical adsorption is the most economical, while cross-linking and covalent bonding are the most expensive (Ogundolie et al. 2024). In physical adsorption, cells attach to carriers through weak forces like hydrogen bonding, dipole interactions, van der Waals forces, or hydrophobic interactions. The type of interaction depends on the properties of both the carrier and the cells (Kumar et al. 2016). Overall, these weak interactions govern the initial attachment behaviour of cells to carrier surfaces.

The entrapment technique involves trapping microbial cells inside a fibre or gel-like material such as alginate, agar, collagen, or synthetic resins (Trelles & Rivero 2013). This creates a protective barrier around the cells, helping them stay alive and active during storage and treatment. It also prevents cell leakage, making it suitable for long-term wastewater treatment, especially in harsh or toxic environments (Najim et al. 2024). Encapsulation is a non-reversible method of immobilization, similar to entrapment, where cells or enzymes are enclosed inside a capsule with a semi-permeable membrane. This membrane allows nutrients and substrates to pass through while keeping the biocatalyst safely inside. The pore size of the membrane affects how well substances can enter and exit (Rathore et al. 2013; Rodrigues et al. 2020). The cross-linking method involves forming strong covalent bonds between cells and a solid surface using a chemical binding agent (crosslinker). The surface must first be chemically treated to allow bonding. While this method works well for attaching enzymes, it is rarely used for whole cells because the chemicals used can be toxic and may damage the cells (Prabhakar et al. 2025). Consequently, chemical bonding has limited applicability for whole-cell immobilization.

Chemical and physical immobilization techniques offer a combination of benefits and limitations, each of which affects their long-term effectiveness in bioaugmentation. These techniques offer several advantages, including enhanced biomass retention, protection from environmental stressors, and improved operational stability. Additionally, they allow for higher microbial concentrations, reuse of biomass, and better resistance to toxic substances (Bouabidi et al. 2019). Importantly, they help to mitigate one of the most common causes of bioaugmentation failure, loss of introduced microorganisms due to washout or protozoan predation (María Múnera Porras et al. 2025). Studies have reported such failures due to inoculum instability in the absence of immobilization (Boon et al. 2000; Chen et al. 2015; Chong et al. 1997; Dueholm et al. 2015; Fu et al. 2009; Park et al. 2008).While periodic dosing of microbial cultures is often used to maintain performance, it significantly increases operational costs. Immobilized cell technology thus provides a practical solution by enhancing microbial persistence and tolerance to fluctuating environmental conditions (Ma et al. 2022). It shows significant potential in treating complex wastewaters containing metals, phenolic compounds, and other refractory organic contaminants (Hou et al. 2024). Thus, cell immobilization is not only beneficial for improving microbial persistence but also crucial for enabling effective treatment of challenging and complex wastewater.

While chemical and physical immobilization methods, particularly those relying on synthetic carriers or chemical crosslinkers, have proven effective in enhancing microbial persistence, they have significant limitations. Diffusional constraints, gradual loss of microbial activity, potential toxicity from chemical crosslinkers, carrier degradation, and operational complexity can reduce their long-term efficiency and increase costs (Najim et al. 2024). Additionally, these approaches often fail to fully exploit the inherent capabilities of microbial communities, such as self-aggregation and biofilm formation. Such limitations highlight the need for more sustainable and biologically compatible strategies that can naturally retain microorganisms, enhance their interactions with native sludge communities, and improve overall bioaugmentation stability.

These limitations have driven increasing interest in more biologically compatible, carrier-free immobilization systems that avoid the challenges associated with artificial matrices. Such approaches aim to achieve microbial retention and stability through natural mechanisms rather than relying on external carriers.

3.2 Microbial aggregation strategies for enhancing bioaugmentation performance

In this context, aggregating bacteria have emerged as promising natural immobilizers, capable of forming self-aggregates or flocs that serve as scaffolds for introduced pollutant-degrading strains. These bacteria not only enhance microbial retention but also support biofilm development and community stability, effectively bridging the gap between conventional immobilization techniques and biologically driven wastewater treatment strategies.

While many pollutant-degrading bacterial strains have limited capacity to form biofilms on carrier surfaces, certain bacteria can act as bridging organisms, facilitating the integration of other microbes into biofilms (Li et al. 2013). Some strains possess strong biofilm-forming and coaggregation abilities, promoting the development of stable, multi-species biofilms that retain and support a diverse microbial community (Rickard et al. 2002). Since the colonization and persistence of inoculated microbes are critical for successful bioaugmentation, leveraging natural coaggregation offers a simple, cost-effective alternative to synthetic immobilization methods for stabilizing functional bacteria in wastewater treatment systems.

Microbial aggregation exists along a continuum, from flocs to biofilms and granules (Fig. 2). Both biofilm and granule formation begin with initial bioaggregation that continues to grow until a threshold size is reached. Once this point is surpassed, these structures may disperse, releasing floccular biomass and initiating a new cycle of aggregation. These microbial aggregates are highly organized and dynamic, capable of adapting to environmental conditions. By engineering these structures, it is possible to create specific microbial niches that support targeted functions, offering a flexible and effective strategy for bioaugmentation and wastewater treatment (Aqeel et al. 2019). Overall, this highlights microbial aggregation as a dynamic process that can be strategically harnessed to create more stable and efficient systems for improving the stability and functionality of engineered treatment processes.

As interest grows in harnessing these naturally aggregating bacteria, understanding the underlying mechanisms that drive their role as natural immobilizers and microbial bridges becomes essential. These mechanisms govern not only how bacteria self-aggregate but also how they integrate other strains into stable biofilms; directly influencing bioaugmentation success.

3.2.1 *Functional drivers of microbial aggregation in bioaugmented wastewater systems*

Understanding the functional drivers of microbial aggregation is essential for elucidating the mechanisms that enhance the success of bioaugmentation in wastewater systems. Numerous aerobic and anaerobic microorganisms exhibit aggregation in response to environmental stressors, including limited oxygen or nutrient availability, shifts in pH, changes in salinity or temperature, and the presence of divalent cations such as calcium and magnesium (Trunk et al. 2018). In wastewater treatment systems, which often contain complex nutrients, toxic chemical intermediates, and high enzymatic demands, microbial aggregation facilitates a division of labour among community members. This enables distinct populations to carry out specialized yet complementary metabolic functions, thereby enhancing the overall functional efficiency and resilience of the treatment process (Rodriguez-Conde et al. 2025). These processes highlight microbial aggregation as a key functional mechanism that strengthens community performance and directly contributes to successful bioaugmentation.

