AquaGuardian
(DIGIT.2. 1. 02. 001)

21 Ružičkini dani
DANAS ZNANOST – SUTRA INDUSTRIJA
9. – 11. rujna 2026. • Vukovar • Hrvatska

AquaGuardian

A Comprehensive AI Powered Early Warning System for Digital Management of Water Supply Security, Resilience, and Sustainability

(DIGIT.2. 1. 02. 001)

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CURRENT CHALLENGES IN DRINKING WATER SYSTEMS: BIOFILMS, MICROORGANISMS AND WATER SAFETY

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From source to safe water

MONITOR · UNDERSTAND · PROTECT

SCAN FOR MORE INFORMATION

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A COMPREHENSIVE AI POWERED EARLY WARNING SYSTEM

for Digital Management of Water Supply Security, Resilience, and Sustainability (AquaGuardian) – DIGIT. 2. 1. 02. 001

SAFE WATER | RESILIENT COMMUNITIES | A SUSTAINABLE TOMORROW

From source to safe water

PROJECT LEADER:
University of Zagreb
Faculty of Chemical Engineering and Technology

PROJECT PARTNERS:

- University of Zagreb Faculty of Civil Engineering
- University of Zagreb Faculty of Electrical Engineering and Computing
- Endress+Hauser Ltd.
- Genos Ltd.

PROJECT DURATION:
1 January 2026 – 30 October 2028

TOTAL ELIGIBLE COSTS:
4,265,369.22 €

GRANT AMOUNT:
3,981,251.41 €

https://aquaguardian.eu/

aquaguardian@fkit.unizg.hr

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AquaGuardian: From Experience to Early Warning

Protecting drinking water quality in distribution systems through science, technology and innovation

1 FCET team

more than 20 years of experience and work on drinking water treatment projects

Knowledge • Expertise • Commitment

2 Pilot areas

across Croatia

Real systems.
Real conditions.
Real data.

3 Key challenges

in disinfection and system operation

- Maintaining disinfection control
- Operational variability
- Infrastructure limitations

4 Hidden risks

threatening water quality and system stability

- Disinfection by-products (DBPs)
- Biofilm formation
- Pipe ageing and corrosion
- Microbial regrowth

These processes are often invisible – until it's too late.

5 The key question

How can we detect early signs of deterioration **before** water becomes unfit for drinking?

From reactive control to **proactive** protection.

6 AquaGuardian solution

Integrated approach for **early warning** and **decision support**

AI

- Real-time monitoring
- Advanced analytics
- Smart decisions

Right information. Right time. Right action.

Our goal: A safe, resilient and sustainable drinking water supply.

- Protect public health
- Strengthen water resilience
- Ensure operational excellence
- Support a sustainable future

AquaGuardian Research Activities (A2–A8)

From advanced monitoring to predictive water safety

A2 — Advanced Monitoring Technologies

- Sensors
- GC-MS/MS
- LC-HRMS
- ICP-MS
- IC
- TOC

A3 — Health Risk Assessment

- HepG2 cells
- Gene expression
- Glycan profiling

A4 — Pilot Monitoring & Real-Time Data

- Pilot sites
- Online monitoring
- Analytical validation

A5 — AI-Based Prediction & Early Warning

- Anomaly detection
- Predictive maintenance
- Drones
- Early warning systems

A6 — Digital Twin & System Integration

- Hydraulic + chemical modelling
- Scenario simulation
- AI-driven decision support

A7 — Platform & Communication Infrastructure

- IoT data collection
- Secure data exchange
- Alerts management
- Web applications

A8 — Field Trials, System Validation & Optimization

- Real-world testing
- Stress tests
- Performance evaluation
- System optimization

DETECTION > **ASSESSMENT** > **PREDICTION** > **DECISION SUPPORT** > **SAFER DRINKING WATER**

Integrated pathway for proactive, intelligent and resilient water safety management

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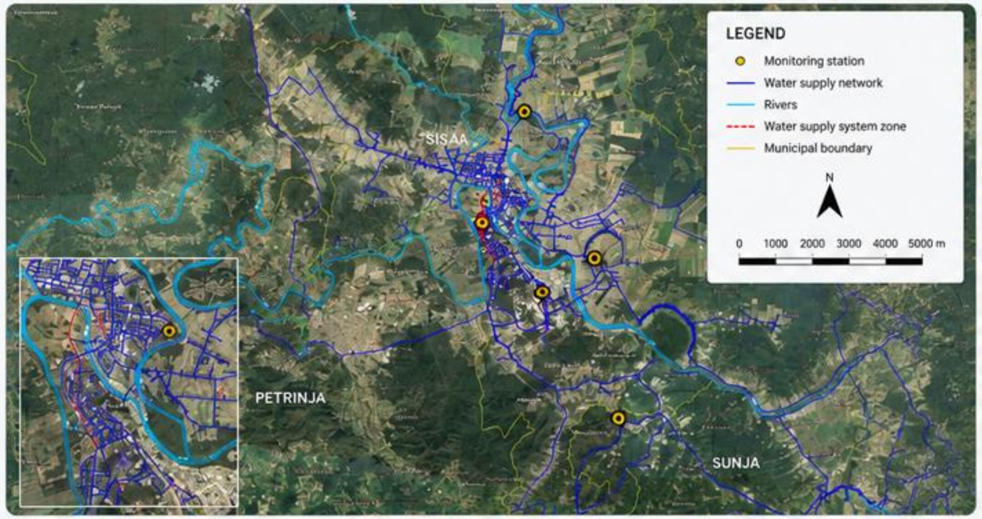
1. Within the fenced premises of Sisački vodovod, next to the Veliko Trojstvo Water Reservoir (Water retention time: 20–24 h)
2. Within the fenced premises of Sisački vodovod, next to the Ivajak Dosing Station (Water retention time: 24–36 h)
3. **Crnac settlement**, Sisak, within the Sisak Wastewater Treatment Plant (48 – 72 h)
4. **Prelošćica settlement**, Sisak. Inspection manhole near the morgue in Prelošćica (Water retention time: 48–72 h)
5. **Lukavec Posavski settlement**, Sisak. Above the inspection manhole next to the road, near the Gušće ferry crossing (> 72 h)
6. Within the fenced premises of Sisački vodovod, next to the abandoned Sunja drinking water treatment facility (Water retention time: > 72 h)

MONITORING STATIONS: LOCATIONS WITHIN THE SISAK WATER SUPPLY SYSTEM



Real-time monitoring of key water quality parameters | Early warning for contamination and system risks | Data-driven decisions for safer drinking water | Supporting sustainable and resilient water management

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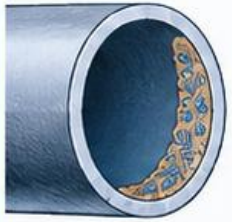
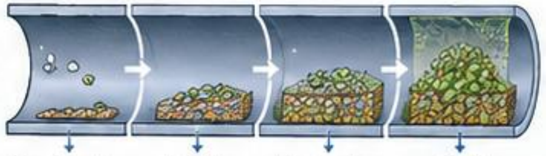
Lecture outline: Biofilms in Drinking Water Distribution Systems

