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Factors Governing Biofilm Formation in Drinking Water Networks: A General Survey

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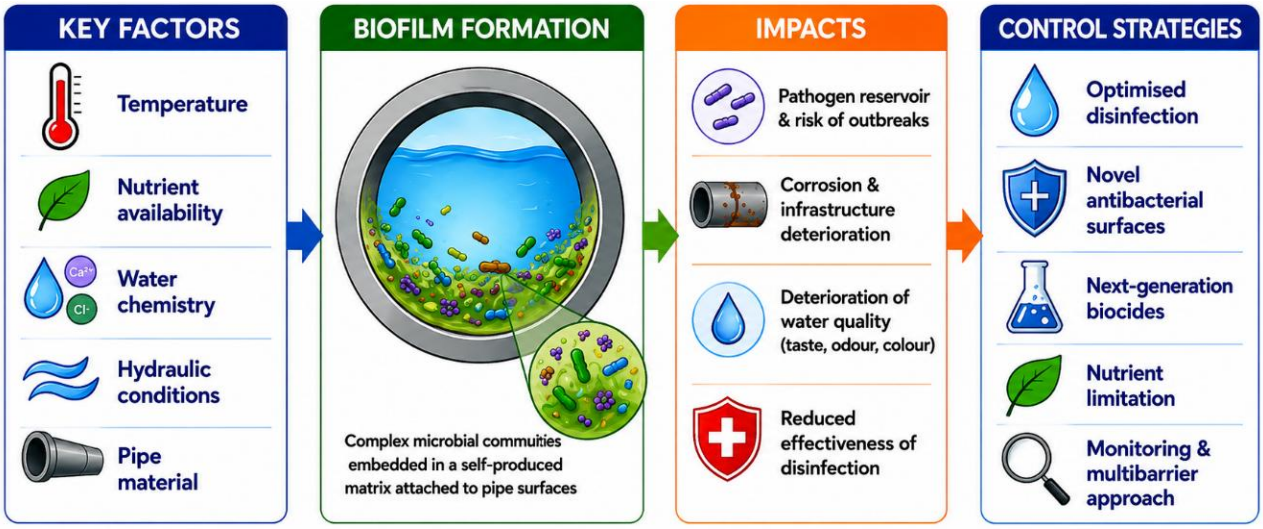
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Graphical Abstract

The drinking water distribution system (DWDS) leads to the consumer's tap. Microbial biofilms can colonize pipe surfaces, act as reservoirs for pathogens, accelerate infrastructure decay, and degrade organoleptic water quality. Their resistance to conventional disinfection, including chlorine, together with their regenerative capacity, makes eradication difficult. Biofilm proliferation is governed by temperature, nutrient availability, water chemistry, hydrodynamics, and pipe material. Consequently, safeguarding water quality requires integrated multi-barrier strategies, including novel antibacterial surfaces, next-generation biocides, and stringent nutrient limitation to effectively control biofilm resilience and protect public health.



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Abstract: The drinking water distribution system (DWDS) is often threatened by the dangerous threat of biofilms as these intricate microbial consortia aggressively colonize pipe surfaces and act as reservoirs for pathogens which accelerate the infrastructure decay and degrade the organoleptic quality. Their resistance to conventional disinfection which includes chlorine, and their powerful regenerative capacity make eradication strategies ineffective. Environmental variables that govern biofilm proliferation include temperature, nutrient availability, water chemistry, and hydrodynamics, as well as the pipe material. The safeguarding of the water quality needs to be transformed by integrated and multi-barrier strategies. This necessitates also the implementation of advanced solutions like novel antibacterial surfaces, next-generation biocides, and stringent nutrient limitation to interrupt the resilience of biofilm and to protect public health.

Keywords: *Biofilm, Drinking water, Disinfection, Factors.*

Introduction

In a DWDS, the bacterial biomass is associated with biofilms (Figure 1) rather than being suspended in the water, where their structure is influenced by numerous factors (hydraulic conditions, pipe materials, etc.) [1]. Moreno *et al.* [2] estimate that 95 % of the total biomass is attached to the pipe walls, compared to only 5 % in the water (Figure 2).

Biofilms act as reservoirs for pathogens, giving rise to outbreaks of infection and incurring considerable healthcare costs [3]. Fragments of biofilm can break away and contaminate water, causing outbreaks of gastroenteritis and other diseases [4]. Studies have shown that a greater bacterial diversity was observed in biofilms compared to planktonic water, with the families *Pseudomonadaceae* and *Nitrospiraceae* being the most common [5]. Biofilms accommodate a variety of pathogens (*Vibrio cholerae*, *Salmonella spp.*, *Escherichia coli*, *Legionella pneumophila*, *Mycobacterium avium*, *Pseudomonas aeruginosa*) as well as antibiotic resistance genes [6], which are responsible for legionellosis, pneumonia and other diseases [7]. It has been suggested that bacteria present in the form of biofilms in the DWDS are metabolically active and culturable, being potentially able to contaminate water with pathogens [8].

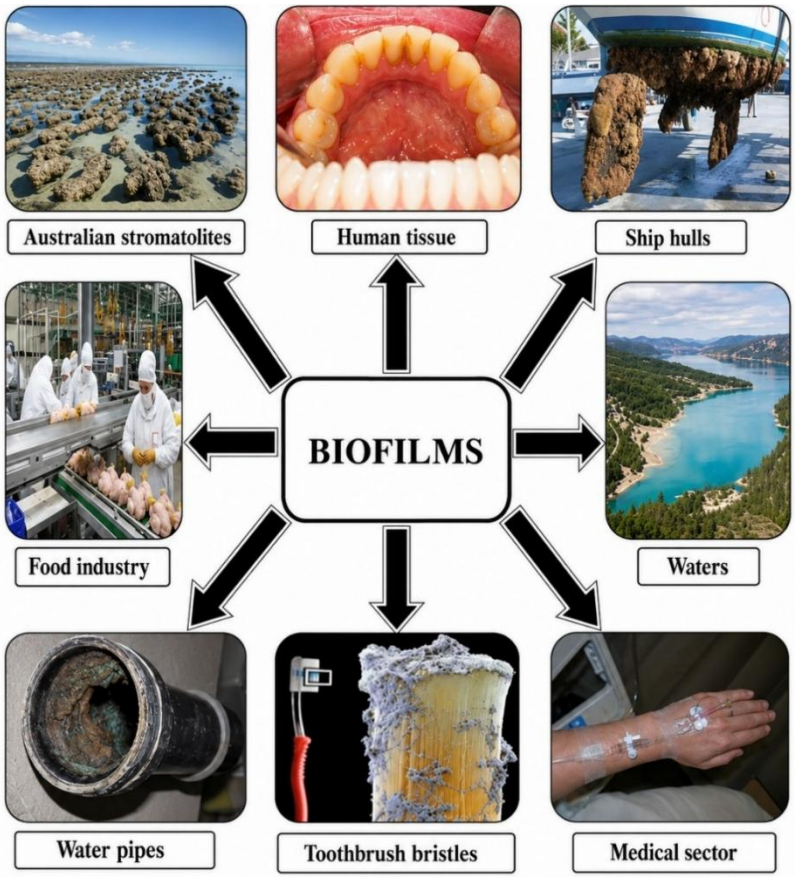


Figure 1. Representative environments where biofilms naturally develop and persist across environmental, industrial, domestic, and clinical settings

Studies note that biofilms are composed of metabolically heterogeneous populations; only some cells are highly active, and others have reduced or dormant metabolism controlled by gradients of oxygen, nutrients, and electron acceptors, determining specific physiological groups of bacteria in the biofilm [9,10]. Copper surfaces and copper-containing surfaces may also reduce the cultivability and metabolic activity of bacteria in biofilms by several orders of magnitude (or log) by eliminating viable and metabolically active cells from the biofilm through membrane damage and contact poison mechanisms [11]. Furthermore, the activity of biofilms can cause undesirable odours, tastes and discolouration in water through the accumulation of organic matter and metals [12].

The formation of biofilms in DWDS (Figure 3) creates technical (especially corrosion and clogging), economic, operational, and maintenance-related challenges [9]. First, biofilms may promote corrosion processes in iron pipes and cement-based materials through electrochemical interactions and the impact of metabolites and specific redox mechanisms [13]. For instance, bacteria can adhere to corroded areas and roughness on the surface of the pipe, leading to enhanced biofilm formation and localized corrosion [13-15]. Second, several studies have revealed that corrosion products from iron materials can facilitate biofilm development, diminishing disinfectant efficacy and promoting microbiologically influenced corrosion [12]. It is reported that such organisms contribute to the deposition of particles, scale, and suspended matter, resulting in clogging, discoloration, and, in extreme instances, total blockage [12]. Specifically, the adhesion of biofilms and inorganic material (e.g., iron and manganese) to the inner walls of the pipes decreases the lumen of the pipe, increasing the pressure while lowering the outflow [12,16]. Such impacts require extensive cleaning and replacement of the corroded and blocked pipes, incurring significant costs.