However, Doloman and Sousa (2024) explained that, while aggregation in microbial communities is

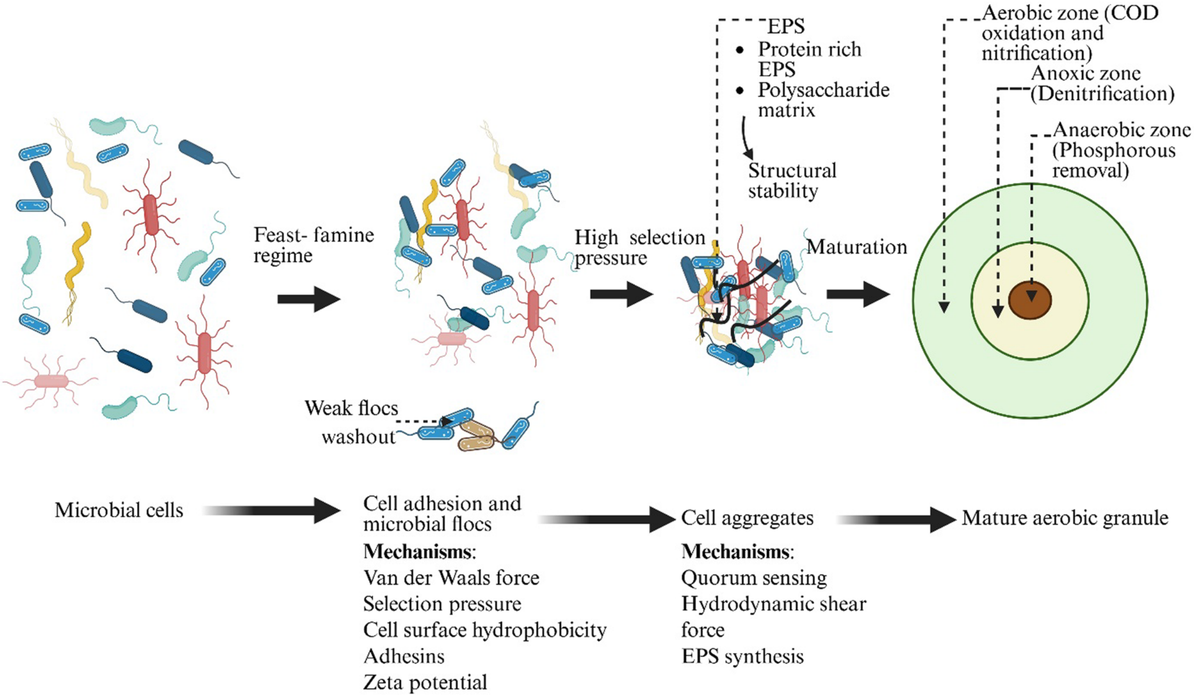

Fig. 2 Schematic representation of aerobic granule formation, illustrating the progressive transition from dispersed microbial cells to mature aerobic granules under operational and hydrodynamic selection pressures. The figure highlights key mechanisms governing each stage

often attributed to metabolic interdependence, where tightly associated populations rapidly exchange essential metabolites, this is not the sole driver of aggregation in wastewater systems. In environments such as wastewater and activated sludge bioreactors, certain microbial species primarily act as bridging organisms, facilitating the co-aggregation of non-aggregating bacteria without directly contributing to metabolic activity. Similarly, Katharios-Lanwermeyer et al. (2014) reported that microbes such as *Sphingomonas sp., Acinetobacter calcoaceticus*, and *Micrococcus luteus* were not directly involved in pollutant degradation. In fact, their primary function was bridging, i.e., co-aggregating to support the coexistence of non-aggregating bacteria within the biofilm (Katharios-Lanwermeyer et al. 2014). Together, these findings demonstrate that microbial aggregation in wastewater systems can be structurally driven by bridging organisms, independent of direct metabolic contributions.

Other representative genera include *Fusobacterium*, *Blastomonas* and *Methylobacterium* (Rickard et al. 2002). These bridging species often exhibit cell surface properties conducive to adhesion, such as high hydrophobicity and slightly negative surface charge, which may promote electrostatic interactions with positively charged microbial partners (Malik et al. 2003). Overall, these surface characteristics enable bridging species to play a key structural role in microbial co-aggregation.

3.2.2 Mechanistic insights into aggregating bacteria-mediated immobilization

A central mechanism underpinning aggregating bacteria-mediated immobilization is their ability to function as *bridging organisms* that physically integrate pollutant-degrading bacteria into developing or established biofilms through coaggregation. Across wastewater treatment studies (Cheng et al. 2014), aggregating bacteria consistently exhibit broad-spectrum coaggregation capacity, enabling interactions with phylogenetically and functionally diverse partners, including strains with limited intrinsic biofilm-forming ability. Rather than relying solely on the attachment capacity of individual degraders, this mechanism embeds functional strains within a shared extracellular matrix, thereby enhancing biomass retention, spatial stability, and sustained access to nutrients and electron donors.

At the community level, microbial aggregation is accompanied by coordinated transcriptional and regulatory responses that distinguish aggregated consortia from planktonic populations. Comparative gene expression analyses have demonstrated that aggregated cells exhibit elevated expression of signal transduction pathways and genes associated with extracellular matrix synthesis relative to dispersed cells (Doloman & Sousa 2024). Across systems ranging from activated sludge flocs to engineered biofilms, these transcriptional shifts consistently distinguish aggregated consortia from planktonic populations, indicating a conserved regulatory response to spatial organization.

Further, these transcriptional shifts promote the production of EPS, surface-associated proteins, and adhesion factors that collectively strengthen intercellular cohesion and enhance the mechanical stability of microbial aggregates. As a result, aggregating bacteria act as natural immobilizers, stabilising functional degraders through EPS production, cell-surface adhesins, and hydrophobic interactions rather than through artificial carrier materials.

Consequently, these transcriptional shifts are not isolated responses but are commonly regulated through quorum-sensing (QS) networks that synchronise collective behaviours across mixed communities (Fu et al. 2025). Quorum sensing has emerged as a key regulatory mechanism coordinating aggregation and stabilisation within naturally immobilised consortia. In wastewater treatment systems, multiple QS signalling molecules often coexist, forming interconnected regulatory networks that synchronise collective microbial behaviours (Hu et al. 2020). These include N-acyl homoserine lactones (AHLs) in Gram-negative bacteria, peptide-based QS systems in Gram-positive taxa, diffusion signalling factors (DSFs), and the interspecies autoinducer-2 (AI-2). Despite differences in signalling chemistry and taxonomic distribution, these QS systems converge on a common functional outcome: the coordinated reinforcement of aggregation, EPS production, and interspecies cohesion. QS-deficient strains consistently exhibit impaired aggregation and reduced biofilm formation capacity, underscoring the central role of QS-mediated coordination in stabilising immobilised microbial communities (Markowska et al. 2024). Overall, quorum sensing acts as an integrative regulatory framework that ensures cohesive and stable aggregation across complex microbial consortia.

Importantly, QS operates in concert with intracellular secondary messengers, particularly cyclic diguanosine monophosphate (c-di-GMP), to regulate the transition from reversible attachment to irreversible adhesion. Elevated intracellular c-di-GMP levels suppress motility while enhancing EPS secretion and cell-surface adhesion, thereby promoting the formation of dense, mechanically stable aggregates (Liu et al. 2020; Wang et al. 2025). Evidence from wastewater biofilms indicates that QS signalling and c-di-GMP regulation form a conserved regulatory axis controlling aggregation, EPS synthesis, and long-term biomass retention (Bao et al. 2025). Through this coordinated regulation, aggregating bacteria facilitate the effective incorporation of functional degraders into stable microbial architectures capable of withstanding hydraulic and ecological stresses.