1 Biofilm definition and relevance



- Complex microbial communities attached to pipe surfaces
- Impacts water quality, health and infrastructure

2 Formation and development in pipes



3 Microbial composition and EPS matrix

- Diverse bacteria, archaea, fungi and protozoa
- EPS matrix (polysaccharides, proteins, eDNA) protects and stabilizes biofilms

4 Factors promoting biofilm growth

- Temperature
- Water quality (nutrients, organic matter)
- Hydraulics (low flow, stagnation)
- Pipe material, age, roughness
- Disinfectant residual and decay

5 Sampling from pipe surfaces

- Representative, quantitative sampling
- Swabs, coupons, brushes and discs

6 Detection and characterization methods

- Culture-based methods
- Microscopy (CLSM, SEM)
- Molecular tools (qPCR, NGS)
- Physicochemical analysis (EPS, ATP)

7 Challenges in detection including VBNC state

- Low biomass and heterogeneity
- VBNC state under stress
- Methodological biases and limits
- Correlating data with risk

8 Control, removal and disinfection strategies

- Optimize disinfectant residuals and monitoring
- Physical removal (flushing, pigging, brushing)
- Advanced oxidation and alternative technologies
- Biofilm-informed management and risk-based approach

From pipe-wall colonization to monitoring, risk assessment and control.

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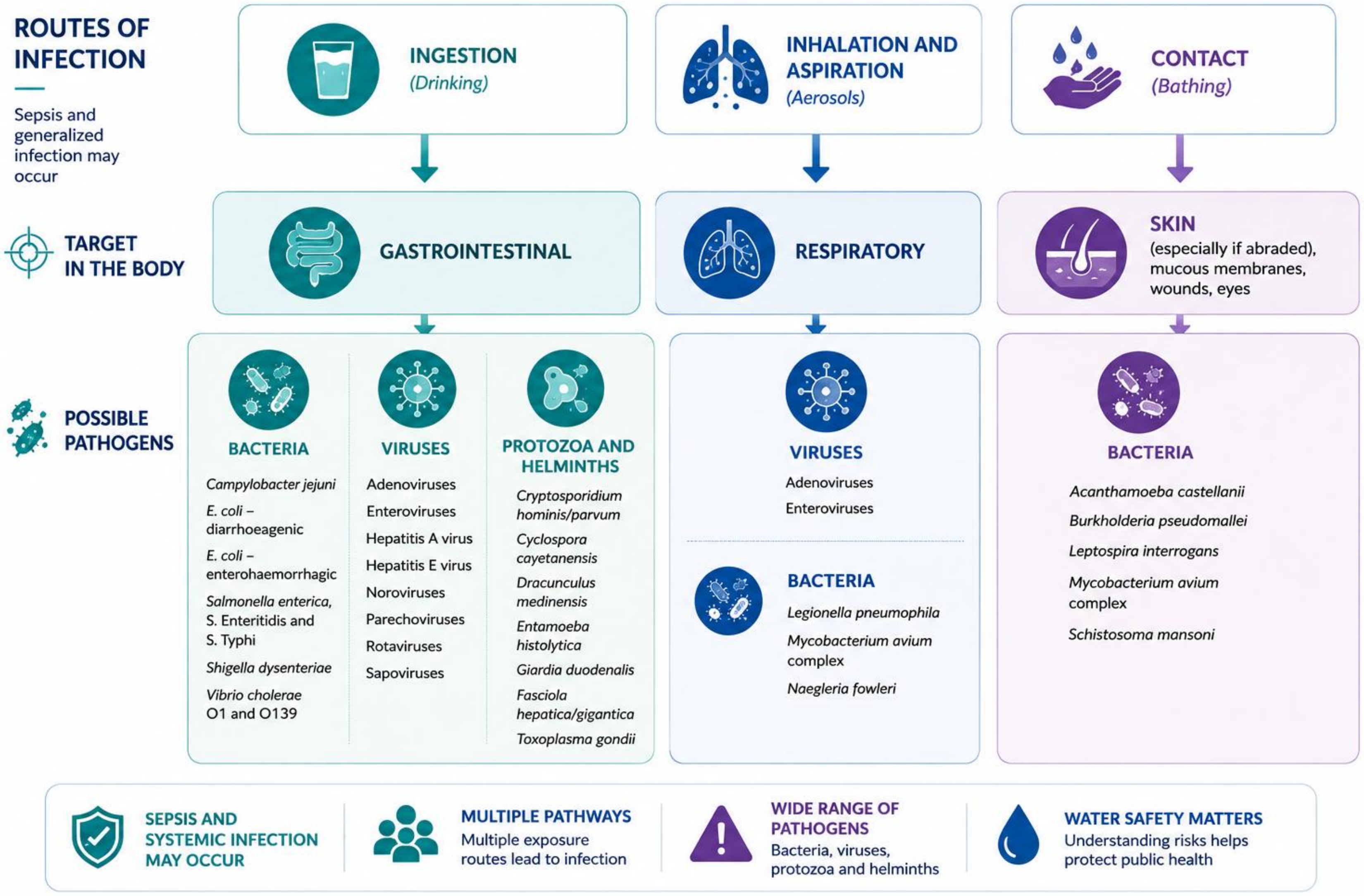
MICROORGANISMS & DRINKING WATER



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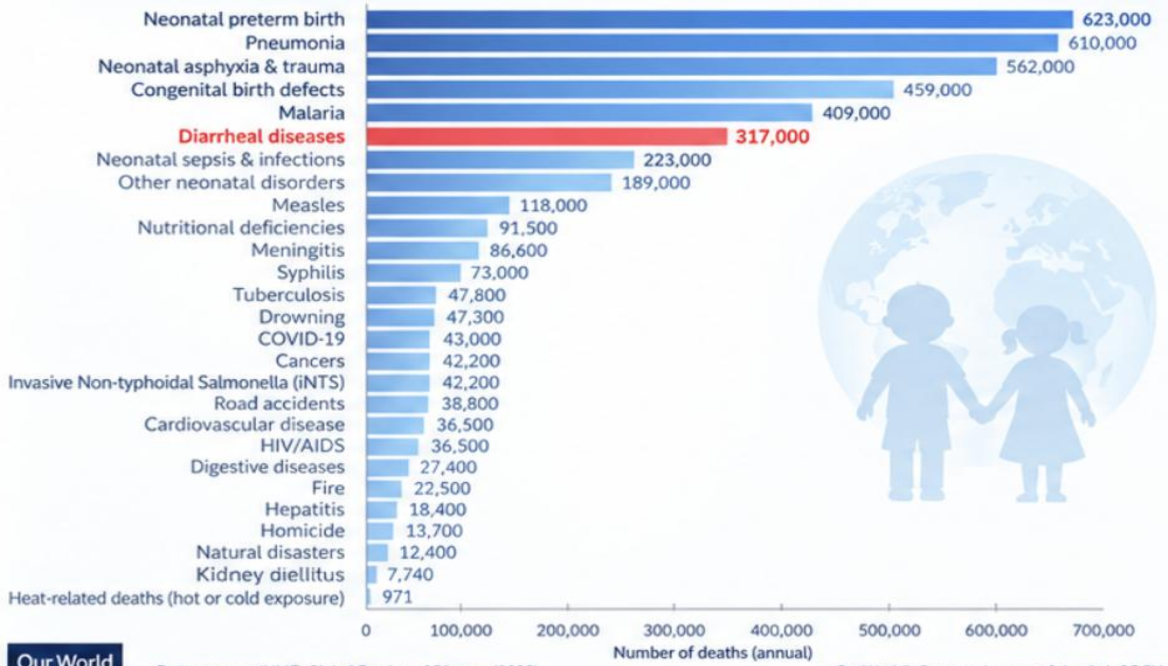
From source to safe water

Causes of death in children under five, World, 2023

The estimated annual number of deaths from each cause. Estimates come with wide uncertainties especially for countries with poor vital registration[1].