In addition, biofilm-covered stagnant water promotes poor water quality and encourages the proliferation of organisms [17,18]. The number of bacteria increases substantially following stagnation. For example, Li *et al.* [19] demonstrated that when exposed to stagnant conditions, bacteria grew rapidly in the pipe biofilm. Finally, the chemical composition of the material influences the characteristics of biofilm as well as the release of dissolved organic carbon into the water [20]. Rahmatika *et al.* [21] discovered that when compared to the initial content (<103 cells/mL), total cell counts (TCC) and dissolved organic carbon (DOC) values rose exponentially (6.0 × 105 cells/mL and 5.2 mg C/L, respectively) in the water stored inside the cross-linked polyethylene.

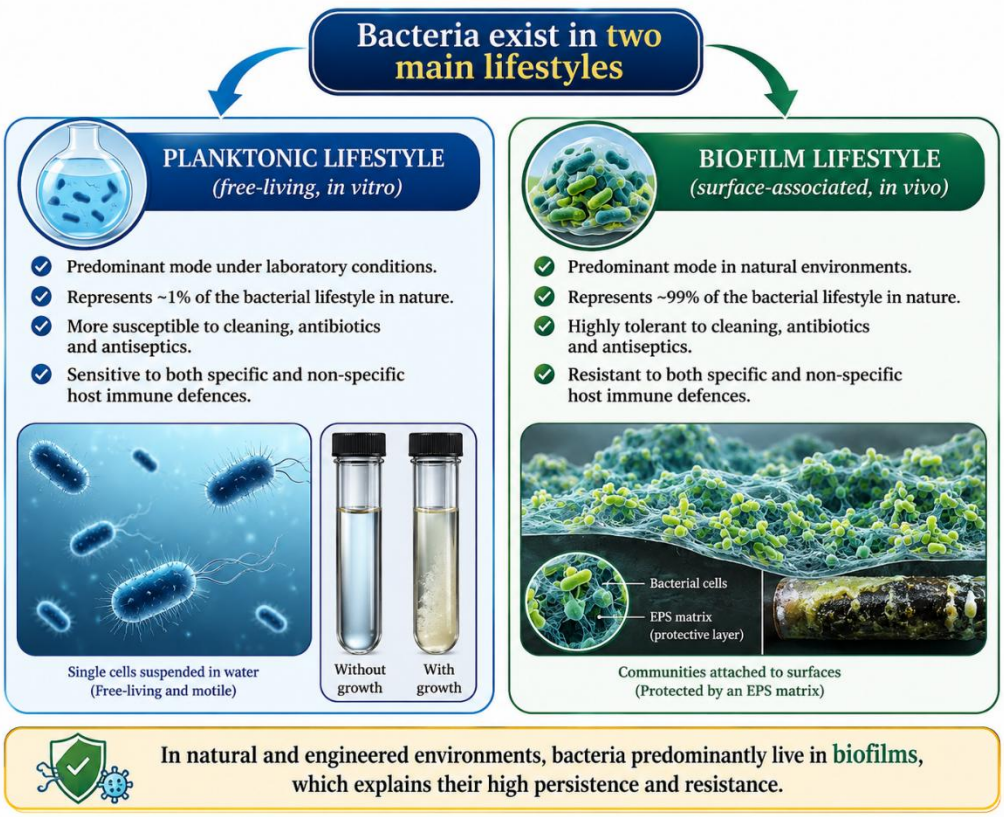


Figure 2. Comparison between planktonic and biofilm lifestyles of bacteria

Biofilms typically possess many defense mechanisms against environmental stress. Their exopolysaccharides (EPS: extracellular polymeric substances) have been shown to provide protection from disinfectants or osmotic stress [4,22]. For instance, biofilms can resist disinfectants such as chlorine in various ways including the EPS diffusion barrier [21], decreased metabolism of cells inside the biofilm [23,24], production of neutralizing enzymes [22,23]. This resistance includes interactions with protozoa that can shelter pathogens (e.g., *Legionella*) are also involved in this resistance [25]. Indeed, *Legionella* was even found to be ubiquitous in biofilms and source water which highlights its importance as an indicator of treatment efficacy [26,27]. Moreover, iron and steel metal pipes are commonly degraded by bacteria, which also decreases the efficiency of disinfectants through chlorine reaction with the metal surface [28]. As a result, biofilms resist the disinfectant action of chlorine and chloramines and other disinfectants allowing the proliferation of chlorine-resistant organisms such as *Mycobacterium spp.* or *Helicobacter pylori* [29]. Therefore, there is a disinfection dilemma with biofilms, as they can regrow even at high chlorine concentrations (10 mg/L).

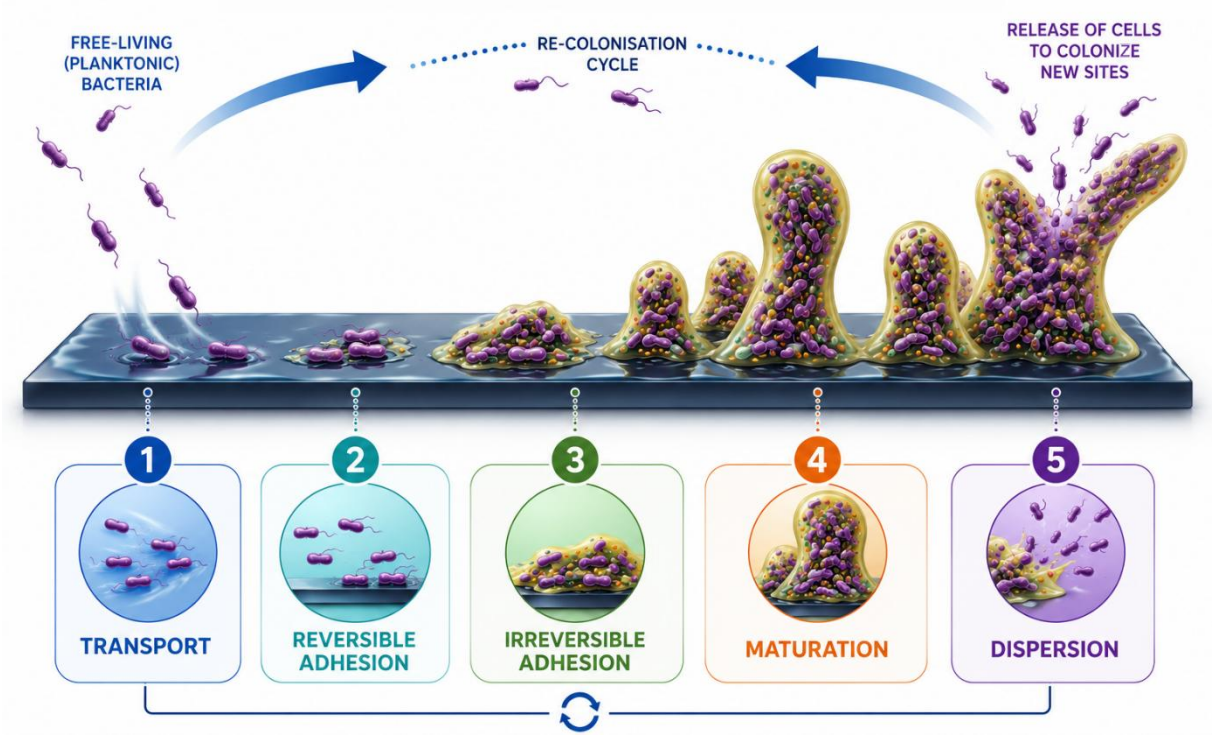


Figure 3. Schematic representation of the sequential stages of biofilm formation and development

Biofilms are a serious threat to public health and infrastructure. There is not enough study of the combined effect of environmental, technological and anthropogenic factors (temperature, chemical composition of water, nutrients, properties of the substrates, disinfectants, and hydrological characteristics) on their formation. Accordingly, this general survey offers a critical analysis of the factors influencing biofilm formation, supplemented by (i) a comparative assessment of recently developed innovative control strategies and (ii) a practical methodology for analysing distribution networks, intended for researchers and managers. The focus is on conventional drinking water systems, excluding industrial networks or domestic hot water systems, although certain principles may apply to them. Consequently, the central question is which factors are decisive for their prevention and control.

Factors governing biofilm formation

Physicochemical factors of water

Temperature

Temperature is a key variable governing biofilm dynamics and the microbiological integrity of water, yet it is not a mere growth promoter [30]. Indeed, it affects microbial communities in a non-

linear manner [31-33]. At the molecular level, it alters the physicochemical properties of the bacterial surface (hydrophobicity, electron-donating character) and directs metabolism towards the production of EPS, which is essential for the structure and resilience of the biofilm [30]. Responses vary by species: some pathogens (e.g. *Salmonella*) maximize biofilm formation at moderate temperatures (about 20–22°C); others (e.g. Vibrio) enhance it at cooler temperatures [34]. At the community level, warming restructures populations by favouring certain genera (*Pseudomonas*, *Fusarium*) and alters the viscosity of EPS, which may paradoxically lead to detachment and downstream dispersal [32,35].

Although health authorities recommend temperatures above 55–60°C to combat planktonic pathogens (notably *Legionella*), biofilms offer substantial protection against such temperatures [36]. The optimum temperature for biofilm growth is not certain. Some studies suggest >45°C, others a peak at 45°C, with these discrepancies attributable to the composition of the communities and the experimental contexts [34]. Furthermore, temperature acts indirectly by promoting the release of dissolved organic carbon (DOC) from pipework materials (notably a 7.2-fold increase in release at 60°C), thereby stimulating microbial regrowth and reducing the efficacy of disinfectants [32]. Figure 4 summarizes these multiple mechanisms.

