Chapter I: Water microbiology

I. Natural waters

Natural waters form a fundamental environment for environmental microbiology, hosting diverse microbial communities essential for biogeochemical cycles, organic matter degradation, and aquatic ecosystem health. They vary in physicochemical composition, such as salinity, dissolved minerals, gases, and organic matter, which directly influences the distribution, abundance, and adaptations of microorganisms (bacteria, archaea, fungi, and viruses). These waters include marine, freshwater, rainwater, and groundwater, each fostering unique microbial assemblages.

a. Marine waters

Marine waters, or seawater, are characterized by their high salinity levels, which typically range from 30 to 40 grams of dissolved salts per liter (often in the form of sodium chloride). This salinity is a result of the evaporation of water and the dissolved salts carried by rivers from the land.

Marine waters are rich in various dissolved minerals such as calcium, magnesium, sodium, and sulfate. Additionally, gases like oxygen, carbon dioxide, and nitrogen are dissolved in seawater, which are essential for aquatic life, including marine microorganisms.

Marine environments are home to a wide diversity of microorganisms, including bacteria, archaea, and fungi. These organisms often exist in various forms:

- **Suspended** in the water column, where they play a key role in nutrient cycling and energy flow within marine ecosystems.
- **Attached** to submerged surfaces such as rocks, coral reefs, or ship hulls, where they form biofilms and participate in the breakdown of organic matter.
- **Sediment-associated**, where they live in the ocean’s sediment, contributing to the biogeochemical processes occurring in the seabed.

The marine environment also hosts a unique set of extremophilic microorganisms that can thrive in extreme conditions. These include:

- **Barophiles**: These microorganisms can withstand the immense pressures found in deep ocean waters, where pressures can exceed 1,000 times that of the atmosphere at sea level.
- **Halophiles**: These organisms thrive in high-salinity environments, such as salt ponds and the saline areas of seawater, often surviving in conditions that would be lethal to most other life forms.

- **Psychrophiles**: These microorganisms are adapted to cold environments, such as the deep ocean or polar regions, where they function optimally at temperatures near or below freezing.

b. Freshwaters

Freshwaters, in contrast to marine waters, have low salinity levels, usually below 0.5 grams of dissolved salts per liter, and are typically found in rivers, lakes, streams, and ponds. These waters support a very different set of ecosystems compared to marine environments due to their lower mineral content and freshwater-specific organisms.

Freshwater ecosystems also contain dissolved organic matter (such as decaying plant and animal material) and dissolved minerals like calcium and iron. These minerals and organic components play a crucial role in supporting the growth of aquatic plants, algae, and microorganisms. The concentration of these elements can vary depending on seasonal or environmental conditions, such as run-off after rainfall, agricultural practices, or pollution.

Freshwater ecosystems experience notable seasonal variations that influence the biological communities within them. Changes in temperature are particularly important, as they can dictate the metabolic rates of aquatic organisms, including algae, fish, and invertebrates. Light availability also changes seasonally, affecting the photosynthesis rates of primary producers, and thus, the overall productivity of the system.

C. Rainwater

Rainwater is a type of precipitation that falls directly from the atmosphere. While it is initially pure, rainwater can collect pollutants from the atmosphere as it falls, especially in urban and industrial areas. The quality of rainwater can vary depending on local environmental factors, including pollution levels and atmospheric conditions.

Rainwater is typically soft and low in dissolved salts. However, in polluted areas, rainwater can contain acidic compounds such as sulfuric acid (H_2SO_4) and nitric acid **(**HNO_3), which result from the combination of water vapor with air pollutants.

Although rainwater is often considered clean, it can become contaminated by pathogens, such as *Escherichia coli, Salmonella, or Cryptosporidium*, when it interacts with contaminated surfaces like rooftops or gutters.

d. Groundwater

Groundwater refers to water that is found below the Earth's surface in aquifers. It is stored in spaces between soil particles and rocks, and it moves slowly through these underground layers. Groundwater is a vital source of freshwater for drinking, irrigation, and industry, especially in regions where surface water is scarce.