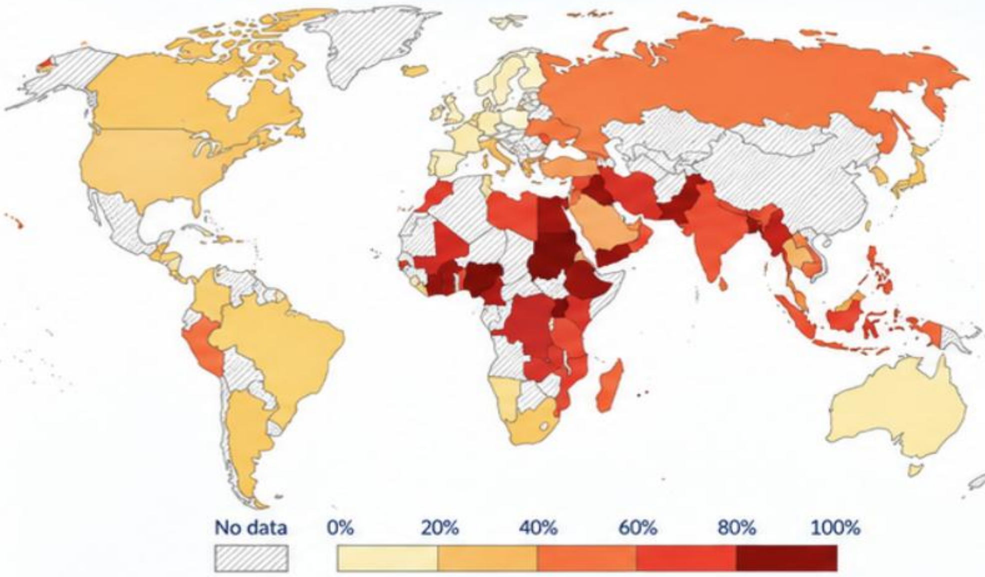
Our World in Data

Data source: IHME, Global Burden of Disease (2025) OurWorldInData.org/causes-of-death | CC BY

1. Civil Registration and Vital Statistics system (CRVS) is an administrative system in a country that manages information on births, marriages, deaths and divorces. It generates and stores vital records and legal documents such as birth certificates and death certificates.

Share of *E. coli* infections resistant to cephalosporins, 2022

Cephalosporins are a class of antibiotics commonly used to treat *E. coli* infections. This shows the estimated share of infections by *E. coli* in the bloodstream that were resistant to 3rd generation cephalosporins.



Antimicrobial resistance is a growing global threat to water and public health.

Data source: World Health Organization OurWorldInData.org/antibiotics | CC BY

Note: Where data for 2022 is unavailable, the value from the closest year between 2018 and 2021 is shown instead.

1. Escherichia coli (E. coli) is a bacterium that normally lives in the intestines as part of the gut's natural flora, where it helps with digestion. However, such as food poisoning, diarrhea, urinary tract infections, diarrhea, uuinary tract infections, pneumonia, and sepsis. Harmful strains, such as E. coli O157:H7, produce travel from diseases. E. coli stm. E. coli strains can also from the intestines to areas like the urinoing tract, causing infections when the immune system is compromised.

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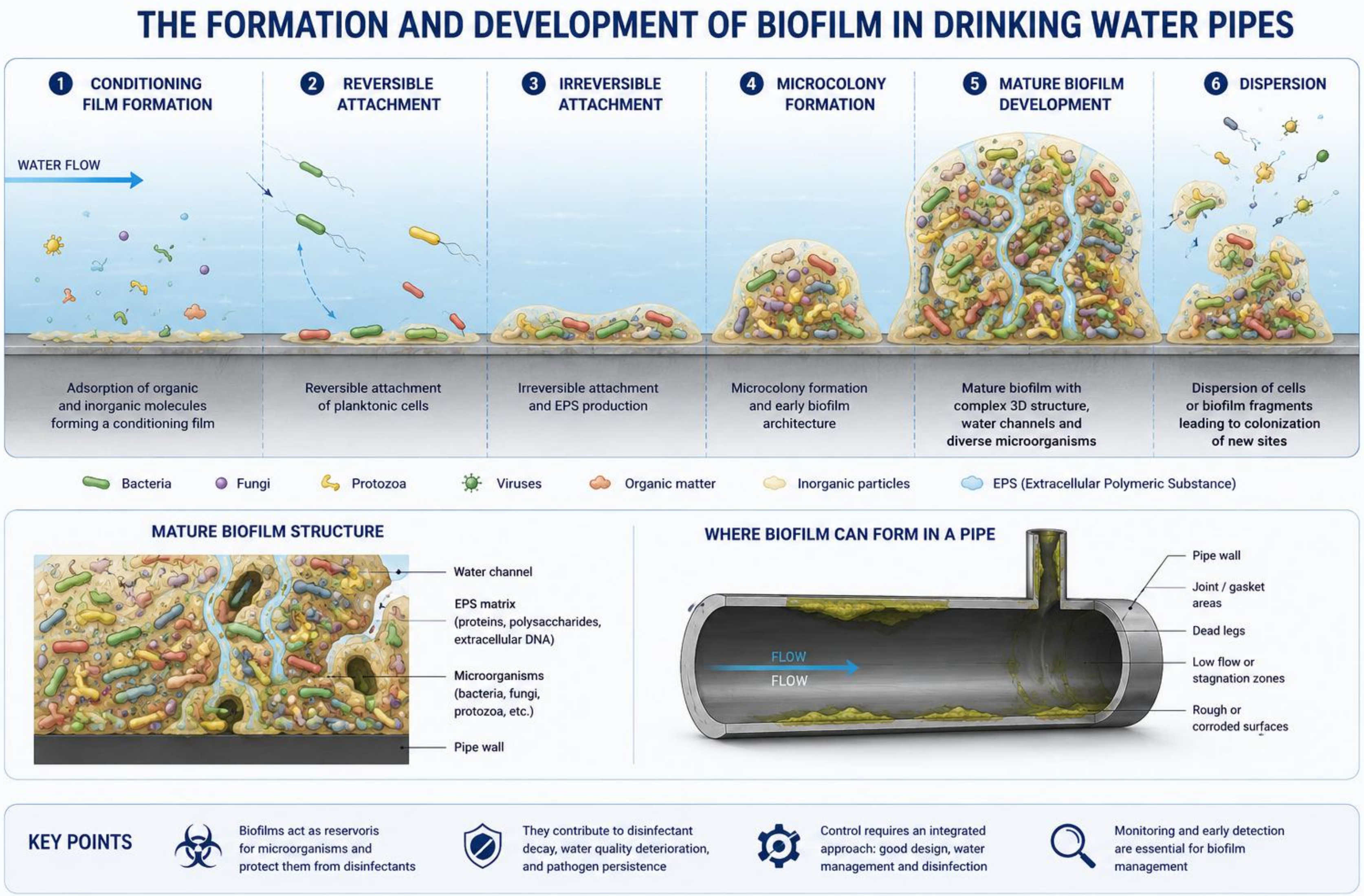
- Biofilms are complex microbial communities attached to pipe surfaces and embedded in an EPS matrix, which provides structural stability and protection against environmental stress and disinfectants.