Thermal management offers targeted solutions among control strategies. For example, Lamas *et al*. [34] demonstrated that maintaining a temperature of 45°C in shower heads effectively reduces active microbial populations and pathogens. But, the application of this approach to all DWDS networks is severely limited by (i) inevitable temperature fluctuations, particularly in secondary sections, and (ii) the complex and non-linear responses of multi-species biofilms to heat. Consequently, this approach is mainly relevant for domestic hot water systems in large buildings (hospitals, hotels) and is not a universal solution for cold-water networks, where temperature control is difficult.

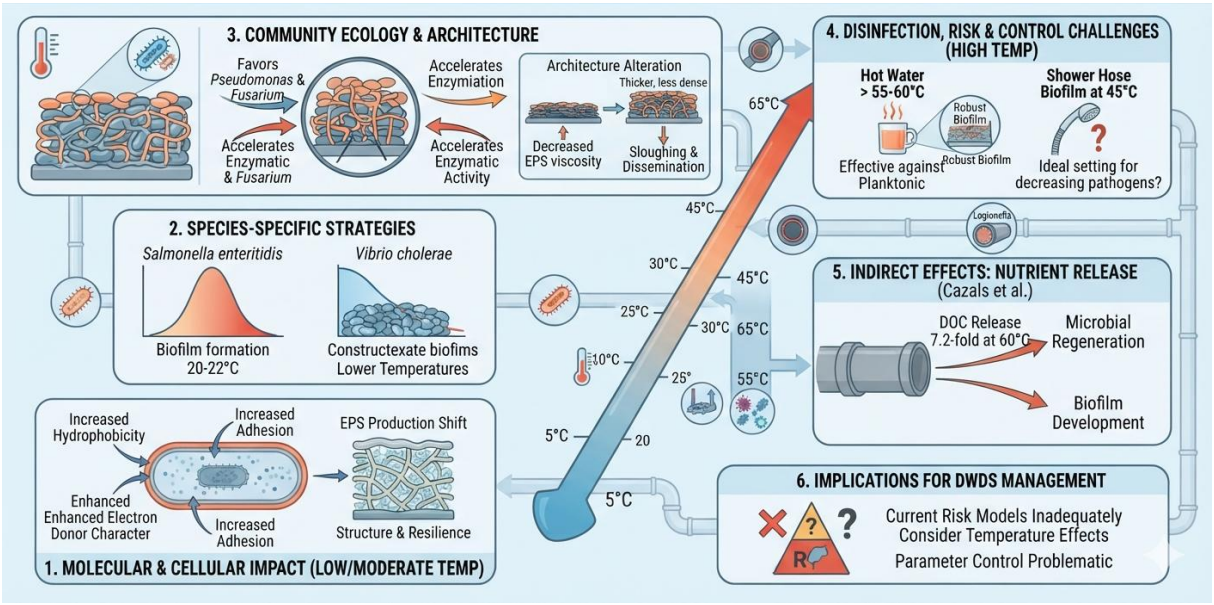


Figure 4. Temperature: a nuanced regulator of biofilm dynamics in drinking water

pH

pH is a key determinant of biofilm behaviour (Figure 5), integrating physicochemical and biological pathways [37]. By altering the electrostatic environment at solid–liquid interfaces, pH influences the chance of bacterial adhesion: as pH approaches the isoelectric point, electrostatic repulsion weakens, reducing the energy barrier to attachment and promoting early colonization [37]. Changes in pH alter surface hydrophobicity, making substrates more adhesive. These physicochemical effects interact directly with metabolic activity: conditions conducive to nitrification, for example, favour autotrophic populations but cause local acidification, which can, paradoxically, enhance adhesion to materials such as PVC [37,38]. This coupling is, however, bidirectional: changes in pH, particularly sudden or extreme ones, disrupt the structural integrity of mature biofilms by weakening the extracellular matrix, causing detachment with direct consequences for water quality [37,39].

Sensitivity to pH is particularly evident in the case of *Salmonella spp.*, where recent studies show that biofilm formation is optimal in a slightly acidic environment (pH 5.5–6.6), whereas extreme conditions (pH 4.4–4.5) completely inhibit it [40]. These findings contradict earlier studies which suggested there was no effect, and highlight the importance of the experimental context and the strains studied.

pH control can be used as a preventive measure. Indeed, Tran *et al.* [39] demonstrated that maintaining a slightly acidic pH of 5.5-6 inhibits formation of *Bacillus subtilis* biofilm, most likely

through altered EPS matrix production. Applicability in real drinking water systems is limited by two factors: (i) regulations call for a pH of 6.5-9.5, excluding too acidic solutions, and (ii) the acidic conditions accelerate corrosion of metal (cast iron, copper, steel) pipes which leads to metal leaching and infrastructure damage. Hence, this approach is restricted to specific applications, e.g., closed-loop industry circuits or pre-treatment steps.

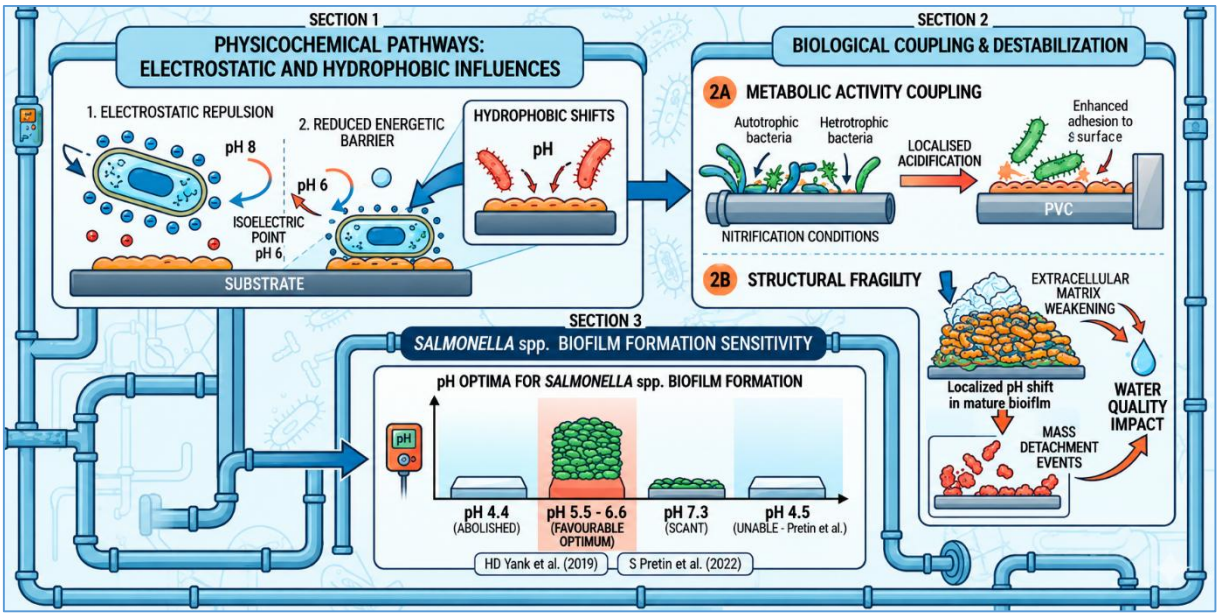


Figure 5. The critical role of pH in biofilm behavior

Nutrient availability

Nutrient availability is a major factor controlling biofilm formation in DWDS (Figure 6). Its role is often reduced to merely stimulating growth, but an approach based solely on the Redfield ratio (C:N:P) is too narrow to understand the mechanisms of microbial adaptation to different nutritional conditions [35,41]. Nutrient limitation does not necessarily reduce biofilm formation; on the contrary, it may select for stress-tolerant populations and favour species with an increased ability to colonize nutrient-rich surface niches [35]. Furthermore, high concentrations of ammoniacal nitrogen (NH_4^+) promote the growth of nitrifying bacteria, significantly altering the composition and biogeochemical functions of the community [38].

The interactions between nutrition and adhesion go beyond the mere supply of nutrients required for colonization. To illustrate, ions present in tap water alter the surface properties of bacteria (hydrophilicity, electron-donating character) and promote adhesion, irrespective of cell density or nutritional status [42,43]. The study by Montoya-Pachongo *et al*. [44] shows that nutritional deficiency reduces EPS production and the adhesive properties of biofilms, in line with the work of Zhang *et al*. [41] and Desmond *et al*. [24]. At the community level, nutrient gradients within the

biofilm allow the coexistence of microorganisms with opposing metabolisms (methanogens/methanotrophs), and the ability to use complex carbon sources (humic substances) via extracellular digestion confers resilience to fluctuations [23,41].