Groundwater is typically mineral-rich because it dissolves minerals from the rocks and soil through which it passes. The quality of groundwater can vary based on its source and the geological layers it moves through. It often contains a wide range of bacteria, viruses, and fungi, although it is usually considered to be microbially sterile when it is deep underground. However, in shallow groundwater systems or areas where aquifers are contaminated by human activities, microbial populations can be significant.

II. Self-purification

Definition

Self-Purification of Natural Waters refers to the integrated physical, chemical, and biological processes by which aquatic ecosystems, such as rivers, lakes, and coastal seas, naturally attenuate pollution and restore water quality following contamination. This innate cleansing mechanism operates through abiotic factors like dilution via water flow, sedimentation of particulates, and photochemical reactions driven by solar UV radiation, alongside biotic interactions dominated by microbial communities. For instance, in a river receiving urban effluents, self-purification degrades excess nutrients (nitrogen and phosphorus) and organic pollutants through bacterial metabolic pathways, preventing eutrophication and hypoxia. From a microbiological perspective, self-purification drives several key outcomes essential for ecosystem health. It achieves a reduction of pathogenic microorganisms via mechanisms including predation by bacteriophages and protozoa, resource competition, and natural die-off under unfavorable conditions (pH shifts, temperature, dissolved oxygen).

Sources of water pollution

Pollution sources determine the contaminants that microbial communities must degrade or inactivate. Key contributors include domestic wastewater from households, laden with human excreta and kitchen wastes; agricultural runoff carrying fertilizers, pesticides, and crop residues; animal manure from livestock operations, which releases high loads during storms; and food industry effluents from dairy, meat processing, and breweries, high in proteins, sugars, and fats. These sources introduce biodegradable organic matter (measured as BOD or COD), fostering rapid bacterial proliferation, alongside microbial contaminants such as bacteria, viruses, protozoa (free-living amoebae), and helminth eggs.

Mechanisms

Self-purification of waters hinges on three key mechanisms: physical (dilution, sedimentation, UV dispersion), chemical (oxidation, hydrolysis, photolysis), and biological (microbial degradation, predation, nutrient cycling), working synergistically to reduce pollutants like organic matter and pathogens, with optimal efficacy under favorable conditions like high flow and temperature.

1. Physical processes

Physical processes form the foundational stage of self-purification, primarily through passive transport and environmental forces that reduce pollutant availability without biological intervention.

A. **Dilution:** Polluted water mixes with cleaner upstream or tributary flows, proportionally decreasing pollutant concentration and microbial density This does not eliminate pollution but disperses it, easing the load on downstream processes.
B. **Sedimentation:** Heavier particles settle in eddies or riverbeds, removing suspended solids, organic debris, and particle-attached bacteria (flocculation via biofilms enhances this).
C. **Solar radiation** (UV Effect): UV penetrate surface waters, causing thymine dimer formation in microbial DNA, inactivating bacteria (*Vibrio*, *Salmonella*), viruses, and protozoa.
D. **Temperature**: Higher temperatures (optimal 20-30°C) boost molecular kinetics, increasing microbial metabolism rates and accelerating physical processes like evaporation; many mesophilic pathogens (*Shigella*) die off above 25°C, while, psychrophiles persist in cold waters.

2. Chemical processes

These abiotic reactions clean water by:

- Redox: Oxygen breaks down organics (e.g., sugars to CO_2).
- Hydrolysis/UV: Water/light degrade pesticides/chemicals.
- Precipitation/Adsorption: Metals/pathogens stick to sediments.
- pH/Salinity: Kill bacteria via acidity or salt stress.