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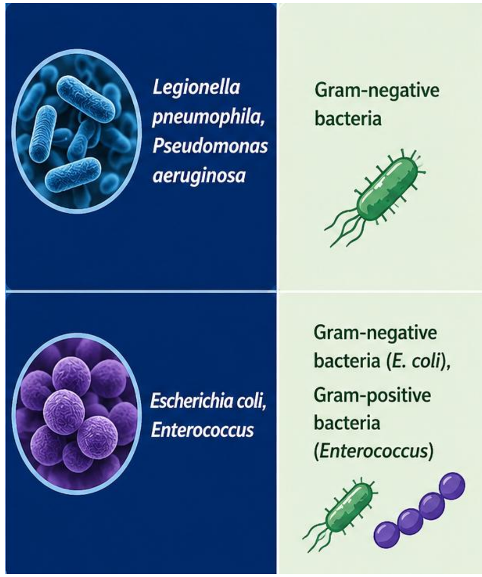
Microbial composition of drinking water biofilms



Microbial group	Typical representatives	Role / relevance in drinking water biofilms
Bacteria	*Pseudomonas*, *Acinetobacter*, *Sphingomonas*, *Aeromonas*, *Bacillus*	Dominant biofilm-forming microorganisms; EPS production, attachment and biofilm maturation
Opportunistic pathogens	*Legionella pneumophila, Pseudomonas aeruginosa*, non-tuberculous *Mycobacterium* spp.	Can persist and proliferate within biofilms; increased resistance to environmental stress and disinfection
Faecal / indicator bacteria	*Escherichia coli, Enterococcus* spp., coliform bacteria	Usually associated with contamination events; may survive or persist within established biofilms
Nitrifying microorganisms	*Nitrosomonas*, *Nitrospira*, *Nitrobacter*	Oxidation of ammonia and nitrite; may contribute to nitrification and disinfectant decay
Iron- and manganese-related bacteria	*Gallionella*, *Leptothrix* and related organisms	Associated with mineral deposition, corrosion products and discoloration
Fungi	Filamentous fungi and yeasts	Participate in organic matter degradation and contribute to structural complexity of the biofilm
Protozoa / free-living amoebae	*Acanthamoeba*, *Vermamoeba* and other protozoa	Graze on bacteria but may also provide protected intracellular niches for pathogens such as Legionella
Archaea	Methanogenic and ammonia-oxidizing archaea	Usually less abundant; contribute to specific nutrient-transformation processes
Viruses / bacteriophages	Bacteriophages and other viruses	Influence bacterial abundance, community structure and horizontal gene transfer

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Water-Quality Indicators Suggesting Biofilm Development

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Acute contamination
sudden event → microbial spike ↑ → *E. coli*/coliforms may appear → turbidity ↑ → event passes / moves downstream

Biofilm development
- gradual Cl_2 decay
- persistent ATP ↑
- FCM cell counts ↑
- recurrent HPC ↑
- periodic turbidity/particle spikes after hydraulic disturbances
- possible Fe/Mn release
- repeated microbial signature at the same location

→ HIGH PROBABILITY OF BIOFILM DEVELOPMENT

Indicator in bulk water	Pattern suggesting biofilm development	Significance
Residual chlorine ↓	Gradual and recurrent decrease in Cl_2 residual along the distribution system / increased chlorine decay	Biofilm, EPS and deposits increase **disinfectant demand**
ATP ↑	Gradual or persistent increase in cATP above the site-specific baseline	Indicates an increase in **active/viable microbial biomass**; cATP > **1 pg mL^{-1}** has been used as an action trigger in some systems, but is not a universal threshold
Total / intact cell count ↑	Sustained increase in FCM cell counts	Indicates increased **microbial biomass and/or regrowth**
HPC ↑	Gradual increase above the established baseline	Supports evidence of **microbial regrowth**, but represents only the culturable fraction and is insufficient as a stand-alone indicator
Turbidity ↑ / recurrent spikes	Intermittent peaks, particularly following changes in flow or pressure	May indicate **biofilm/deposit detachment and resuspension**
Particle count ↑	Peaks associated with hydraulic disturbances	May indicate **detachment of biofilm, EPS and deposits**
Fe/Mn ↑	Periodic metal mobilization coinciding with increased turbidity/particle counts	May indicate **corrosion–deposit–biofilm interactions**
TOC/DOC/AOC changes	Increased organic carbon availability or systematic changes along the distribution network	Provides substrate for **microbial growth** and may be associated with biofilm/EPS dynamics
ORP ↓	Persistent shift toward lower oxidation–reduction potential	May indicate conditions increasingly favorable for **microbial activity**
Specific microbial signals	PCR/qPCR/16S analyses show recurrent increases in specific microorganisms	Supports the presence of an **established microbial community** rather than a single transient contamination event

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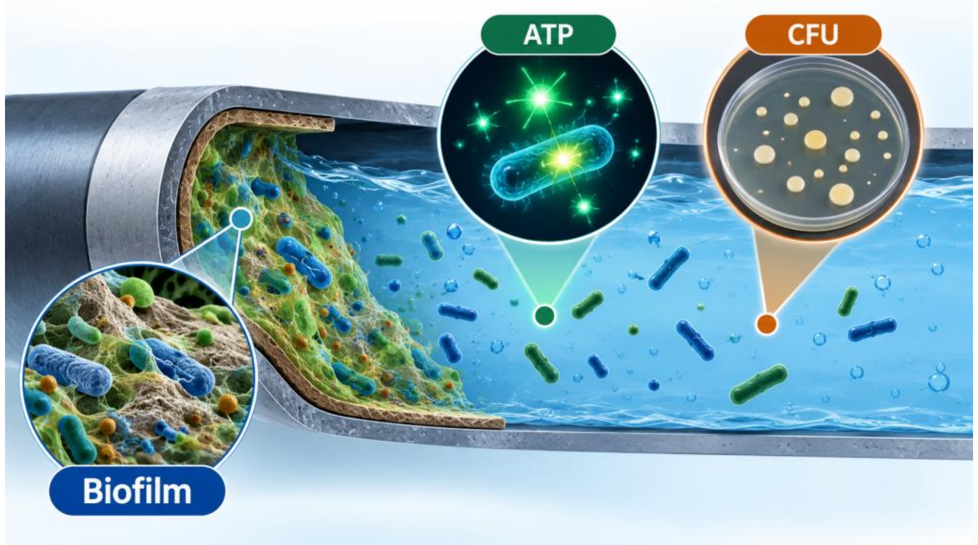