At the molecular scale, aggregation-mediated immobilization arises from a combination of extracellular matrix production and highly specific surface interactions. While polysaccharide-rich EPS and protein adhesins have traditionally been viewed as the dominant drivers of microbial aggregation, increasing evidence suggests a more diverse repertoire of structural components. Proteinaceous adhesins, often localised on fimbriae or distributed along the cell envelope, mediate highly specific adhesin-receptor interactions between neighbouring cells, frequently involving complementary saccharide moieties. These interactions enable selective yet stable interspecies aggregation, supporting the persistence of genetically distinct bacteria within shared biofilm structures (Doloman & Sousa 2024; Stevens et al. 2015). However, despite their recognised importance, detailed molecular characterisation of adhesin-mediated interactions in environmental aggregates remains limited, highlighting a key knowledge gap in understanding aggregation-driven immobilization in engineered systems (Afonso et al. 2021). Addressing this gap is essential for rationally designing and optimising aggregation-based immobilization strategies.

Beyond conventional EPS and adhesin mediated immobilization mechanisms, as extensively documented in previous studies (Hasan et al. 2024;

Ibrahim et al. 2022; Karygianni et al. 2020; Kim et al. 2020; Li et al. 2024),recent research has identified unconventional matrix components that contribute to immobilization. S-layer protein-mediated structuring, as reported by Wong et al. (2023) in anammox biofilms, illustrates how these proteins function as structural scaffolds distributed across cell surfaces and the extracellular matrix to facilitate interspecies immobilization within complex microbial networks. This dual localisation demonstrates how specific structural proteins can simultaneously act as cell-surface adhesins and matrix-embedded scaffolds. Collectively, these findings challenge EPS-centric models of aggregation and indicate that immobilised consortia rely on multiple, partially redundant structural mechanisms whose relative importance may shift depending on community composition and environmental conditions.

As a functional consequence of the regulatory and structural mechanisms described above, aggregation-related features enable tight cell-to-cell adhesion and the formation of cohesive microbial clusters on surfaces or in suspension. Coaggregation, auto-aggregation, and interspecies adherence among genetically distinct bacteria therefore represent key emergent properties of aggregating consortia and are critical for the development of stable, multi-species biofilms. By embedding pollutant-degrading strains within a shared extracellular matrix, these interactions enhance biomass retention, promote metabolic cooperation, and improve pollutant removal efficiency and overall system robustness.

Comparative investigations of coaggregation dynamics indicate that aggregation behaviour is both species-specific and temporally regulated, reflecting differences in cell-surface chemistry, regulatory responses, and growth kinetics. Time-dependent aggregation patterns, as reported by Ramalingam et al. (2013) in drinking water-derived bacterial isolates, demonstrate that minimal initial auto- or coaggregation can evolve into pronounced aggregation over time, with *Methylobacterium* exhibiting the highest aggregation potential after 72 h. Moreover, the formation of dual-species consortia consistently resulted in more robust biofilms than corresponding monocultures, illustrating how synergistic interspecies interactions rather than the aggregation capacity of individual strains alone, govern biofilm stability and persistence in engineered systems.

These general principles are further illustrated in pollutant-specific bioaugmentation contexts. In p-nitrophenol (PNP) degradation systems, Yue et al. (2018) demonstrated that co-aggregating support strains (*Bacillus megaterium* T1 and *Bacillus cereus* G5) establish highly specific molecular interactions with the PNP degrader *Methylobacterium* sp. C1. These interactions, mediated by protein–sugar recognition involving cell-surface adhesins and lipoteichoic acids, promote rapid coaggregation and early-stage biofilm nucleation. Once co-aggregated, the resulting consortia facilitate early biofilm development, enhance the retention of functional degraders, and stabilise EPS-rich matrices that provide both structural support and chemical protection by adsorbing toxic compounds and buffering environmental stress.

Critically, the functional benefits of coaggregation extend beyond physical immobilization. In sequencing batch reactors, PNP removal efficiencies increased from approximately 80% in monoculture systems containing *Methylobacterium* sp. C1 alone to over 99% following the introduction of co-aggregating *Bacillus* strains, demonstrating that aggregation-driven immobilization substantially enhances biodegradation performance (Yue et al. 2018). High-throughput sequencing further revealed that these co-aggregated biofilms exhibited increased community diversity and functional redundancy, promoting metabolic cooperation and long-term stability. Collectively, these findings demonstrate that aggregating bacteria function as both structural and functional anchors within bioaugmented consortia, replacing artificial carriers by forming biologically driven immobilization matrices that enhance degrader persistence, resilience, and treatment efficiency.

Through this naturally mediated immobilization process, aggregating bacteria overcome the wash-out problem often observed with free cell bioaugmentation and reduce reliance on synthetic carriers. The outcome is a more resilient and self-organizing microbial community, capable of withstanding fluctuations in environmental and operational conditions while maintaining high pollutant removal efficiency (Fig. 3).

These findings reinforce the potential of leveraging coaggregation to promote robust, multi-species biofilms in bioaugmentation strategies. As previously