3. Biological processes

Biological processes represent the core of self-purification, driven by microbial communities that actively metabolize, compete against, and prey on pollutants. These dominate after physical/chemical priming, relying on diverse bacteria, protozoa, algae, and biofilms in a succession from high-organic to oligotrophic conditions. They achieve >90% organic matter removal in well-oxygenated rivers.

***<u>3.1. Biodegradation of organic matter</u>*:**

Heterotrophic bacteria secrete enzymes (proteases, amylases, lipases) to hydrolyze complex organics (proteins → amino acids → NH_4^+; carbs → sugars → CO_2). fungi aid lignocellulose breakdown in sediments.

$$\text{Organic matter} + O_2 \rightarrow CO_2 + H_2O + \text{Energy}$$

<u>3.2. Nutrient cycling and succession:</u>

Nutrient cycling restores balance by transforming key elements: nitrogen via nitrification ($NH_4^+ \rightarrow NO_3^-$) then denitrification ($NO_3^- \rightarrow N_2$), preventing eutrophication; phosphorus gets fixed into biomass by bacteria and algae; carbon cycles through methanotrophs converting CH_4 to CO_2. Microbial succession follows, shifting from fast-growing copiotrophs (*E. coli*) in nutrient-rich early stages to efficient oligotrophs (*Caulobacter*) in cleaner waters, boosting diversity and stability.

- ***Nitrification***: It converts toxic ammonium (NH_4^+) from organic pollution (e.g., sewage, manure) into less harmful nitrates (NO_3^-), reducing free ammonia while supplying oxygen acceptors for heterotrophs.

Performed by slow-growing autotrophic bacteria, it inhibits pathogens indirectly by competing for oxygen.

Reactions (two-step, energy-yielding for bacteria):

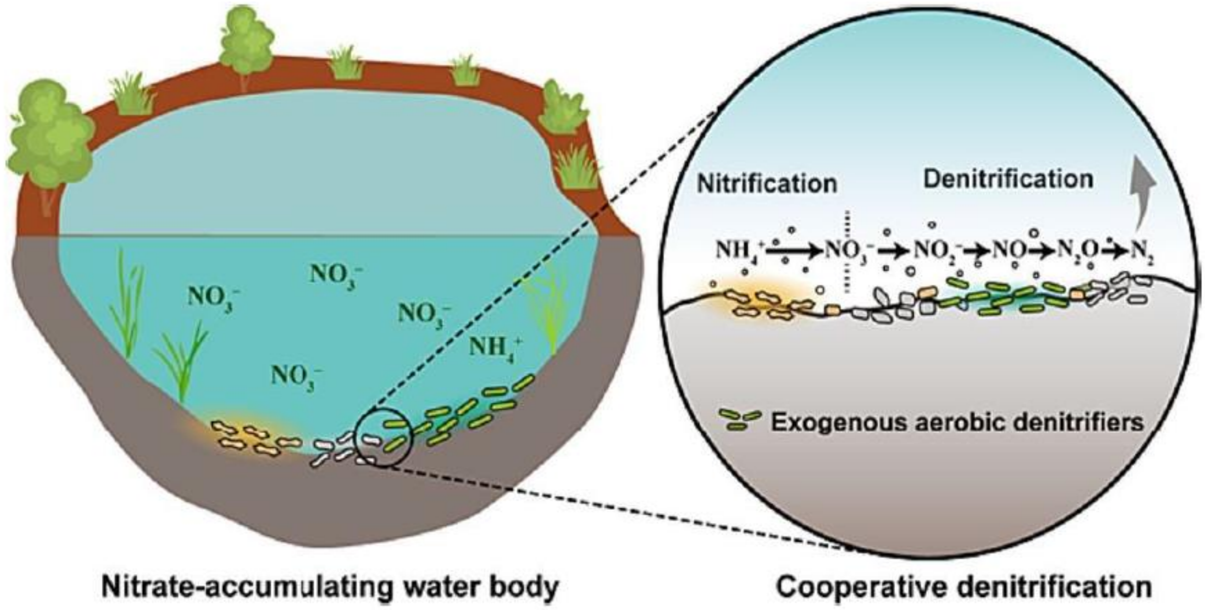
  - Ammonia-oxidizing bacteria: *Nitrosomonas europaea*, *Nitrosospira* → NH_4^+ to NO_2^-.
  - Nitrite-oxidizing bacteria: *Nitrobacter hamburgensis*, *Nitrospira* → NO_2^- to NO_3^-.
- ***Denitrification***: It removes excess nitrates (eutrophication risk) by converting to inert N_2 gas, closing the nitrogen cycle and recovering alkalinity lost in nitrification. If nitrate accumulates due to incomplete self-purification often from excess agricultural runoff or insufficient anoxic zones, it triggers eutrophication, fueling explosive algal blooms that deplete oxygen, kill fish, and produce toxins. It also pollutes groundwater, rendering aquifers unsafe for drinking as NO_3^- exceeds 50 mg/L thresholds. Denitrification counters this by using facultative anaerobes (*Pseudomonas*, *Paracoccus*) to remove nitrate into harmless nitrogen gas via nitrate reductase enzymes, effectively returning nitrogen to the atmosphere and restoring balance in sediments or biofilms.