Parameter	CFU / HPC	ATP
What does it measure?	Microorganisms capable of forming colonies under defined culture conditions	ATP associated with microbial biomass
Result	CFU/mL	RLU or ATP concentration, depending on the method
Detects VBNC cells?	**No**	**ATP from metabolically active non-culturable cells can be detected**
Time to result	1–3+ days	**Minutes**
Dependence on culture medium / incubation conditions	**Very high**	**Low compared with culture-based methods**
Information on microbial activity / biomass	Limited	**Yes – particularly microbial/cellular ATP**
Biofilm-specific?	No	No

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FACTORS AFFECTING BIOFILM FORMATION

Key environmental and operational parameters in drinking water systems

PARAMETER	EFFECT ON BIOFILM	VALUES / CONDITIONS REPORTED IN STUDIES
Temperature	↑ microbial activity	4 – 25 °C (optimal 20 – 30 °C) Significant biofilm increase > 15 °C
Water age / stagnation	↑ growth	24 h – 7 days Noticeable growth after > 24 – 48 h
Flow velocity	↑ growth (low flow) ↓ detachment (high flow)	Growth dominant: < 0.1 m s^{-1} Detachment observed: > 1.0 m s^{-1}
pH	microbial selection	6.0 – 8.5 (most studies) Optimal growth near neutral pH (6.5 – 7.5)
AOC/DOC/TOC	nutrient availability	AOC: 10 – 500 µg C L^{-1} TOC: 0.5 – 5 mg C L^{-1}
N, P	nutrient limitation / growth	N: 0.01 – 1.0 mg N L^{-1} P: 0.001 – 0.1 mg P L^{-1}
Residual chlorine	biofilm control	0.1 – 1.0 mg L^{-1} (control range) > 1.0 mg L^{-1} effective biofilm reduction
Fe/Mn	supports deposits / biofilm	Fe: 0.05 – 1.0 mg L^{-1} Mn: 0.01 – 0.20 mg L^{-1}
Turbidity	protection + attachment	0.1 – 5 NTU Higher turbidity → stronger attachment
Pipe material	attachment / growth	PVC, PE, HDPE (high attachment) Cast iron (corrosion + deposits ↑ biofilm)

BIOFILM FORMATION IS INFLUENCED BY:

- Hydraulic conditions
- Water quality and nutrients
- Disinfectant residuals
- Pipe material and surface properties

KEY MESSAGE: Biofilm development is a result of the complex interaction of hydraulic, chemical and microbiological factors. Control requires a **balanced optimization of multiple parameters.**

Influence of Pipe Material on Biofilm Formation

Material properties shape initial attachment, microbial community and biofilm stability

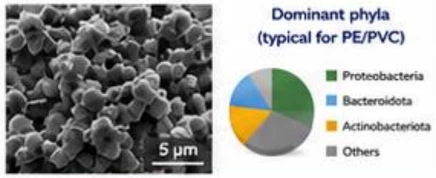
MATERIAL / CHARACTERISTIC	HOW IT INFLUENCES BIOFILM FORMATION	KEY FINDINGS FROM LITERATURE
Surface roughness & porosity	More attachment sites, protection from shear stress, enhanced initial colonization.	• Rough and porous surfaces support higher attached biomass.
Cast iron / iron-based pipes	Corrosion increases roughness, creates deposits and tubercles; iron release can support microbial growth and biofilm development.	• Iron corrosion, increased roughness and porosity contribute to increased attached biomass. • Iron acts as a nutrient for microorganisms.
Corrosion products (Fe deposits)	Provide more surface area and micro-niches; limit disinfectant penetration.	• Deposits are hotspots for microbial attachment and biofilm persistence.
PVC / PE / HDPE	Smooth, non-corrosive surfaces but biofilm still forms readily.	• Generally lower biomass than corroded metals, but not biofilm-free.
Leaching from polymeric materials	Organic compounds leached from some plastics can provide biodegradable substrates and promote microbial growth.	• Organic carbon release from plastics can fuel biofilm development.
Pipe ageing	Ageing increases surface defects, roughness and accumulation of deposits.	• Older pipes show higher attachment potential and thicker biofilms.
Surface chemistry / hydrophobicity	Influences cell–surface interactions and determines which microorganisms initially attach.	• Hydrophobicity and functional groups affect adhesion of different taxa.
Material-specific microbial selection	Different materials favour different microbial taxa and community structures.	• Water quality and hydraulics can sometimes override material effects.

CAST IRON – POSITIVE FEEDBACK LOOP

26 Fe — Iron corrosion, increased roughness and porosity contribute to increased attached biomass. Iron can act as a nutrient for microorganisms.

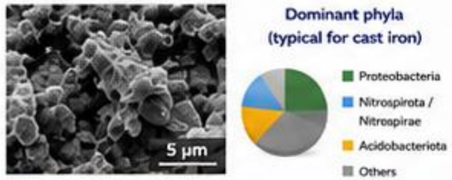
BIOFILM IS PRESENT ON ALL MATERIALS, BUT THE MICROBIAL COMMUNITY DIFFERS

PE-HD / PVC-U (plastic pipes)



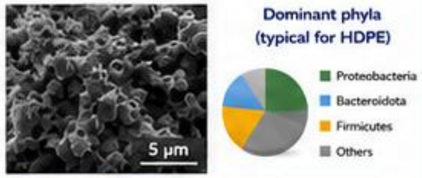
- Biofilm develops, but generally lower total biomass than corroded iron pipes.
- Communities often dominated by *Proteobacteria* (e.g., *Pseudomonas, Acinetobacter, Sphingomonas*).

Cast iron (iron-based pipes)



- Communities more often enriched in nitrifiers (e.g., *Nitrospira, Nitrosomonas*) and bacteria linked to iron corrosion (e.g., *Gallionella, Sideroxydans*).
- Higher potential for corrosion and deposit formation.

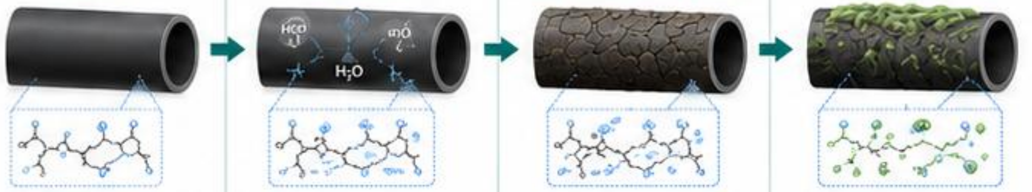
HOPE (plastic pipes)



- Biofilm present and adapts to the surface.
- Specific taxa may be associated with polymer degradation and organic compound release.

Water quality, flow regime, temperature, nutrients and disinfectant residual strongly interact with material effects.

HOPE – SURFACE DEGRADATION AND ORGANIC LEACHING



New HDPE surface
- Smooth and hydrophobic
- Limited anchoring sites

Long-term contact with water
- Water penetration
- Chemical aging (oxidation)
- Additives leach out

Surface becomes rougher and degraded
- Increased roughness
- Micro-cracks and pits
- Higher surface energy

Biofilm development & organic release
- Biofilm facilitates further degradation
- Organic compounds migrate from material to water

Biofilm and microorganisms can accelerate HDPE surface degradation and are associated with the migration of organic compounds from the material into the water.