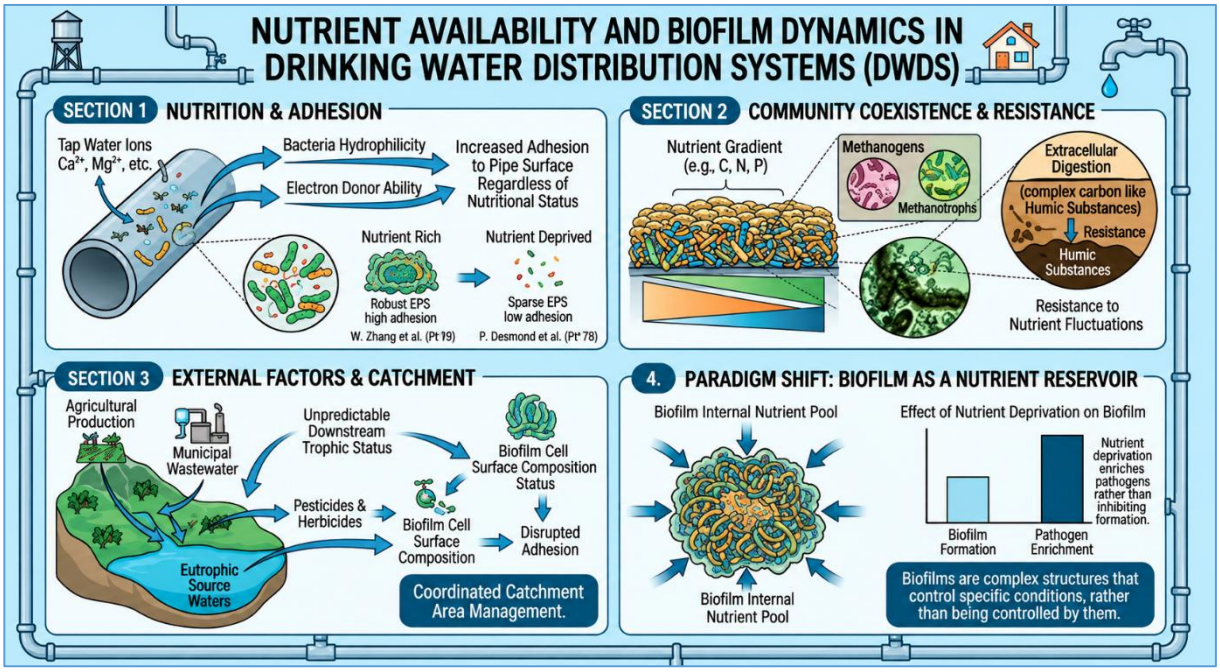


Figure 6. Nutrient availability and biofilm dynamics in DWDS

The nutrient load from source waters (eutrophication, agricultural discharges) directly influences the trophic status of downstream biofilms [42,43,45]. The intrusion of pesticides and herbicides into groundwater can also alter the chemical composition of cell surfaces and disrupt cell adhesion, highlighting the need for integrated catchment management [42,43,45]. Nutrient deficiency does not necessarily inhibit biofilm formation, but may promote the enrichment of pathogens, particularly as the biofilm itself acts as a nutrient reservoir regardless of the quality of the circulating water.

Limiting the availability of nutrients – though difficult to achieve in a DWDS – can be enhanced by careful choices of materials. The use of materials with low dissolved organic carbon (DOC) leaching is a promising avenue, as illustrated by Cazals *et al*. [32], who observed a 7.2-fold increase in DOC leaching at 60°C. Anti-leaching coatings, currently under development, could offer a complementary solution for reducing the bioavailability of carbon in pipes, although their durability and long-term effectiveness under real-world conditions remain to be demonstrated.

Operational and treatment factors

Water chlorination

Reliance on disinfectant residues as the primary barrier against microbial proliferation in DWDS ignores the adaptability of biofilms. The matrix of extracellular polymeric substances (EPS) greatly impedes the penetration of biocides [4,22]. Further, the natural degradation of disinfectants through reaction with organic matter and the biofilm itself makes regrowth inevitable [49]. The persistence of biofilms under continuous disinfection pressure, evidenced by recurrent deterioration in water quality (turbidity, colour, biocorrosion), exposes a fundamental flaw in strategies that treat bulk water chemistry as a substitute for microbial control of surfaces [46,47].

Disinfection procedures also have a selective effect on microbial communities: multi-species biofilms show greater tolerance to chlorine than pure cultures, probably through synergistic interactions [46]. Thus, monochloramine may promote the proliferation of opportunistic pathogens such as *Legionella spp.* or nitrifying bacteria, depending on the concentrations applied [47]. Chlorinated solutions, tend to homogenize the composition of microbial communities [26]. At sublethal concentrations (0.75–1 mg/L), chlorine can paradoxically stimulate the adhesion and cultivability of Pseudomonas aeruginosa, suggesting adaptive responses that precondition the cells for increased resistance [48,49]. Exposure to chlorine can induce antibiotic resistance through mutagenesis and horizontal gene transfer, or generate viable but non-cultivable (VBNC) forms capable of re-emerging at a later stage [50]. Finally, the formation of by-products and undesirable flavours may affect consumer confidence [16].

Disinfection reveals complex trade-offs: free chlorine and chlorine dioxide act quickly but have poor penetration; monochloramine penetrates better but is generally less effective [47]. Concentrations below 2 mg/L are insufficient, and changes in disinfectant (e.g. replacing chlorine with chloramine) can destabilize existing biofilms and mobilize contaminants and pathogens [51]. Furthermore, upstream treatments influence biofilm potential: coagulation and filtration reduce bacterial loads, but granular activated carbon (GAC) filters can enrich antibiotic resistance genes (ARGs) and biofilm-forming genera such as Pseudomonas, thereby seeding downstream systems [50,52].

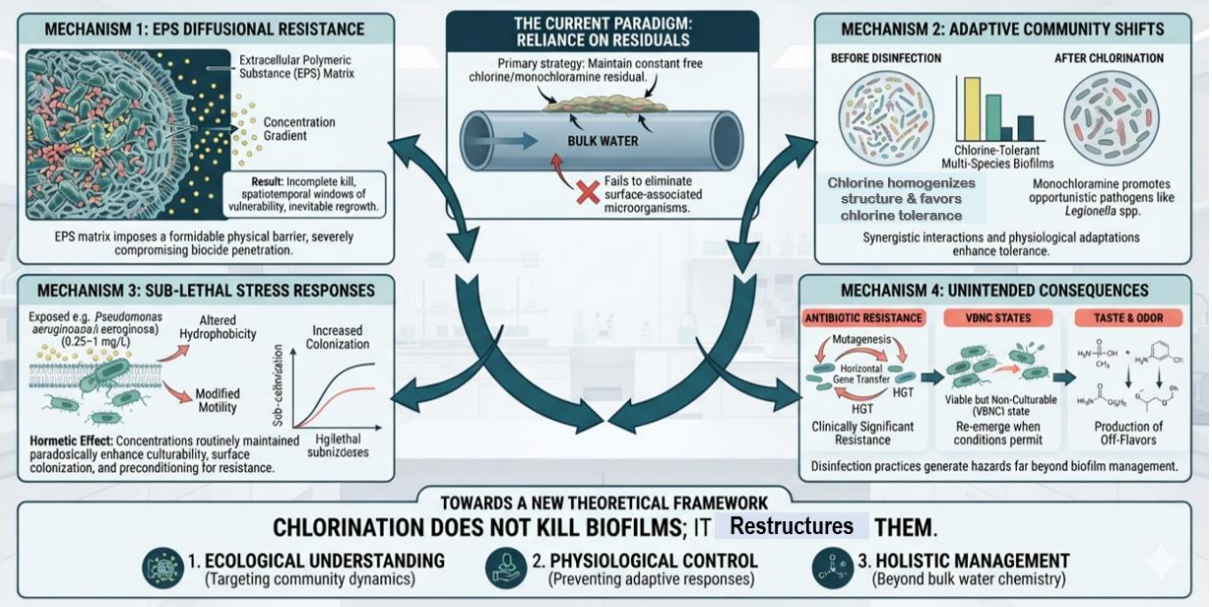


Figure 7. Rethinking disinfection: the paradox of biofilm adaptation in drinking water systems

Beyond traditional biofilm-control strategies, a paradigm shift is needed. Rather than seeking to 'kill' biofilms, emerging approaches aim to weaken them or prevent their formation. Table 1 shows that, to date, no innovative strategy (Advanced physical disinfection (UV-LED, cold plasma, electroporation), Combined disinfection approaches (alternating chlorine/monochloramine, chlorine + UV)) offers a universal solution. Their effectiveness, often demonstrated in the laboratory, has yet to be validated under real-world network conditions, where the diversity of strains, the presence of organic matter and hydraulic fluctuations complicate the results. These approaches should be regarded as complements to basic treatment, rather than as alternatives. Figure 7 summarizes this paradox: disinfection, as currently practised, does not 'kill' biofilms but 'restructures' them, promoting the emergence of resistant strains and VBNC states, which necessitates a rethinking of control paradigms [6,52].

Hydrodynamics

Hydraulic conditions are the dominant physical driver of the ecology and integrity of biofilms in drinking water networks. The interaction between flow rate, pressure and flow regime determines the fundamental balance between biofilm accumulation and stability [53,54]. The shear stresses generated by turbulent flow exert a strict selective pressure, leading to compact and stable communities that are, however, more resistant to chemical removal [53]. Conversely, areas of low flow (end points of the network, dead spots) promote particle sedimentation and the accumulation

of nutrients, creating reservoirs of microbial proliferation that are protected from residual disinfection [17]. The decoupling of hydrodynamics from disinfectant transport makes these stagnant zones critical points for the regrowth of opportunistic pathogens.