Microorganisms : Facultative anaerobes like *Paracoccus denitrificans*, *Pseudomonas denitrificans*, *Bacillus* use NO_3^- as terminal electron acceptor.

- Reactions (multi-step reduction):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Final product: Nitrogen gas (N_2) escapes into atmosphere



Cooperative denitrification by exogenous aerobic denitrifiers and indigenous sediment microbiota in nitrate-accumulating waterbodies **(Huang et al., 2024)**

- ***Carbon Cycle***

Heterotrophic bacteria (*Pseudomonas*, *Bacillus*) and methanotrophs degrade organic matter (BOD) via respiration (organics → $CO_2 + H_2O$) or fermentation (to CH_4, then oxidized to CO_2), reducing hypoxia; phototrophs like cyanobacteria (*Anabaena*) fix CO_2 into biomass via photosynthesis, fueling primary production.

- ***Phosphorus Cycle***

Polyphosphate-accumulating bacteria (*Achromobacter*) and algae (*Chlorella*) uptake soluble PO_4^{3-} from wastewater, storing it intracellularly or precipitating as apatite-like minerals; mineralization by phosphatases releases it slowly, preventing eutrophication spikes.

- ***Sulfur Cycle***

Sulfate-reducing bacteria (*Desulfovibrio*) in anoxic sediments convert SO_4^{2-} to H_2S (using organics as electron donors); sulfur-oxidizers (*Thiobacillus*) reoxidize H_2S to SO_4^{2-} aerobically, detoxifying black sediments from organic pollution.

Microbial succession in natural water self-purification

It refers to the sequential shifts in microbial communities as polluted water recovers quality through aerobic degradation, predation, and ecological succession. This process restores oxygen levels and reduces organic pollutants via distinct phases involving aerobes, copiotrophs, oligotrophs, protozoa, and bacteriophages.

- ***Initial aerobic phase***

High organic pollution (BOD > 20 mg/L) initially supports copiotrophs fast-growing, bacteria like *Pseudomonas*, *Aeromonas,* and *Bacillus* that aerobically oxidize carbon sources to CO_2 and H_2O. Dissolved oxygen (DO) drops below 5 mg/L, but these aerobes respire aerobically until substrates deplete, producing biomass and excreting enzymes (cellulases, proteases).

As DO recovers (>5 mg/L), protozoa (e.g., amoebae, ciliates like *Vorticella*) emerge via predation on free bacteria, reducing bacterial numbers by 90% and recycling nutrients.