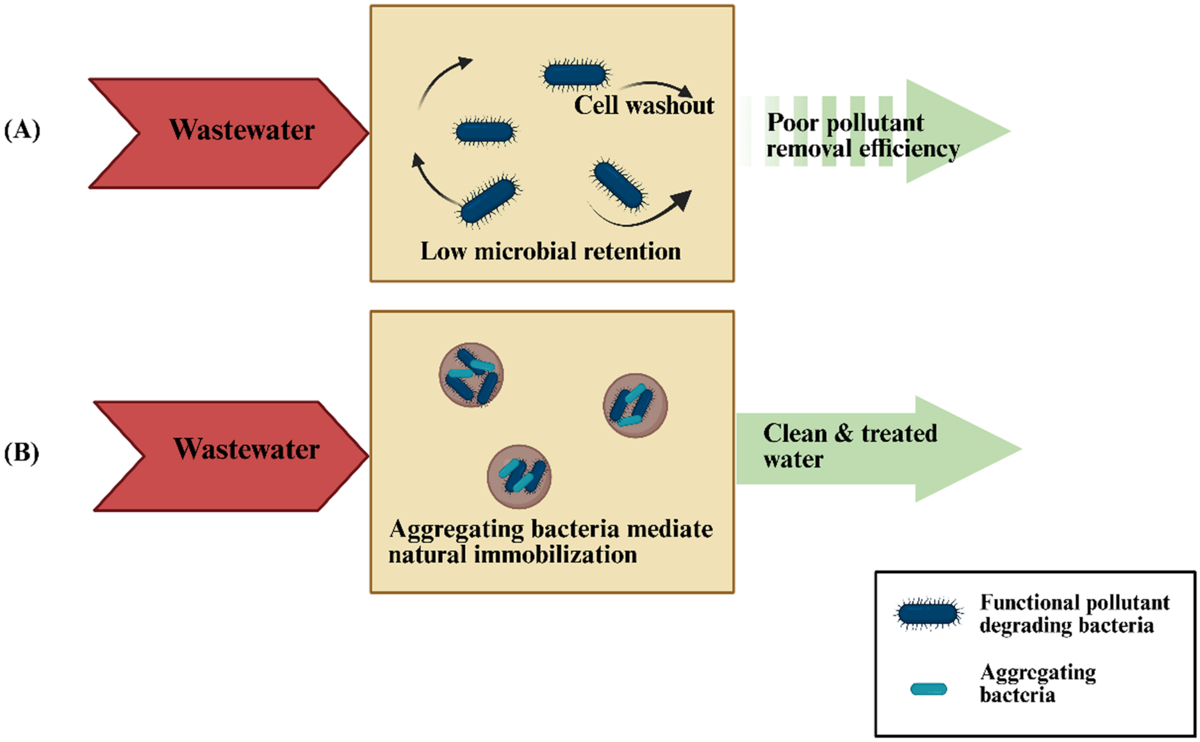

Fig. 3 Comparison of bioaugmentation approaches: **a** Free-cell bioaugmentation and cell washout, **b** Aggregating bacteria-mediated immobilization

discussed for *Methylobacterium* sp. C1 and its coaggregation with *Bacillus* strains (Yue et al. 2018), Table 1 further summarises other key studies that demonstrate the enhancement of pollutant removal through the use of aggregating bacteria in wastewater treatment systems, providing a comparative overview of bioaugmentation strategies incorporating degraders, enriched biofilms, and aggregating bacteria to improve the removal of emerging and conventional pollutants. In most cases, functional pollutant-degrading bacteria alone achieved moderate removal efficiencies, which were significantly improved when co-cultured with aggregating bacteria. For example, iron and manganese-oxidising strains (*Acinetobacter* sp., *B. megaterium*, and *Sphingobacterium* sp.) achieved 68–78% removal, and when paired with the aggregator *Bacillus mojavensis* M1, removal efficiencies exceeded 99% in lab-scale biofilters (Li et al. 2016). Enhanced degradation kinetics were also observed in phenol-degrading systems: the degrader *Propioniferax*-like PG-02 achieved complete phenol removal in 53 h, whereas co-culturing with the aggregator *Comamonas* sp. PG-08 accelerated removal to 29 h (Jiang et al. 2006). Improved resilience under shock-loading conditions was reported by (Cheng et al. 2015), where the degrader *Comamonas testosterone* A3 removed 70–72% of 3,5-dinitrobenzoic acid, but removal increased to 84% upon addition of the aggregator *B. cereus* G5 in a lab-scale SBR. For other xenobiotics, the degrader *Rhodococcus rhodochrous* BX2 removed only 27% of acetonitrile, whereas coaggregation with *Bacillus* spp. (E2, E3, N4) increased removal to 64–72% (Li et al. 2013). Likewise, the degraders *Acinetobacter* BCaL1 and *Stenotrophomonas* BCaL2 removed only 7.9–25.4% of polyethoxylated nonylphenol, but removal increased to 42.3% when paired with the aggregator *Bacillus* VA160 (Di Gioia et al. 2004). A similar trend was observed in constructed wetland biofilms, where Iasur-Kruh et al. (2011) showed that augmenting wetland biofilms with estrogen-degrading *Novosphingobium* JEM-1 led to complete removal of endocrine-disrupting chemicals (EDCs) within three days and significantly enhanced estrogen removal rates, demonstrating the

Table 1 Comparative summary of bioaugmentation strategies incorporating degraders, enriched biofilms, and aggregating bacteria to enhance the removal of emerging and conventional wastewater pollutants

Functional pollutant-degrading bacteria	Aggregating bacteria	Pollutant / Nutrient	Removal by degrader alone	Removal after adding aggregators	Operating system	Key findings	Limitations	Reference
Methylobacterium sp. C1	*Bacillus megaterium* T1, *B. cereus* G5	p-Nitrophenol	80%	> 99%	SBR	-Enhanced degrader immobilization and pollutant removal through coaggregation	-Limited to a single pollutant -Long-term stability in continuous systems not evaluated	Yue et al. (2018)
Acinetobacter sp., B. megaterium, Sphingobacterium sp.	*Bacillus mojavensis* M1	Fe, Mn	Fe: 72.87–78.45%; Mn: 67.93–69.42%	Fe: 99.87%; Mn: 99.62%	Lab-scale biofilters	-Biofilm-forming bacterium enhanced immobilization of Fe/Mn-oxidizing strains -Improved Fe and Mn removal efficiency in biofilters	-Real wastewater complexity not assessed	Li et al. (2016)
Acinetobacter calcoaceticus I6, *Candida tropicalis* I9	*Bacillus thuringiensis* I2	Phenol (500 mg/L)	I2: 0.29 I8:0.44 I9: 0.22 (mg phenol/mg CDW/h)	I2 + I6: complete removal in 24–26 h with higher biomass production	Batch serum bottles	Demonstrated strong auto- and co-aggregation between strains, improving phenol tolerance	-No clear quantitative data on co-cultures -Batch tests only	Adav et al. (2008)

Table 1 (continued)

Functional pollutant-degrading bacteria	Aggregating bacteria	Pollutant / Nutrient	Removal by degrader alone	Removal after adding aggregators	Operating system	Key findings	Limitations	Reference
Comamonas testosterone A3	*Bacillus cereus* G5	3,5-Dinitrobenzoic acid	70–72%	84%	Lab-scale SBR	-*Comamonas* stayed dominant and stable in the reactor when both strains were added -Aggregator promoted better sludge retention and faster degradation	Long-term stability beyond experimental duration was not fully evaluated	Cheng et al. (2015)
Rhodococcus rhodochrous BX2	*Bacillus subtilis* E2, E3, N4	Acetonitrile	27%	64–72%	SBR	-Improved nitrile removal due to enhanced co-aggregation and increased biomass retention	-Continuous operation was not tested -Shock load tests were limited to acetonitrile concentration changes	Li et al. (2013)
Propioniferax-like PG-02	*Comamonas* sp. PG-08	Phenol (250 mg/L)	100% in 53 h (2.94 mg/L/h)	100% in 29 h (6.30 mg/L/h)	Batch tests	-Co-culture tests and co-bioaugmentation tests performed -Coaggregation mechanism identified	-No pilot or full-scale validation -Long-term stability of co-culture not assessed under fluctuating conditions (pH, load, shear) -Quorum-sensing role suggested but not fully explored	Jiang et al. (2006)

Table 1 (continued)