The destabilizing impact of transient events (flushing, pressure surges, daily variations in flow rate) is underestimated. These disturbances can dislodge mature biofilms, causing detachments that are not limited to the resuspension of inert particles: they release metabolically active microorganisms into the water, directly compromising biological stability and manifesting as problems with turbidity and colour [63]. Hydraulic management therefore manifests competing effects: flow regimes that favour accumulation create the potential for subsequent severe mobilizations [54,55].

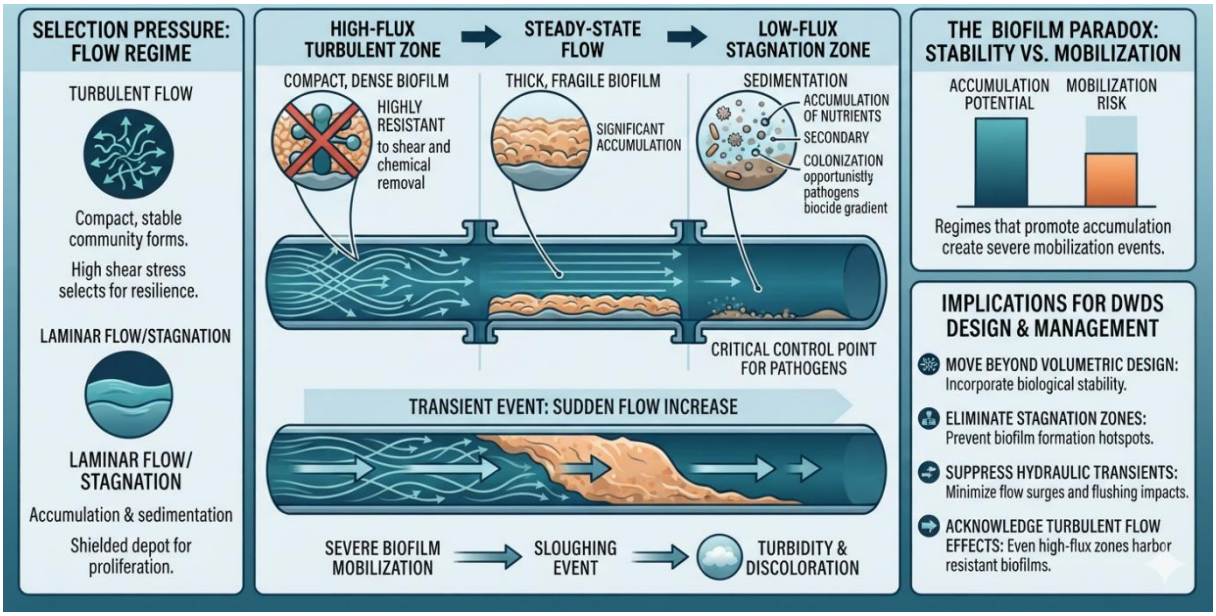


Figure 8. Hydraulic control: the dominant driver of biofilm ecology in DWDS

Recent advances in biofilm control have focused on active hydraulic management approaches. For example, Zeidan and Ostfeld [54] propose the use of scheduled flushing using controlled hydraulic transients to dislodge biofilms. This requires controlled variations in flow velocity, the kinetic energy of which detaches sessile communities without causing significant deterioration in water quality. The effectiveness of these approaches depends on the critical velocity specific to each type of biofilm and material, and their implementation requires accurate hydraulic modelling of the network to avoid excessive discharges or damage to infrastructure. These strategies have not yet been validated on a large scale.

Hydraulic design assumptions must incorporate biological stability criteria. The suppression of transients and the elimination of stagnation zones are key measures for reducing the risk of biofilm development, as summarized in Figure 8.

Material and infrastructural factors

The surface properties of pipes determine the structure of the ecological community [27,56]. Metal surfaces, because of conditions created by corrosion, promote greater bacterial growth that is qualitatively different from that observed on polymers [14]. The nodules formed on corroded metals provide favourable niches for long-term proliferation. Corrosion alters the physicochemical parameters of the water (disinfectant consumption, metal leaching) and promotes nitrification [15]. Conversely, polymer surfaces, although harbouring generally lower bacterial densities, can selectively enrich hydrophobic pathogens such as *Legionella*, whose adhesion is favoured by the surface chemistry [57]. The leaching of plasticizers and antioxidants can also provide nutritional resources for microbial communities [58]. Furthermore, the antimicrobial properties of copper are temporary: they reduce biomass in the short term, but bacteria such as *Pseudomonas aeruginosa* can enter a VBNC state and then re-emerge once the stress has been removed, and corrosion ultimately undermines this ability [11,22]. The age of infrastructure universally amplifies these effects, with corrosion products, organic deposits and mature biofilms creating heterogeneous environments that resist conventional measures [46,47]. Finally, surface roughness and the conditioning film interact with the material's properties to determine colonization, yet this film gradually masks the initial characteristics, rendering surface-level distinctions less relevant as the biofilm matures [26,56].

The selection of materials can therefore only be optimized in conjunction with active control strategies.

Anti-corrosion coatings with controlled release of biocides represent a promising control strategy. Emelyanenko *et al*. [59] demonstrated that copper- or silver-based coatings, deposited electrolytically, could reduce attached biomass by 2 to 3 logs under experimental conditions. Their main limitation remains ageing and the gradual loss of effectiveness, with performance declining after 1 to 2 years under real-world conditions. A promising approach combines these coatings with superhydrophobic surfaces that limit primary adhesion, but this technology still requires long-term validation before it can be widely adopted. The choice of materials for DWDS networks cannot be easily resolved, but rather requires a trade-off between different risks, influenced by the age of the infrastructure and the chemistry of the water (Figure 9).

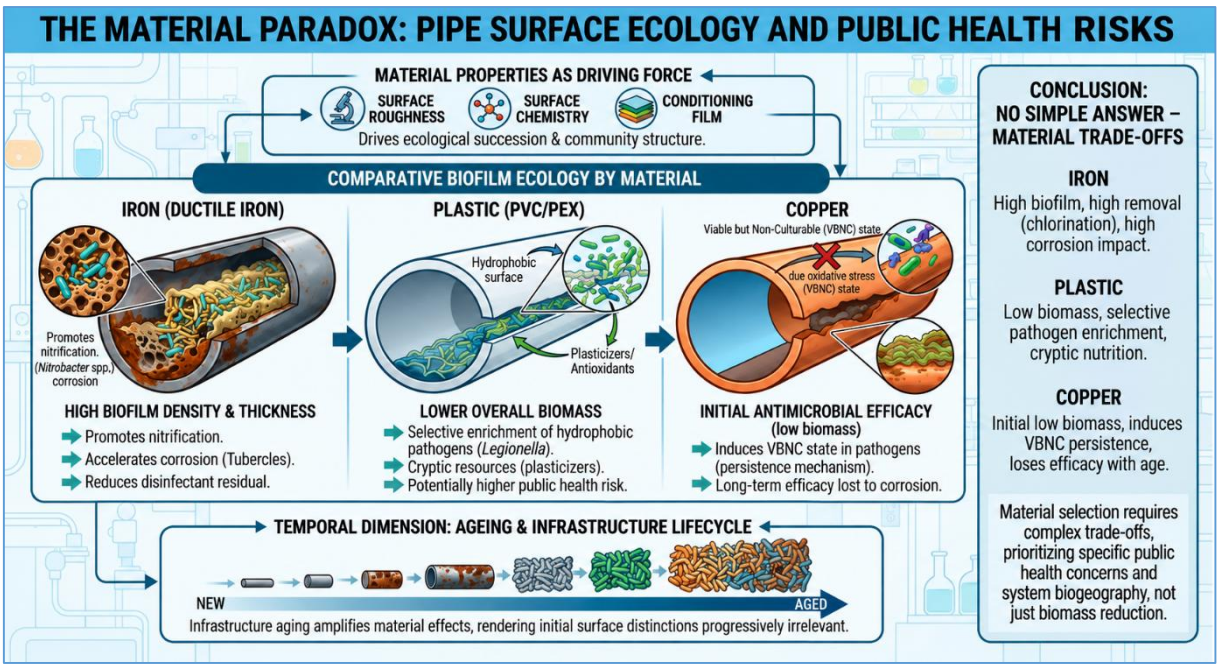


Figure 9. The material paradox: Pipe surface ecology and public health risks

Biological and ecological factors

Sensitive systems of intercellular communication govern the formation and structural dynamics of biofilms; e.g., quorum sensing, which regulates collective behaviour and community organization [60]. Extracellular polymeric substances (EPS) play a key role in providing a nutrient-rich habitat and enhancing the biofilm's resistance to environmental fluctuations [60,61]. Studies conducted on single-species biofilms limit our mechanistic understanding of EPS dynamics in a multi-species context, where bacterium-fungus interactions alter the physicochemical properties of the biofilm [62].