Pipe material is a key factor, but not the only one controlling biofilm development.

Understanding material–biofilm interactions is essential for the AquaGuardian project: integrated monitoring of biofilm, water quality, disinfection by-products and health risks.

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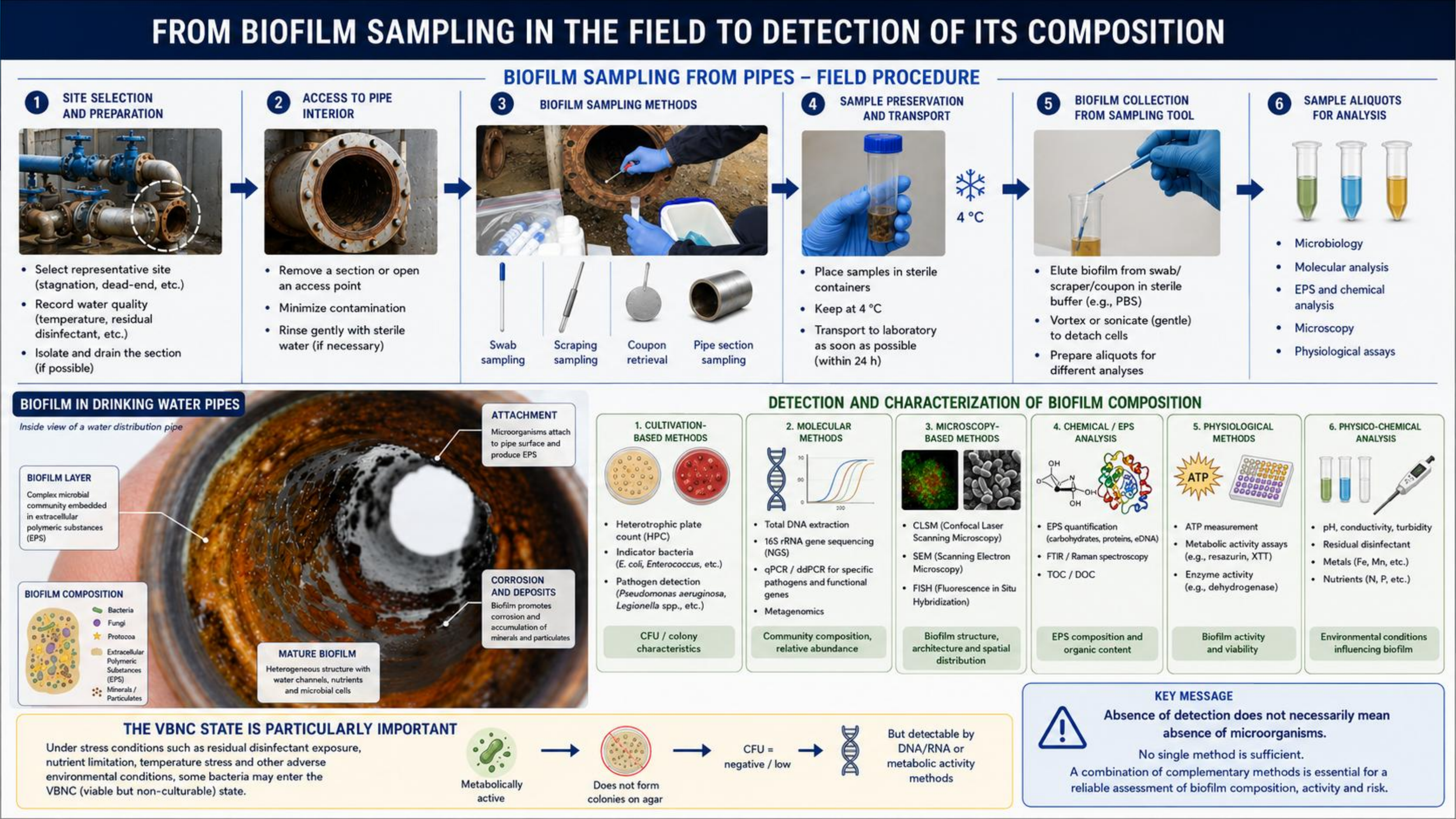
AI EARLY WARNING SYSTEM FOR SAFE & RESILIENT WATER SUPPLY


FROM BIOFILM SAMPLING IN THE FIELD TO DETECTION OF ITS COMPOSITION
BIOFILM SAMPLING FROM PIPES – FIELD PROCEDURE
1 SITE SELECTION AND PREPARATION
• Select representative site (stagnation, dead-end, etc.)
• Record water quality (temperature, residual disinfectant, etc.)
• Isolate and drain the section (if possible)
2 ACCESS TO PIPE INTERIOR
• Remove a section or open an access point
• Minimize contamination
• Rinse gently with sterile water (if necessary)
3 BIOFILM SAMPLING METHODS
Swab sampling
Scraping sampling
Coupon retrieval
Pipe section sampling
4 SAMPLE PRESERVATION AND TRANSPORT
4 °C
• Place samples in sterile containers
• Keep at 4 °C
• Transport to laboratory as soon as possible (within 24 h)
5 BIOFILM COLLECTION FROM SAMPLING TOOL
• Elute biofilm from swab/ scraper/coupon in sterile buffer (e.g., PBS)
• Vortex or sonicate (gentle) to detach cells
• Prepare aliquots for different analyses
6 SAMPLE ALIQUOTS FOR ANALYSIS
• Microbiology
• Molecular analysis
• EPS and chemical analysis
• Microscopy
• Physiological assays
BIOFILM IN DRINKING WATER PIPES
Inside view of a water distribution pipe
BIOFILM LAYER
Complex microbial community embedded in extracellular polymeric substances (EPS)
ATTACHMENT
Microorganisms attach to pipe surface and produce EPS
CORROSION AND DEPOSITS
Biofilm promotes corrosion and accumulation of minerals and particulates
MATURE BIOFILM
Heterogeneous structure with water channels, nutrients and microbial cells
BIOFILM COMPOSITION
Bacteria
Fungi
Protozoa
Extracellular Polymeric Substances (EPS)
Minerals / Particulates
DETECTION AND CHARACTERIZATION OF BIOFILM COMPOSITION
1. CULTIVATION-BASED METHODS
• Heterotrophic plate count (HPC)
• Indicator bacteria (E. coli, Enterococcus, etc.)
• Pathogen detection (Pseudomonas aeruginosa, Legionella spp., etc.)
CFU / colony characteristics
2. MOLECULAR METHODS
• Total DNA extraction
• 16S rRNA gene sequencing (NGS)
• qPCR / ddPCR for specific pathogens and functional genes
• Metagenomics
Community composition, relative abundance
3. MICROSCOPY-BASED METHODS
• CLSM (Confocal Laser Scanning Microscopy)
• SEM (Scanning Electron Microscopy)
• FISH (Fluorescence in Situ Hybridization)
Biofilm structure, architecture and spatial distribution
4. CHEMICAL / EPS ANALYSIS
OH
OH
OH
• EPS quantification (carbohydrates, proteins, eDNA)
• FTIR / Raman spectroscopy
• TOC / DOC
EPS composition and organic content
5. PHYSIOLOGICAL METHODS
ATP
• ATP measurement
• Metabolic activity assays (e.g., resazurin, XTT)
• Enzyme activity (e.g., dehydrogenase)
Biofilm activity and viability
6. PHYSICO-CHEMICAL ANALYSIS
• pH, conductivity, turbidity
• Residual disinfectant
• Metals (Fe, Mn, etc.)
• Nutrients (N, P, etc.)
Environmental conditions influencing biofilm
THE VBNC STATE IS PARTICULARLY IMPORTANT
Under stress conditions such as residual disinfectant exposure, nutrient limitation, temperature stress and other adverse environmental conditions, some bacteria may enter the VBNC (viable but non-culturable) state.
Metabolically active
Does not form colonies on agar
CFU = negative / low
But detectable by DNA/RNA or metabolic activity methods
KEY MESSAGE
Absence of detection does not necessarily mean absence of microorganisms.
No single method is sufficient.
A combination of complementary methods is essential for a reliable assessment of biofilm composition, activity and risk.