Functional pollutant-degrading bacteria	Aggregating bacteria	Pollutant / Nutrient	Removal by degrader alone	Removal after adding aggregators	Operating system	Key findings	Limitations	Reference
Acinetobacter BCaL1, *Stenotrophomonas* BCaL2	*Bacillus* VA160	Polyethoxylated nonylphenol (98 mg/L)	BCaL1- 7.9%; BCaL2-15%; consortium -25.4%	Three-member consortium -42.3%	Lab-scale cultures	-*Bacillus* VA160 enhanced degradation by promoting aggregation -Demonstrated a natural immobilization strategy	-No reactor-scale (SBR/MBBR) or pilot-scale validation	Di Gioia et al. (2004)
Novosphingobium JEM-1(EDB-LI1	Wetland biofilm community	endocrine-disrupting chemical (EDC) residues	full removal of 80 mg liter^{-1} EDC: 3 days	Augmented biofilms showed significant estrogen removal:(17.6 and 16 mg L^{-1} g^{-1} gravel day^{-1})	Constructed wetlands biofilm	Successful colonization of biofilm, enhanced EDC removal -Altered community composition -performance dependent on pond stage	Biofilm type strongly influenced outcomes -No evaluation under engineered reactor conditions -short-term batch incubation (24 h) limits understanding of long-term stability	(Iasur-Kruh et al. 2011)

Table 1 (continued)

Functional pollutant-degrading bacteria	Aggregating bacteria	Pollutant / Nutrient	Removal by degrader alone	Removal after adding aggregators	Operating system	Key findings	Limitations	Reference
Sphingomonas sp., *Zoogolea*, *Labrys*, *Koazkia*, *Asprobacter*, unclassified *Cyclobacteriaceae* (ELB16-189), *Bryobacteraceae* (Fen-178)	Biofilms on biocarriers	Pesticides: N, N-diethyl-meta-toluamide (DEET), Aminotriazole (AMT), Atrazine (ATZ) Antibiotics: Sulfamethoxazole (SMX), Trimethoprim (TMP) Pharmaceuticals and personal care products: Carbamazepine (CBZ), Lidocaine (LDC)	No significant removal in non-acclimated biofilms without carbon supplementation	DEET, SMX: > 50% AMT,LDC,TMP,ATZ: 0.024~0.031% per week	Sludge derived biofilms in glass bottles	-Enhanced pollutant removal with carbon addition -Pollutant-specific degraders enriched -Degradation pathways identified	-Roles of individual degraders unclear due to complex biofilm interactions -Slow degradation rates caused by limited biofilm biomass -Metabolite analysis incomplete (only positive metabolites screened) -Batch assays may underestimate performance compared to continuous systems	(Li et al. 2025)

compatibility of aggregating degraders with natural biofilm communities.

More recently, Li et al. (2025) provided new insight into the role of sludge-derived biofilms in micropollutant removal under carbon-amended conditions. Their study identified several pollutant-degrading taxa; *Sphingomonas, Zoogloea, Labrys, Kaistia, Asprobacter,* and unclassified members of *Cyclobacteriaceae* (ELB16-189) and *Bryobacteraceae* (Fen-178), that became enriched within biofilms grown on biocarriers exposed to a mixture of pesticides (DEET, amino triazole, atrazine), antibiotics (sulfamethoxazole, trimethoprim), and pharmaceuticals (carbamazepine, lidocaine). While non-acclimated biofilms showed minimal removal without supplementary carbon, acclimated communities achieved above 50% removal of DEET and sulfamethoxazole, and measurable (though slow) weekly degradation rates for amino triazole, lidocaine, trimethoprim, and atrazine. Carbon supplementation facilitated both biomass development and pollutant-specific enrichment, supporting the activation of metabolic pathways associated with these taxa. However, the authors noted that the functional roles of individual degraders remained partly unresolved due to complex interspecies interactions within the biofilms, and that slow biofilm growth and batch-mode limitations may have underestimated true removal potential compared to continuous systems.

Besides these studies, the application of bacteria exhibiting broad-spectrum coaggregation capabilities in bioaugmentation systems remains relatively underexplored. Thus, further study is needed to understand the relationship between these functional and aggregating bacteria in bioaugmentation applications.

The coaggregation ability of certain bacteria plays a mechanistic role in immobilization by enabling physical bridging between species, promoting the formation of multispecies cell aggregates, and supporting structured biofilm development that enhances pollutant degradation. Cheng et al. (2014) demonstrated that *Bacillus cereus* G5 and *Bacillus megaterium* T1 possess broad-spectrum coaggregation capabilities, forming stable aggregates with 90.5% and 76.2% of the tested strains, respectively. Such high compatibility indicates that these *Bacillus* strains act as biological “bridging organisms,” providing adhesive interfaces that integrate diverse bacteria into early-stage aggregates. Microscopy analyses further showed that *B. cereus* G5 and the 3,5-dinitrobenzoic acid-degrading strain *Comamonas testosterone* A3 formed a well-organized dual-species biofilm, demonstrating how coaggregation-driven adhesion translates directly into structured immobilization frameworks. These mechanistic interactions highlight that coaggregation is not merely an ecological trait but a functional process that anchors degraders within a biofilm matrix, thereby enhancing their persistence and degradation efficiency. Although the application of this directly in wastewater treatment has not been reported, it clearly shows that aggregating bacteria can mediate the integration of exogenous degrading bacteria into biofilms and contribute to bioaugmentation treatment. Achieving better colonization involves ensuring that bioaugmented bacteria can efficiently aggregate and form robust biofilms under various environmental conditions. This is particularly important for overcoming challenges such as slow microbial colonization at low temperatures, which can hinder the start-up and overall efficiency of treatment processes (Li et al. 2022a, b). Overall, these findings demonstrate that coaggregation-driven immobilization is a key mechanism enabling effective integration, retention, and functional stability of exogenous degraders within biofilms, highlighting its potential to enhance bioaugmentation performance under diverse environmental conditions.

3.2.3 Synthetic biology strategies to stabilize co-cultures of aggregating bacteria and pollutant degraders

While naturally occurring aggregating-degrading bacterial partnerships can enhance the stability and retention of functional degraders in wastewater systems, their performance often depends on the compatibility of microbial traits and environmental conditions. Although certain aggregating strains selected primarily for their bridging or structural properties may not be highly specialized degraders, aggregating bacteria as a broader group make important contributions to biodegradation through direct metabolic activity and by stabilizing syntrophic microbial networks. In contrast, degrading bacteria remove nutrients or degrade pollutants but are prone to detachment or being washed out. However, if there is no strong mutual benefit, the degrading bacteria may not stay in the aggregates for long. Synthetic biology offers a means to rationally design and optimize such consortia, enabling predictable and robust formation of biofilms or granules that integrate structural stability with high degradation capacity.

Drawing on principles from syntrophic interactions, co-cultures can be engineered Rodriguez-Conde et al. (2025) explained, engineered metabolic interactions have been successfully applied in the production of biopolymers, natural products, and biofuels through co-cultures so far. While studies (Angeles-de Paz et al., 2023; Shan et al. 2023) on engineering syntrophic co-cultures involving aggregating bacteria and specialized pollutant degraders in wastewater treatment bioaugmentation remain limited, emerging research (Brettfeld et al. 2023; Saini et al. 2023; Yang et al. 2025) indicate that such synergistic microbial interactions, particularly those facilitating biofilm and granule formation, hold significant promise for improving microbial community stability and enhancing contaminant removal efficiency.