Biofilm maturity and species richness influence its tolerance to disinfectants, with mature biofilms being significantly more resistant than younger ones [63]. The physiological diversity of resident populations, including metabolically inactive and viable but non-cultivable (VBNC) cells, contrasts with studies relying on cultivability [10]. Furthermore, biofilms constitute a major reservoir of human pathogens and antibiotic resistance genes (ARGs), the spread of which via horizontal gene transfer remains poorly understood [52]. Interactions with higher trophic levels, notably grazing by protozoa, can increase the persistence of pathogens by creating conditions favourable to their development, as illustrated by the case of *Acanthamoeba* harbouring *Legionella pneumophila* and mycobacteria [64]. Finally, the presence of algae and invertebrates alters the structure of bacterial

communities and creates micro-scale heterogeneities that influence biogeochemical processes, suggesting that biotic interactions may, in some cases, override abiotic factors [10,62,63].

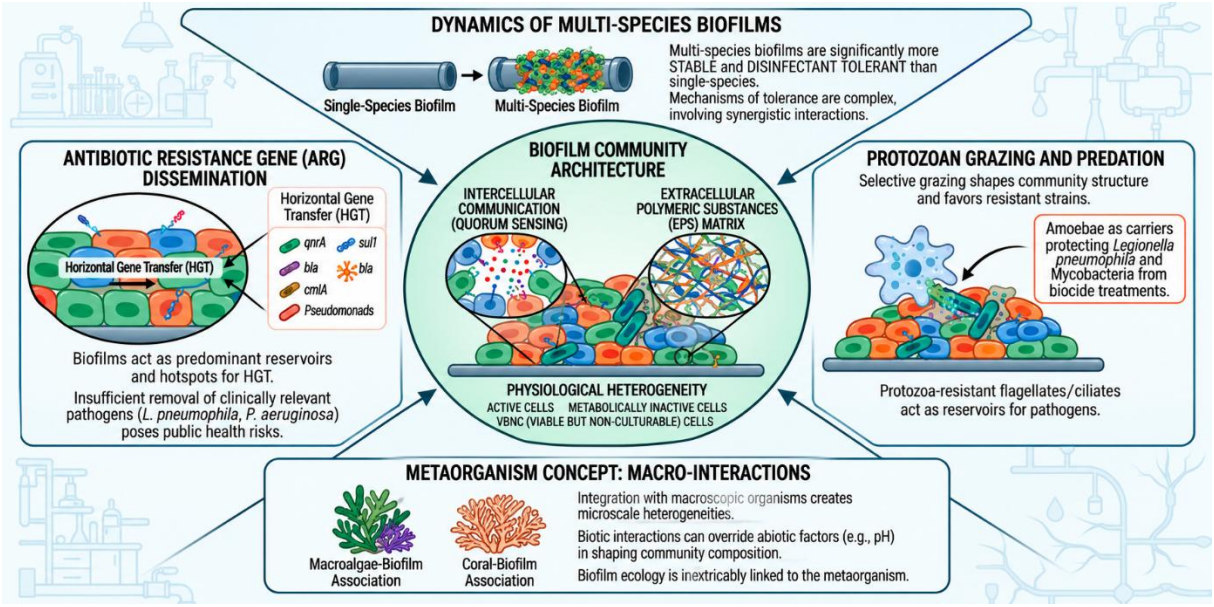


Figure 10. Complex biofilm ecology: From microscale interaction to multi-trophic dynamics

Targeting biotic interactions is an area yet to be explored. The use of predatory protozoa (e.g., *Tetrahymena* or *Acanthamoeba*) to control bacterial populations is discussed in the literature but their use is risky since some protozoa can be reservoirs of pathogens such as *Legionella*, which can worsen the health situation. On the other hand, the use of competing bacteria (probiotics) can lead to a preferential colonization of surfaces allowing for the diminishment of the potential for pathogens to colonize, as there are fewer available resources in terms of energy and space. This approach, which relies on community biology concepts, has not yet been tested in network settings where diversity leads to unpredictable results. Figure 10 summarizes the complexity of multi-trophic relationships which should not be overlooked in predictive biofilm formation models.

External environmental factors

Biofilm formation in DWDS is an adaptive and resilient process, resulting from the complex interaction between hydrogeochemical factors and anthropogenic influences. The quality of the source water is the main factor influencing the potential for microbial colonization, particularly in rural systems and small municipalities where treatment is less extensive [27]. The distinction between surface water and groundwater is not insignificant: systems supplied by surface water

harbour taxonomically richer and metabolically more diverse biofilms, highlighting the role of organic carbon availability as a selective pressure [65].

Climate change aggravates these effects by acting on physical and biological mechanisms. Warm water favours the proliferation of opportunistic pathogens and reduces disinfectant efficacy against *Legionella spp.* and non- tuberculous mycobacteria [66]. Warm water associated with lower rainfall and higher evaporation increases salinity, lowers dissolved oxygen levels and favours stagnation promoting biofilm formation and stabilization [61]. Extreme weather events (heavy rainfall, flooding) increase turbidity and the organic load, reducing the effectiveness of disinfectants but promoting bacterial colonization [67].

The chemical composition of water, which is determined by both natural processes and human activities, can have profound effects on the characteristics of biofilms. For example, mining activities alter the ionic composition of water, promoting colonization through changes in surface charge and adhesion [42,43]. Similarly, saline intrusion into coastal drinking water reservoirs affects the properties of biofilms forming on the surfaces of pipes [68]. Particulate matter and sediments constitute another underestimated factor: they provide both a habitat and a source of bacteria that are qualitatively different from those found in the water, enabling anaerobic processes to occur in an aerobic environment [56]. Furthermore, adsorbed pollutants (pesticides, heavy metals, toxins) can alter their morphology [22,45]. The protective effect of sediments and associated biofilms against disinfectants and predators is well established, but this resistance factor remains poorly accounted for in water quality management [45]. Conversely, factors such as such as pH and light, exert only a marginal influence in buried or opaque drinking water networks [27,56].

In the context of climate change, adaptation measures are being taken, such as increased chlorine dosing during warm periods or even the use of real-time sensors (turbidity, temperature, pH). These measures do not address the central issue of established biofilms, where viable microorganisms are sheltered. Thus, an integrated strategy, which considers upstream factors, active surveillance, and targeted intervention, is required to manage the impending challenges to water microbiology. Figure 11 shows the interrelationships between various processes, including the key role of source water quality and climate change in biofilm development, which are frequently overlooked in the context of drinking water management.

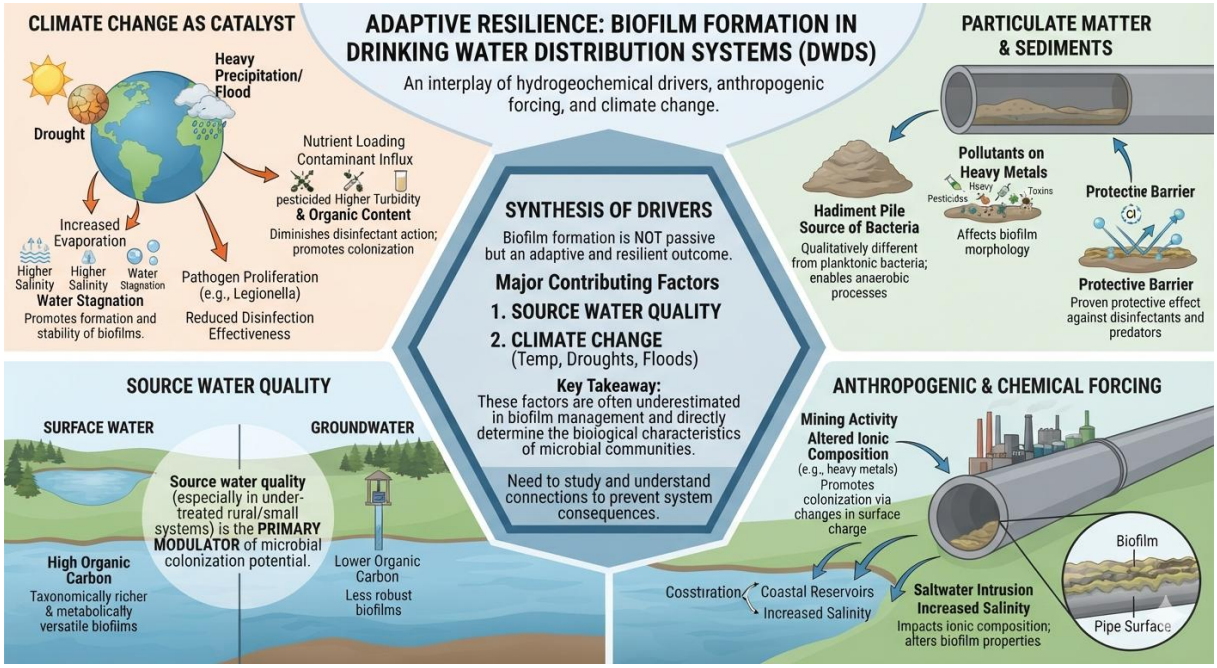


Figure 11. Adaptive resilience: Biofilms formation in DWDS

Simple visualisation

Table 2 shows the main factors influencing biofilms, summarizing their effect, whether or not the operator can control them, and the main known side effects. This simple guide makes it possible to prioritize the issues and highlight the importance of the synergistic interactions, which will be detailed in the following section.