Challenges in Biofilm Detection and Characterization

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Detection challenge	Why it matters
Non-culturable microorganisms	Many biofilm microorganisms do not grow on standard media.
VBNC cells	Cells remain viable but do not form colonies.
Low-abundance pathogens	Microorganisms may be present below the detection limit.
Heterogeneous biofilm structure	One sample may not represent the whole pipe biofilm.
EPS matrix protection	Cells may be strongly embedded and difficult to detach.
PCR inhibition	EPS, metals and organic matter can reduce molecular detection efficiency.
Dead-cell DNA	qPCR may detect DNA from non-viable cells.

The VBNC State Is Particularly Important

Viable ≠ Culturable

- Under stress conditions such as **residual disinfectant exposure, nutrient limitation, temperature stress and other adverse environmental conditions**, some bacteria may enter the **VBNC (viable but non-culturable) state**.

Such cells may remain:

- **Metabolically active → Unable to form colonies on conventional culture media → CFU = low/negative → Still detectable by DNA/RNA-based or metabolic activity methods**

This phenomenon is particularly relevant for microorganisms of concern in drinking water systems, including ***Legionella*** **spp.**, ***Pseudomonas*** **spp.**, ***Escherichia coli***, and other bacteria.

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Biofilm Control and Removal in Drinking Water Systems

- These values - **literature-reported treatment ranges rather than universal operational recommendations**.
- The actual disinfectant concentration and required contact time depend on **pipe material, pH, temperature, organic load, biofilm age and thickness, and drinking water quality requirements**.

Method	Typical conditions*	Main effect
Free chlorine / NaOCl	~10–50 mg L^{-1}, 0.5–2 h	Oxidation and microbial inactivation
ClO_2	~0.5–2 mg L^{-1}, 1–4 h	Oxidation; activity within biofilm
Monochloramine	~3–10 mg L^{-1}, ≥2 h	Slower action, but prolonged penetration
H_2O_2	~5–50 mg L^{-1}, 0.5–2 h	Oxidative damage; generally, requires higher exposure
Peracetic acid	~5–20 mg L^{-1}, 0.5–1 h	Strong oxidation and microbial inactivation
High-velocity flushing	Increased shear stress	Detachment of loosely attached biofilm/deposits
Pigging / mechanical cleaning	Physical contact with pipe wall	Removal of mature biofilm, EPS and deposits
Combined treatment	Cleaning → disinfection → flushing	Usually most effective approach

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Challenges During Pipe Disinfection

Formation of Disinfection By-Products (DBPs)

BIOFILM + DISINFECTION → DBP FORMATION

Biofilm / EPS
polysaccharides • proteins • extracellular DNA • cellular material
↓
Disinfectant exposure
chlorine / other oxidants
↓
Biofilm oxidation & cell damage
↓
Release of dissolved organic matter
↓
Reaction with disinfectant
↓
DBPs
THMs • HAAs • chlorite/chlorate • bromate • other transformation products

Issue	Why it occurs	Potential consequence
Formation of disinfection by-products (DBPs)	Chlorine and other oxidants react with natural organic matter, biofilm components and pipe deposits	Formation of THMs, HAAs and other halogenated/oxidation by-products
High disinfectant demand	Biofilm, corrosion products, Fe/Mn deposits and organic matter consume disinfectant	Reduced effective disinfectant residual and need for higher doses
Release of biofilm fragments	Oxidation and hydraulic shear detach biomass from the pipe wall	Temporary increase in turbidity, microorganisms and organic matter
Mobilization of metals/deposits	Changes in redox conditions and pH may destabilize corrosion scales	Increased Fe, Mn and potentially other metals in flushed water
Residual disinfectant in flushing water	High concentrations used for shock disinfection remain after treatment	Potential toxicity to aquatic organisms if discharged directly
Re-growth after treatment	Inactivation does not necessarily remove the EPS matrix	Surviving cells may recolonize the remaining biofilm structure
Oxidative degradation of plastic pipes	NaOCl and especially ClO_2 can promote oxidative ageing of HDPE/PE, leading to polymer-chain scission, surface roughening and deterioration of mechanical properties	Release of micro- and nanoplastics, increased leaching of organic compounds/additives, enhanced surface roughness and potentially greater biofilm attachment

AquaGuardian shifts the focus from monitoring disinfectant residual alone to understanding the entire chain:
biofilm → organic precursors → disinfection → DBPs → potential health risk.

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Challenges During Pipe Disinfection