When designing such systems, a key principle is to first ensure coexistence under shared environmental conditions before introducing engineered communication modules (Jia et al. 2016). In the case of aggregating-degrading pairs, this means confirming that both partners can co-aggregate and persist in reactor conditions, and only then incorporating communication circuits to strengthen cooperation. QS systems can connect the process by which aggregating bacteria produce EPS with the process where degrading bacteria turn on their catabolic genes. For example, aggregating bacteria-derived QS signals could activate pollutant degradation pathways in functional degrading bacteria, while degrading bacteria-derived signals stimulate EPS synthesis in aggregating bacteria (Fig. 4). Bidirectional interactions can be rationally engineered by combining pairs of unidirectional regulatory elements, a strategy that has been successfully applied in synthetic microbial consortia to promote cooperation and stabilize community function (Deter & Lu 2022). This design principle supports the feasibility of the reciprocal signalling circuit proposed in our study. A similar design principle has been demonstrated by (Alnahhas et al. 2020), who constructed a bidirectional co-repressive quorum-sensing consortium in which each strain modulates gene expression in the partner strain. This provides strong precedent for the feasibility of our proposed reciprocal signalling circuit, in which aggregating and degrading bacteria exchange QS molecules to activate EPS production and pollutant degradation.

In this context, understanding the natural QS mechanisms that regulate aggregation is critical for designing robust co-cultures. In major aggregating species such as *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Burkholderia thailandensis*, and *Bradyrhizobium sp.*, specific QS genes (e.g., *LuxS*, *lasI/lasR*, *rhlI/rhlR*, *BtaI1/BtaR1*) have been shown to regulate EPS production and microbial aggregation (Maddela et al. 2019). For instance, in *Burkholderia thailandensis*, the QS gene pair *BtaI1/BtaR1* controls the production of the acyl-HSL signal C8-HSL and is essential for self-aggregation in minimal medium (Chandler et al. 2009). Similarly, in Gram-positive aggregating bacteria such as *Bacillus sp.*, the ComQXPA quorum-sensing system regulates expression of EPS and matrix genes (*epsA-O*, *tasA*) that drive cell aggregation (Comella & Grossman 2005). Leveraging these QS-regulated pathways in synthetic co-cultures offers a promising strategy to enhance EPS secretion, promote stable cell aggregation, and facilitate biofilm or granule formation, thereby improving the structural stability and retention of pollutant-degrading bacteria in wastewater treatment systems.

In summary, synthetic biology provides a powerful framework for integrating natural aggregation mechanisms with engineered quorum-sensing circuits to stabilize degrader-aggregator co-cultures, strengthen biofilm or granule formation, and ultimately enhance the reliability and efficiency of bioaugmentation in wastewater treatment systems.

4 Sources and application of aggregating bacteria for immobilization-based bioaugmentation

4.1 Sources of aggregating bacteria for bioaugmentation

Aggregating bacteria can be sourced from a variety of natural and engineered environments where microbial communities exhibit strong self-aggregation, coaggregation, or biofilm-forming capabilities. These sources are valuable for identifying strains that enhance immobilization in bioaugmentation applications without relying on synthetic carriers. Common environments include municipal sewage, activated

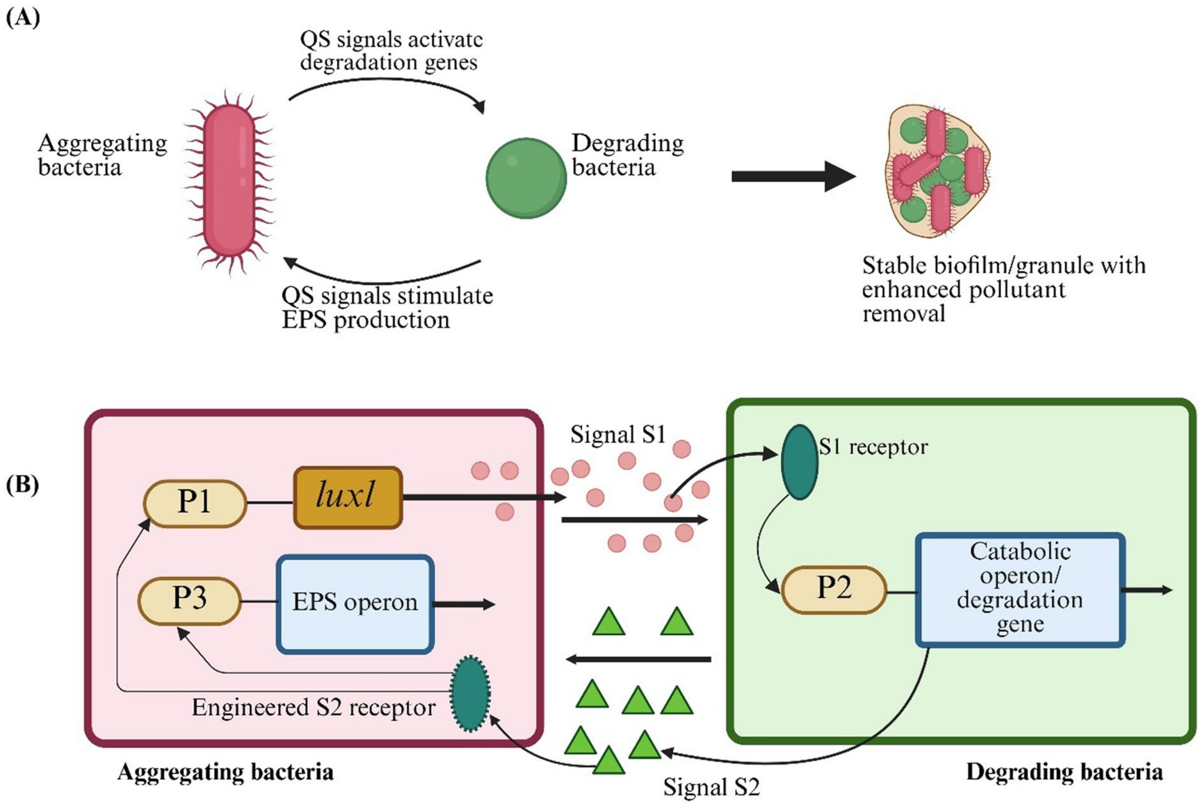

Fig. 4 Conceptual synthetic quorum-sensing circuit engineered to stabilize co-cultures of aggregating bacteria and pollutant degraders. **(A)** Schematic overview of the proposed bidirectional signalling between aggregating bacteria and pollutant-degrading bacteria. QS signals produced by the aggregating strain activate pollutant degradation pathways in the degrader, while feedback QS signals from the degrader stimulate EPS production, **(B)** Conceptual detailed genetic circuit diagram of the two-strain communication system. In aggregating strain, an engineered S2-responsive receptor activates promoter P1 to drive luxI-mediated synthesis of the QS signal S1, and simultaneously activates promoter P3 to induce the EPS biosynthesis operon. Signal S1 diffuses to degrading strain, where it binds the S1 receptor and activates promoter P2, inducing expression of the catabolic operon responsible for pollutant degradation. Degradation activity produces the feedback signal S2, which returns to the aggregating strain and reinforces both S1 production and EPS synthesis