Table 1: Summary of the main factors influencing biofilms in drinking water systems:

Factor	Effect on the biofilm	Can the operator control it?	Known side effect	Related Innovative Strategies	Intervention priority
Temperature	Strong, nonlinear	Difficult (except for water heaters)	Accelerated decline in chlorine levels	Pulsed heat treatment (45°C)	Medium (hot water systems)
pH	Moderate to strong, depending on the species	Yes (treatment)	Affects corrosion	Targeted adjustment (pH 5.5-6)	Low (regulatory constraints)
Nutriments (C, N, P)	Strong, but micro-niches	Partial (pre-treatment)	Eutrophication of springs	Low-release materials, pre-treatment	High (upstream)
Residual disinfectants	Moderate, selective	Yes, but limited	Selection of resistors, VBNC	Combined disinfection (chlorine + UV)	very high
Hydrodynamics	Very strong	Yes (network design)	Stagnation vs. shear	Scheduled purges, transient modeling	Very high (design)

Pipe material	Moderate to strong	During installation	Leaching, corrosion, aging	Antibacterial coatings, superhydrophobic surfaces	High (renovation)
Biological interactions (mixed cultures, VBNC, amoebae)	Very strong (often underestimated)	Not directly	Increased durability	Probiotics, dispersing enzymes	Low (Search)
Climate change	Indirect amplifier	No	Water source contamination	Adaptive strategies, enhanced monitoring	Low (Mitigation)

Interactions between factors and synergistic effects

The factors that influence formation and growth of biofilms in DWDS (water characteristics, materials, hydraulics) determine whether or not the biofilm will proliferate within the distribution network. Water quality parameters are synergistic, determining both the deficiency and the probability of survival of micro-organisms [27,56]. Seasonal variations in turbidity, pH and temperature actively compromise the disinfection barrier [69]. Exceeding the turbidity limit of 30 NTU protects bacteria from disinfectants, rendering the inactivation processes in treatment works ineffective [70]. This finding, combined with the fact that seasonal changes in water chemistry require a significant adjustment to the disinfection regime, argues against the adequacy of the current approach.

Although it is established that higher temperatures accelerate microbial growth, the direct link with the threshold at which pathogens emerge is less clear. For example, a water temperature below 18°C does not allow sufficient growth of *Legionella pneumophila* to pose a significant health threat; it is only at higher temperatures that the bacterium proliferates intensively [71]. Furthermore, rising temperatures promote corrosion and the inactivation of chlorine in a non-linear manner, thereby enhancing biofilm growth [72]. The physicochemical properties of pipework materials also influence microbial growth: as the temperature rises, bacteria adhere more strongly to various materials, making it more difficult to control biofilm growth [33].

The debate on pipework materials must go beyond their biofilm-forming propensity. Cast iron pipes, for example, are not only difficult to disinfect; they promote recalcitrant biofilms through corrosion-mediated protection, rendering even high residual chlorine levels ineffective [12]. Conversely, the apparent advantage of copper is only conditional; it is only evident in nutrient-poor water with a constant level of free chlorine, which is rarely the case in real-world systems [4]. Thus, the performance of materials depends on the specific properties of the water and its flow regime, which must be taken into account when selecting materials for pipework.

Although the risk of stagnation is often highlighted, its interaction with water age is more complex than it appears. Stagnation does not simply create conditions favourable to biofilm growth; it alters the system's microbiome. Water age in certain zones favours the establishment of Nitrospira and the depletion of monochloramine; a low water age favours the growth of pathogens and the accumulation of disinfectants [18]. This highlights the link between the proper organization of water flow and the maintenance of a stable microbiome.

Finally, the ability of *Bacillus cereus*, *Sphingomonas spp.* and *Acinetobacter spp.* to colonize biofilms is a result of residual disinfection [73]. The occurrence of these bacteria is linked to changes in water composition, EPS content, aggregation and the potential for surface modification [74]. Ignoring the mechanisms of biofilm formation means that residual disinfection cannot prevent disease, acting merely as a method of combating the consequences of contamination.

Significant gaps in our understanding weaken our ability to control biofilms: the reactivation of VBNC bacteria following stress, the destabilizing role of transient hydraulics, and the complex interactions between mixed materials within a single network remain poorly understood. We remain unable to predict health risks reliably based solely on measurements of biomass or diversity. Crucially, the actual effectiveness of next-generation strategies (phages, enzymes, specialized coatings) has not been demonstrated outside the lab – in the dynamic conditions of real-world distribution networks.

Innovative biofilm control strategies: a comparative assessment

The limitations of conventional disinfection approaches have prompted the development of alternative and complementary strategies targeting different stages of biofilm formation, from initial adhesion to mature community disruption. Table 2 shows a structured comparative assessment of the most promising innovative strategies, evaluating their principle, reported efficacy, limitations, and practical applicability to drinking water networks.

Table 2. Innovative biofilm control strategies: comparative assessment

Strategy	Principle	Mechanism of Action	Reported Efficacy	Key Limitations	Applicability to DWDS	Stage of Development
Phagotherapy	Use of specific bacteriophages to lyse biofilm bacteria	Phages infect and replicate within host bacteria, causing cell lysis and biofilm disruption	2–3 log reduction in laboratory settings [46]	High host specificity; selection of resistant mutants; inactivation by residual chlorine; limited penetration into mature biofilm	**Low** – requires prior identification of target strains; best suited for point-of-use applications or emergency responses	Preclinical / Laboratory
Dispersing enzymes (DNase, proteases, glycosidases)	Enzymatic degradation of the EPS matrix, weakening biofilm structure	Hydrolysis of extracellular DNA, proteins, and polysaccharides, releasing cells from the biofilm	Variable efficacy (30–80% reduction) depending on biofilm type and maturity [23]	High cost; limited stability in water; ineffective against mature biofilms; potential interference with water quality	**Low to moderate** – targeted applications in specific hotspots (e.g., reservoirs, storage tanks)	Laboratory / Pilot
Anti-quorum sensing molecules (furans, AHL analogues, brominated furanones)	Inhibition of bacterial cell-to-cell communication, preventing maturation and virulence	Interference with acyl-homoserine lactone (AHL) signalling pathways, reducing EPS production and biofilm formation	50–80% biomass reduction in laboratory settings; effective against Gram-negative bacteria [23]	Uncertain stability under real-world conditions; non-specific effects on beneficial bacteria; potential toxicity; limited field validation	**Low** – continuing research; no large-scale validation in DWDS	Laboratory / Early research
Innovative antibacterial surfaces (polymer grafting, nanoparticles, superhydrophobic coatings)	Prevention of primary adhesion or controlled release of biocides	Modification of surface physicochemical properties (hydrophobicity, charge) or slow release of antimicrobial agents (Cu, Ag, Zn)	60–90% reduction in adhesion under controlled conditions [11,22]; 2–3 log reduction with Cu/Ag coatings [11,22]	Ageing and loss of efficacy over time (decline after 1–2 years); fouling; high cost; limited validation in real networks	**Moderate** – applicable to new installations or during pipe renovation; requires long-term durability studies	Pilot / Early deployment
Advanced physical disinfection (UV-LED, cold plasma, electroporation)	Inactivation via physical (non-chemical) mechanisms	DNA damage (UV), generation of reactive species (plasma), or	Highly effective against planktonic bacteria (>4 log reduction); limited	Limited penetration depth (mm range); high energy costs; integration challenges in existing	**Low to moderate** – best suited for point-of-use applications (hospitals, buildings)	Pilot / Commercial (point-of-use)

		membrane disruption (electroporation)	penetration into biofilm matrix [11,22]	networks; point-of-use only	or treatment plant outlets	
Probiotic / competitive bacteria	Competitive exclusion of pathogens by beneficial bacteria	Colonization of surfaces by non-pathogenic strains, occupying ecological niches and limiting pathogen establishment	Promising in laboratory settings; limited field data	Risk of introducing non-native species; strain stability; regulatory hurdles; unknown long-term effects	**Very low** – remains speculative for DWDS; requires extensive safety validation	Early research
Combined disinfection approaches (alternating chlorine/monochloramine, chlorine + UV)	Synergistic effects of multiple disinfectants to reduce resistance selection	Sequential or simultaneous application of disinfectants with different mechanisms of action	Reduced selection of resistant strains; improved biofilm control compared to single disinfectants [47]	Complex management; requires precise dosing; potential for increased disinfection by-products	**Moderate to high** – applicable to existing networks with operational adjustments	Operational (some utilities)
Targeted thermal treatment (45°C maintenance)	Heat-induced reduction of active microbial populations	Elevated temperatures (45°C) reduce culturability and metabolic activity of biofilm bacteria	Effective reduction of active populations and pathogens in shower hoses [31]	Limited to hot water systems; temperature fluctuations in networks; energy costs	**Low** – applicable only to domestic hot water systems (hospitals, hotels, large buildings)	Operational (specific systems)
Hydraulic management (programmed flushing using controlled transients)	Detachment of biofilms via controlled hydraulic transients	Generation of brief, controlled increases in flow velocity to dislodge sessile communities without massive water quality degradation	Effective in laboratory and pilot studies; site-specific efficacy [54]	Requires precise hydraulic modelling; efficacy depends on biofilm and material-specific critical velocities; risk of excessive contaminant mobilization	**Moderate** – applicable to networks with identified stagnation zones; requires validation	Pilot / Early deployment

The comparison shows that there is no ideal solution since all options have considerable inconvenience regarding efficiency, cost, complexity, and application. The best performers are those disinfectants that use several mechanisms of action, creating a multi-barrier. Although laboratory tests have demonstrated the strategies' effectiveness, they are not always successful in real settings because of complications (e.g., chlorine, penetration, heterogeneity, and water quality). Hence, only combined disinfection and specific thermal treatments are now applied in practice. The third group of strategies includes materials that could make surfaces antibacterial or self-sterilizing. Some are already used in novel construction or reconstruction to replace old ones; but, these are expensive, frequently inefficient, and rapidly degrade after one or two years. In contrast, hydraulic management through programmed flushing using controlled transients [54] is the most immediately useful strategy, requiring no major infrastructure changes yet relying on precise hydraulic modelling, particularly in networks with identified stagnation zones. Ultimately, combined approaches integrating complementary interventions (e.g., antibacterial surfaces, residual disinfection, hydraulic optimization) within a network-wide management plan offer the best prospect for sustainable biofilm control.