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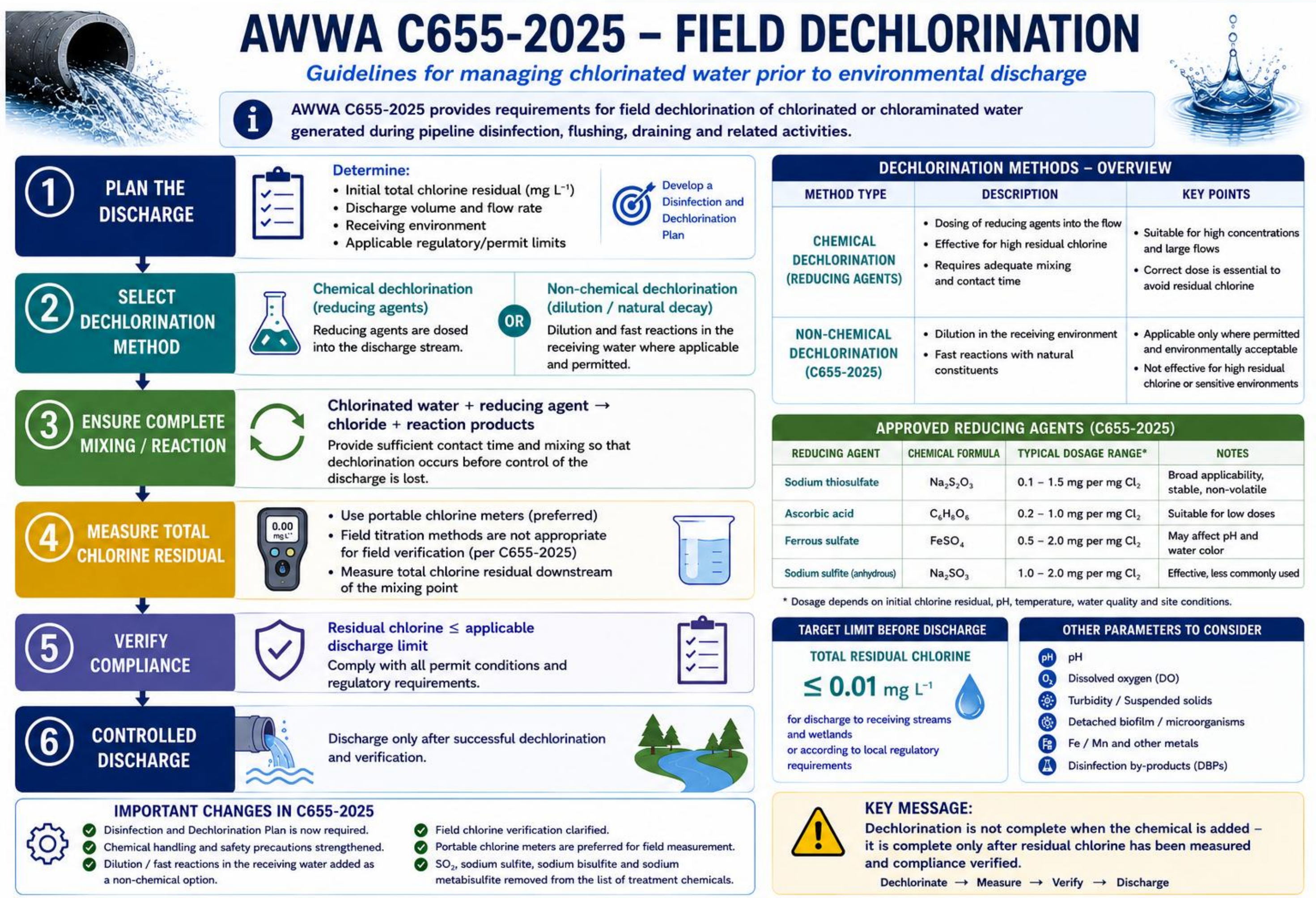
- **USA / AWWA approach**

TRC ≤ 0.01 mg L^{-1} for receiving streams/wetlands
↓
Dechlorinate → Measure → Verify → Discharge

- **EU approach**

No single harmonized EU limit specifically for pipe-disinfection flushing water
↓
Water Framework Directive + EQS framework + national discharge requirements



Case Study: Biofilm Formation in the Drinking Water Treatment Plant Gaj (Lipik/Pakarac), Croatia

1 Location – Vodocrpilište Gaj (Lipik/Pakarac)

2 Drinking Water Treatment Plant Gaj

- Groundwater treatment plant Gaj (Lipik/Pakarac)
- Removal of manganese, iron and ammonium
- Gravity filtration (sand filters) and disinfection
- **Due to biofilm formation, filters are currently backwashed daily**

Target parameters:

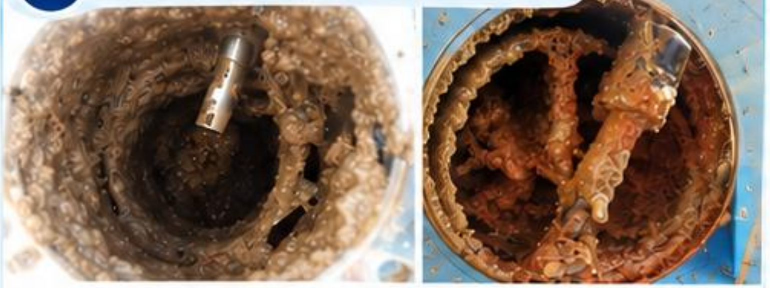
- Manganese (Mn)
- Iron (Fe)
- Ammonium (NH_4^+)

Safe Drinking Water for a Healthier Community

3 Biofilm Formation on Equipment Surfaces

- Mucilaginous biofilm layer on pipes and mixer components
- Biofilm growth on internal surfaces of equipment

4 Biofilm and Sludge in the System



- Thick, slimy biofilm deposits
- Biofilm on the inner walls of the equipment
- Reduced hydraulic capacity and operational issues

5 Filter Media and Biofilm

- Biofilm on the surface of filter media
- Slime-like, gelatinous deposits
- Indicative of iron- and manganese-oxidizing and ammonia-oxidizing microbial communities

Groundwater characteristics

- Elevated Mn and Fe
- Presence of ammonium
- Favourable conditions for microbial growth

Biofilm development

- Growth of iron-, manganese- and ammonia-oxidizing bacteria
- Formation of mucilaginous biofilm on equipment and filter media
- Accumulation of deposits and clogging

Impact

- Reduced efficiency of Mn, Fe and NH_4^+ removal
- Increased head loss
- More frequent cleaning (daily backwashing)
- Risk of deterioration of drinking water quality

Case Study: Biofilm Formation in the Drinking Water Treatment Plant Zakučac – Omiš, Croatia

1 Location – Omiš (Zakučac)

- Drinking water treatment plant Zakučac (Omiš)
- Supplied by the Cetina River

2 Source – Cetina River

- Karstic river with variable water quality
- Important source of drinking water for the Omiš region

3 Drinking Water Treatment Plant Zakučac

- Conventional treatment (filtration and disinfection)
- Rapid sand filters

4 Filter Medium and Biofilm Formation

- Biofilm developed on the surface of filter sand
- Visible layer on the filter medium

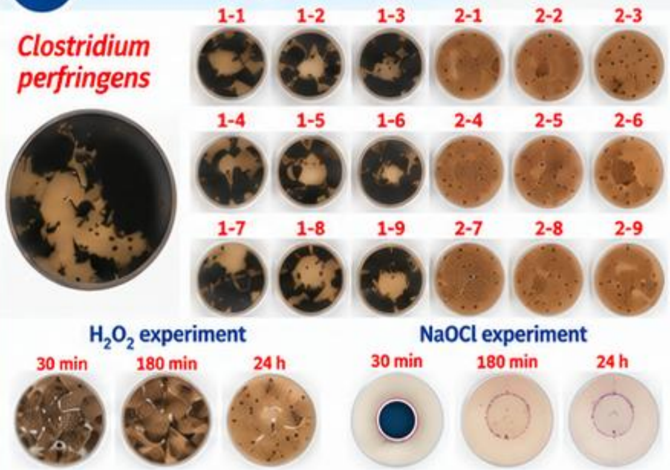
5 Sampling and Field Analysis

- Sampling of filter medium and water
- On-site measurements and monitoring

6 Pilot-scale Studies

- Pilot filtration experiments
- Testing of cleaning and operational strategies

7 Microbiological Analysis



- Presence of *Clostridium perfringens* on filter sand (black colonies)
- Evaluation of disinfection/cleaning methods (H_2O_2 and NaOCl)

Thank You
for Your attention!

CLEAN WATER
SAFER COMMUNITIES
BRIGHTER TOMORROW

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A Comprehensive AI Powered Early Warning System for Digital Management of Water Supply Security, Resilience, and Sustainability

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