sludge, biofilm reactors, natural aquatic sediments, soils, and composted organic matter (Cheng et al. 2014). Further, aerobic granular sludge (AGS) is a particularly valuable source of aggregating bacteria, as aerobic granules are natural, complex, self-assembled microbial aggregates characterized by EPS production and tight cell-to-cell adhesion. Wan et al. (2023) demonstrated that aerobic granules have a spatially ordered microbial colonization, consisting of outer, middle, and inner layers, each with distinct microbial populations performing specific functional roles. Notably, microorganisms in the inner layer exhibited significantly stronger adhesion properties, suggesting that bacteria residing in this zone are likely the initiators of microbial aggregation, an essential trait for successful bioaugmentation. This was supported by the higher abundance of pili genes in inner-layer microbes, which contribute to superior attachment capabilities critical for stable colonization and biofilm formation in carrier-free bioaugmentation systems (Wan et al. 2023). Collectively, these findings highlight aerobic granular sludge and similar environments as strategic reservoirs of aggregation-competent bacteria for effective carrier-free bioaugmentation.

Further supporting this, Li et al. (2022a, b) demonstrated that microbes from the inner core (IC) of aerobic granules exhibited higher aggregation and adhesion capabilities under famine conditions (substrate-limited phase) compared to those from the outer layer (OL). Specifically, under feast conditions (substrate-rich phase), inner-layer microbes showed 30.04 ± 0.06% aggregation, which decreased to 18.97 ± 0.59% under famine stress, while outer-layer microbes aggregated at 23.47 ± 1.12% and 20.95 ± 1.03%, respectively. These results indicate that IC microbes are better suited for initiating and stabilizing granulation during the start-up phase, reinforcing the potential of inner-layer bacteria from aerobic granules as robust candidates for bioaugmentation strategies focused on immobilization.

Although aerobic granules provide an excellent source of aggregating bacteria, a key limitation is the relatively long start-up time typically required for their formation. However, Liu and Tay (2015) demonstrated that by applying strong hydraulic selection pressure (HSP) and overstressed organic loading rates (OLR), aerobic granules could form within 24 h. They further recommended optimizing OLR and aeration rates to ensure long-term granule stability. While the key microbial players involved in this rapid granulation remain unidentified, this finding highlights the possibility of accelerating granule formation, thereby enhancing the practicality of using aerobic granular sludge as a source for isolating aggregating strains for immobilization-based bioaugmentation.

4.2 Application of aggregating bacteria isolated from aerobic granules for immobilization-based bioaugmentation

Aerobic granular sludge technology, in addition to not requiring a carrier, is a continuation of the biofilm technology (Li et al. 2022a, b). There are few studies (Adav & Lee 2008; Jiang et al. 2006; Wan et al. 2014) available on the screening of aggregating bacteria from aerobic granules and utilizing them in bioaugmentation for wastewater treatment with functional bacteria. Jiang et al. (2006) isolated two bacterial strains with co-aggregation characteristics, *Propioniferax*-like PG-02 and *Comamonas* sp. PG-08, for use in bioaugmentation targeting phenol degradation. Importantly, each isolate exhibited only one of the two desirable traits: PG-02 possessed strong degrading ability, whereas PG-08 demonstrated strong aggregating ability but limited degradation performance. When co-cultured, the consortium degraded phenol more rapidly than either strain alone, providing early mechanistic evidence of a division-of-labour strategy in AGS systems, where aggregators supply structural anchoring while degraders perform metabolic transformations, as outlined in Sect. **3.1.1**. Quantitatively, PG-02 (the degrading strain) removed phenol at 2.94 mg/h, while PG-08 (the aggregating strain) showed a much lower rate of 0.95 mg/h. In contrast, the co-culture exhibited a markedly enhanced removal rate of 6.30 mg/h, completely degrading 250 mg/L of phenol in a significantly shorter time. Wan et al. (2014) isolated an aerobic granular sludge (AGS)—derived aggregating consortium comprising four bacterial strains with high aggregating ability (*Pseudomonas putida* X-1, *Acinetobacter sp.* X-2, *Alcaligenes sp.* X-3, and *Comamonas testosteroni* X-4). They further monitored their granulation process in shaken reactors and reported in the later stage of the process, elevated intracellular levels of c-di-GMP accelerated flagellum synthesis while inhibiting bacterial swarming motility, thereby promoting the formation of large aerobic granules. Their results linked aggregation behaviour to intracellular signalling that provides a direct mechanistic connection between cellular regulatory pathways and aggregation-driven immobilization.

Similarly, Adav and Lee (2008) studied aerobic phenol-degrading granules and isolated three strains (*Bacillus thuringiensis* I2, *Acinetobacter calcoaceticus* I6, and *Acinetobacter sp.* I8) with high aggregation ability (auto-aggregation between 40–60%). Their phenol-degrading capacities varied, with specific degradation rates of 0.29, 0.44 and 0.31 mg phenol/mg cell dry weight / h for I2, I6 and I8, respectively. When co-cultured, I2 and I6 degraded an initial phenol concentration of 500 mg/L more rapidly as a consortium, achieving complete removal within 24–26 h, compared with 28–30 h for the individual strains, along with higher biomass production. These findings reinforce that AGS harbours bacteria with intrinsic surface adhesion properties conducive to forming dense, immobilized cell clusters (Adav & Lee 2008). Overall, this example illustrates how aggregation-competent strains from AGS can be effectively exploited for carrier-free bioaugmentation applications.

However, there are limited studies on bioaugmentation applications using the aggregating bacteria isolated from aerobic granules with specific functional pollutant-degrading bacteria. This gap highlights the need for further research to explore how aggregating bacteria from aerobic granules can be effectively leveraged to enhance immobilization-based bioaugmentation strategies for targeted pollutant removal.

4.3 Role of aggregating bacteria and immobilization in treating emerging pollutants in wastewater

Emerging pollutants (EPs), also referred to as contaminants of emerging concern (CECs), comprise a chemically diverse group of compounds including pharmaceuticals, personal care products, pesticides, endocrine-disrupting chemicals, industrial additives, and certain inorganic contaminants such as heavy metals and nanomaterials that are increasingly detected in wastewater at trace to moderate concentrations (Kanadasan et al. 2025). A unifying challenge across these compounds is their environmental persistence, bioaccumulation potential, and poor removal efficiency in conventional biological wastewater treatment systems. Their complex molecular structures and inherent toxicity frequently impose stress on free-living microbial cells, resulting in reduced degradation rates, instability of bioaugmentation cultures, and increased susceptibility to washout under continuous-flow conditions (Melo et al. 2022; Saeed et al. 2022). Taken together, these characteristics render EPs particularly challenging targets for effective and sustainable biological treatment.