Table 3. Practical recommendations for researchers and managers

Strategy	Principle	Reported Efficacy	Limitations	Applicability to Networks
Phagotherapy	Use of specific bacteriophages to lyse biofilm bacteria	2–3 log reduction in laboratory settings	Host specificity, selection of resistant mutants, degradation by chlorine	Low (requires prior identification of target strains)
Dispersing enzymes (DNase, proteases, glycosidases)	Degradation of the EPS matrix, weakening of the biofilm structure	Variable efficacy depending on biofilm type	High cost, limited stability, ineffective against mature biofilms	Low to moderate (targeted applications)
Anti-quorum sensing molecules (furans, AHL analogues)	Inhibition of bacterial communication, preventing maturation	Effective in laboratory settings; 50–80% biomass reduction	Uncertain stability under real-world conditions, non-specific effects	Low (continuing research)
Innovative antibacterial surfaces (polymer grafting, nanoparticles)	Prevention of primary adhesion or controlled release of biocides	60–90% reduction in adhesion under controlled conditions	Ageing, fouling, high cost	Moderate (new installations or renovation)
Advanced disinfection (UV-LED, cold plasma, electroporation)	Inactivation via physical (non-chemical) mechanisms	Effective against planktonic bacteria; limited biofilm penetration	Limited penetration depth, high cost, integration challenges	Low to moderate (point-of-use applications)

This comparison (Table 3) shows that moving from traditional to modern methods of biofilm control requires a shift from single-factorial to multi-barrier approaches. In addition, it is necessary to consider each of the network's qualities: water qualities, materials, flow conditions, and target microorganisms. The methodology described in the next section can help in this task. It was created for evaluating the options for drinking water networks.

Methodological framework for the analysis of a drinking water network

A review of the factors identified enables us to propose a structured approach for assessing an existing network. This methodology comprises five stages:

Step 1: Mapping and preliminary diagnosis

- Identify areas of stagnation (extremities, loops, reservoirs).
- Raise the age of water in each section.
- Document the materials present and their age.
- Identify any history of coloration, tastes, or bacteriological problems.

Step 2: Physico-chemical characterisation of water

- Measure on several points of the network (and continuously): temperature, pH, turbidity, residual chlorine, dissolved organic carbon, nitrogen, and phosphorus.
- Analyze seasonal variations.

Step 3: Hydraulic evaluation

- Model flow regimes (flows, pressures, shear).
- Identify potential hydraulic transients.

Step 4: Biological characterisation

- Collection of biofilms by coring or cutting samples (where possible)
- Molecular analyses (qPCR, metabarcoding) to identify major populations and pathogens
- Assessment of the presence of VBNC bacteria

Step 5: Summary and action plan

Table 4. Summary of key parameters to be evaluated in a given network

Parameter	Level of impact on biofilm	measurement method	critical threshold	possible courses of action	Priority
Temperature	Very high	In situ probes	>18°C (*Legionella* risk)	Thermal management, insulation	High
residual chlorine	Very high	Colorimetric DPD	<0.2 mg/L (free) / <0.5 mg/L (monochl.)	Dose adjustment, chloration points	Very high
Age of water / Time of stay	Very high	Hydraulic modelling	>24-48h	Scheduled purges, shutdown	Very high
Turbidity	High	Nephelometry	>1 NTU	Upstream filtration, removal of deposits	High
Material	Moderate to high	Inventory	Iron / cast iron > plastic	Replacement / coating	Medium (long term)
Dissolved organic carbon	High	Persulphate - UV method	>2 mg/L	Pretreatment, low release materials	High
pH	Moderate	pH meter	Outside 6.5-8.5	Adjust Processing	Average
Nutriments (N,P)	Moderate	Colorimetry	N > 1 mg/L, P > 0.05 mg/L	Upstream pretreatment	High amount
Flow / Shear	Very high	Modelization	Stagnant areas / very low flow rates	Closed network, purges	Very high

Conclusion

Sustainable control of biofilms in drinking water networks cannot be achieved through isolated interventions; it requires a systemic and precisely targeted strategy. The complexity of the interacting factors can be simplified into a clear operational hierarchy: hydrodynamics (stagnation and water age), effective residual chlorine and temperature are the main levers for action. All other factors act as modulators. Success therefore depends on the simultaneous and deliberate manipulation of these three levers, synchronized with an integrated management plan at the network level.

Beyond the standard recommendations, a number of innovative strategies are emerging that offer real potential for drinking water networks:

1. Scheduled hydraulic flushing, using controlled transients to dislodge biofilms, which is particularly effective in identified areas of stagnation.
2. Superhydrophobic or copper/silver-based antibacterial coatings, which are effective in the laboratory but whose ageing under real-world conditions has yet to be assessed.
3. Dispersing enzymes (DNase, proteases), which show promise in the laboratory but are still costly and have not been validated in a network setting.
4. Combined approaches (alternating chlorine and monochloramine), which limit the selection of resistant bacteria but require rigorous management.
5. Targeted heat treatment at 45°C, applicable to domestic hot water systems.

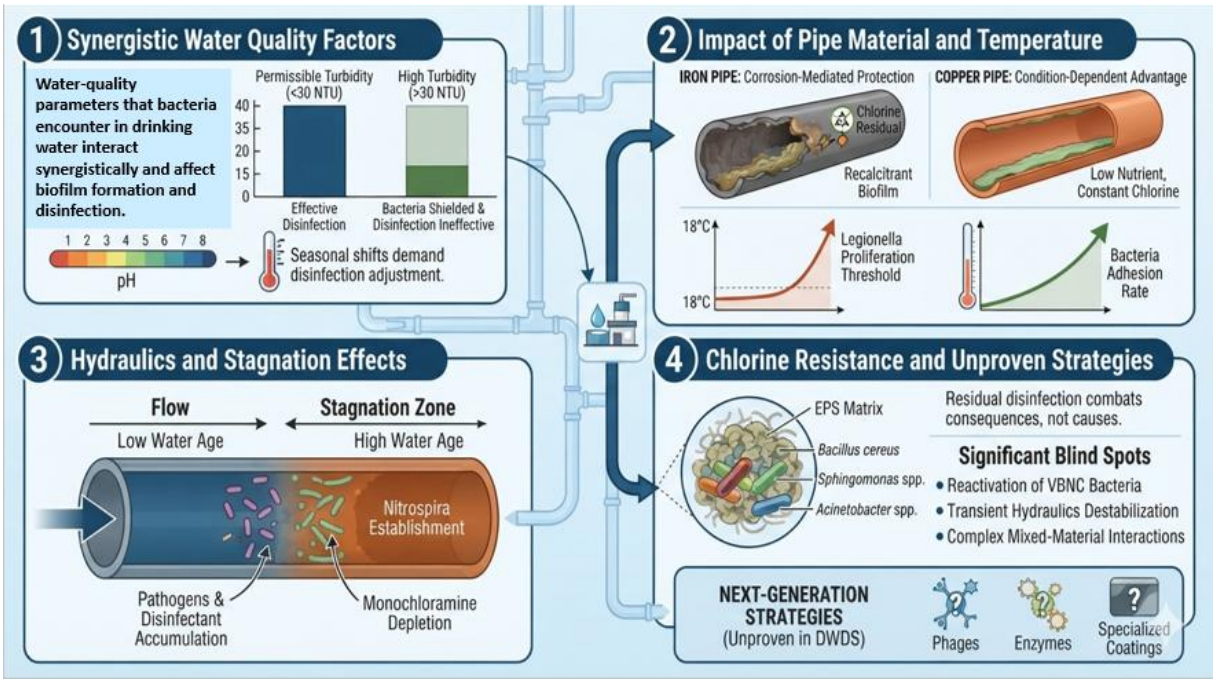


Figure 12: Mechanisms of biofilm formation and persistence in DWDS

Figure 12 shows the need to combine these different approaches to form a multi-barrier strategy, in which each plays its part and works together with the others. The key issue is to shift from managing individual factors to managing their interaction. A systems-based approach, targeting the most cost-effective and influential levers, is the only way of ensuring the microbiological safety of water supplies for consumers.

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- AI use: During the preparation of this work, the authors used Deepseek in order to enhance the English writing. After using this tool/service, the authors reviewed and edited the content as needed and they take full responsibility for the content of the published article.

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