Across diverse studies (Najim et al. 2024; Torresi et al. 2017; Wang et al. 2018), immobilized and aggregation-based microbial systems have consistently demonstrated better performance compared to free-cell systems for the removal of CECs, including antibiotics, pesticides, endocrine disruptors, and other synthetic organic compounds. Rather than being attributable to a single mechanism, this enhanced performance emerges from the combined structural and functional properties of biofilms, aerobic granules, and other aggregated consortia. These systems provide a protective extracellular matrix, promote biomass retention, and create physicochemical microenvironments that mitigate toxic stress while facilitating pollutant capture and transformation.

Aggregation-driven immobilization has important implications for emerging pollutants, wherein EPS mediate interactions between contaminants and aggregated biomass. Protein-rich EPS fractions have been identified as key mechanisms governing the retention and partitioning of pharmaceutical compounds within the biofilm matrix (Wang et al. 2018). Study (Wang et al. 2018) on antibiotic transport and fate in MBBR systems demonstrate that EPS-dominated biofilms can retain structurally diverse antibiotics, with adsorption accounting for 14.5%, 88.2%, and 13.1% of the total concentrations of sulfamethizole, tetracycline, and norfloxacin, respectively, during biodegradation. In addition, EPS-mediated antibiotic retention, as reported by Wang et al. (2018), illustrates how aggregation prolongs pollutant residence time within biologically active microenvironments, thereby reducing acute toxicity to microbial communities and enhancing the probability of subsequent enzymatic transformation. Taken together, these findings highlight the broader role of EPS-mediated retention in explaining the superior performance of biofilms, aerobic granules, and other aggregated biomasses relative to suspended cultures.

Beyond chemical affinity, the physical architecture of aggregated biomass plays a decisive role in governing pollutant transport and bioavailability. Comparative studies of micropollutant sorption and diffusion in MBBRs have demonstrated that biofilm thickness, porosity, and charge characteristics strongly influence solid–liquid partitioning behaviour (Torresi et al. 2017). Positively charged compounds exhibit preferential sorption through electrostatic interactions with EPS and biofilm solids, while thicker and more porous biofilms facilitate enhanced diffusion and pollutant penetration. These findings collectively indicate that aggregation not only enhances pollutant retention but also modulates mass transfer processes in ways that can either limit or promote biodegradation depending on biofilm structure.

In addition to sorption and transport effects, EPS-rich aggregates confer significant physiological protection to microbial communities exposed to toxic emerging pollutants. EPS matrices contain a wide array of reactive functional groups, including hydroxyl, carboxyl, sulfhydryl, and phosphate-amine moieties, which can interact with diverse classes of micropollutants (Melo et al. 2022). These interactions buffer microbial cells from oxidative stress and chemical toxicity, enabling both indigenous and bioaugmented degraders to maintain metabolic activity under conditions that would otherwise inhibit free-living cells. As a result, aggregation-mediated immobilization enhances not only pollutant capture but also microbial resilience and process stability.

The advantages of aggregation-driven immobilization extend beyond organic micropollutants to include inorganic contaminants such as heavy metals. In biofilm-based systems, EPS-mediated biosorption, complexation, and biomineralization collectively underpin metal sequestration while enabling aggregated, metal-tolerant microbial communities to maintain high removal efficiencies under elevated metal loads (Jeong et al. 2019; Kondakindi et al. 2024). In addition, this synergy was exemplified by Jeong et al. (2019) using the biofilm-forming *Methylobacterium hispanicum* EM2 strain, demonstrating how EPS-mediated Pb(II) removal facilitates multilayer adsorption and enhanced microbial tolerance under high contaminant stress. Overall, these interconnected mechanisms highlight the effectiveness of aggregation-driven immobilization for stabilizing biological metal removal processes.

Collectively, evidence across organic and inorganic emerging pollutants indicates that aggregation-driven immobilization enhances treatment performance through the integration of sorption, diffusion control, and microbial stress mitigation. Rather than functioning solely as passive biomass retention structures, EPS-rich aggregates actively shape pollutant bioavailability and microbial resilience, providing a strong mechanistic foundation for the development of robust bioaugmentation strategies targeting chemically complex wastewaters.

5 Research needs and future directions

The integration of aggregating bacteria into bioaugmentation processes represents a significant advancement in addressing the limitations of conventional approaches, particularly in enhancing the retention, activity, and synergistic interaction of functional microorganisms in wastewater treatment systems. These bacteria act as natural scaffolds, promoting co-aggregation and biofilm formation, thereby improving the stability and resilience of microbial communities under dynamic operational conditions.

Despite encouraging progress, several knowledge gaps and technical challenges remain. The mechanisms governing microbial aggregation, compatibility between aggregators and functional strains, and the influence of reactor type and wastewater composition on co-aggregation efficiency require further investigation. Screening of bacteria with coaggregation ability and exploration of such microbial resources may have significant potential in bioaugmentation treatment (Cheng et al. 2014), and as highlighted in this review, aerobic granules have emerged as a promising source of such microbial resources. Moreover, there is a pressing need for the development of standard protocols for screening, isolating, and characterizing aggregating bacteria with desirable traits for bioaugmentation. Furthermore, the design and optimised operation of bioreactor systems constitute a critical determinant of the efficacy of bioaugmentation treatments.

Future research is recommended to emphasize multi-omics and imaging-based tools to better understand microbial dynamics, as well as pilot-scale studies to evaluate performance in real wastewater matrices. Importantly, the concept of “bridging bacteria” opens new avenues for designing synthetic consortia tailored to specific treatment goals, such as nutrient removal, micropollutant degradation, or granule formation. Exploring the potential to apply this strategy in low-cost, non-reactor-dependent systems could be particularly valuable for resource-constrained settings, though further validation is needed. Harnessing the full potential of aggregating bacteria will require interdisciplinary collaboration, combining microbiology, environmental engineering, and systems biology to translate laboratory insights into scalable, sustainable wastewater solutions.

6 Conclusions

This review has examined bioaugmentation in wastewater treatment, covering both conventional approaches and innovative strategies using microbial immobilization and natural aggregation. Aggregating bacteria enhance the structural and functional integrity of microbial communities by forming stable biofilms and co-aggregating with pollutant-degrading strains, supporting sustained activity, improved contaminant removal, and operational robustness. Aerobic granules provide a valuable source of these bacteria, facilitating biofilm formation and functional synergy. Building on these insights, a structured framework is proposed to guide future bioaugmentation design, integrating robust selection of aggregating and functional bacteria, optimization of microbial interactions, and tailoring of reactor conditions to support stable biofilms and granules. By emphasizing iterative testing and performance monitoring, this approach can translate laboratory successes into scalable, sustainable wastewater treatment strategies.

Despite progress, challenges remain in understanding microbial interactions, optimizing immobilization, and scaling up to full-scale systems. Addressing these gaps is crucial for advancing resilient and efficient bioaugmentation systems capable of meeting diverse wastewater treatment demands and contributing to sustainability goals.

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Declarations

Conflict of interests The authors declare no competing interests.

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