- ***Transition to oligotrophic phase***

Organic load decreases, favoring oligotrophs, slow-growing bacteria like *Caulobacter*, *Hyphomicrobium*, and *Sphingomonas* adapted to low nutrients (<1 mg/L BOD). They mineralize recalcitrant compounds (lignin derivatives, xenobiotics).

Bacteriophages (viruses like T4-like phages) peak here, lysing 20-50% of hosts via lytic cycles, releasing lysates for further degradation.

- ***Protozoa and predation dynamics***

Free-living protozoa (*Paramecium*, flagellates) dominate mid-succession, grazing selectively on copiotrophs while sparing oligotrophs. This predation boosts DO by 2-3 mg/L via reduced bacterial respiration and mineralization of protozoan wastes.

Metazoans (rotifers, nematodes) follow, further structuring communities; biofilms on sediments amplify this via extracellular polymeric substances (EPS) trapping pollutants.

- ***Stabilization and biodiversity recovery***

Final phase sees balanced communities: nitrifiers (*Nitrosomonas*, *Nitrospira*) oxidize ammonia to nitrate, denitrifiers (*Pseudomonas denitrificans*) reduce it anaerobically in sediments. Fungi (*Aspergillus*) and algae contribute via surfactants aiding pollutant flotation

II. Water pollution

II.1. Definition:

Water pollution refers to any chemical, physical, or biological alteration that makes water unfit for use. Domestic discharges (untreated wastewater) introduce coliforms and enterococci via excreta. Agricultural activities spread nitrates, phosphates, and pathogens through fertilizers and manure, causing eutrophication and bacterial blooms.

Agro-food industries and livestock operations release high organic loads (BOD > 300 mg/L), favoring *Clostridium* and *Vibrio*. Stormwater runoff carries diffuse pollutants from contaminated soils; poorly managed treatment plants release residual viruses.

Key pathogens

Indicator bacteria: *E. coli* (recent fecal contamination), total coliforms (general deterioration). Pathogens: *Salmonella*, *Shigella*, *Leptospira* (leptospirosis).

Viruses: enteroviruses, adenoviruses, hepatitis A, persistent due to small size (20-300 nm). Protozoa: *Cryptosporidium* oocysts resistant to chlorine. Cyanobacteria (*Microcystis*) produce hepatotoxic microcystins.

II.2. Parameters for measuring water pollution

II.2.1. Biochemical Oxygen Demand over 5 days (BOD5)

It quantifies the oxygen consumed by aerobic microorganisms to degrade organic matter in a sample incubated at 20°C for 120 hours, providing a proxy for biodegradable pollution levels (e.g., typical values range from <5 mg/L in clean water to >20 mg/L in polluted effluents).

II.2.2. Chemical Oxygen Demand (COD)

COD, determined via strong chemical oxidation (e.g., dichromate method), measures total oxidizable organic and inorganic matter, offering a faster, broader assessment of pollution load, often 1.5-2.5 times higher than BOD5 due to non-biodegradable fractions.

II.2.3. Total Suspended Solids (TSS)

It measures the dry weight of particles larger than 2 microns suspended in water that are retained by a filter (1.2-2 µm pore size) after filtration, evaporation, and drying at 105°C, expressed in mg/L.

Comparing BOD5/COD ratios helps classify wastewater treatability: ratios >0.5 indicate good biodegradability, while <0.3 signal recalcitrant pollutants requiring advanced treatment. These metrics, alongside pH, TSS, and nutrients, are essential for monitoring self-purification and treatment efficacy in aquatic systems.

II.3. Eutrophication

Eutrophication is a complex, multi-stage process driven by nutrient enrichment in aquatic ecosystems, profoundly influencing microbial ecology and leading to severe water quality degradation.

a. Stage 1: Nutrient enrichment

Excess nitrogen (N) and phosphorus (P) from agricultural runoff, sewage, or atmospheric deposition enter water bodies, shifting baseline nutrient levels from oligotrophic (<0.01 mg/L P) to eutrophic (>0.03 mg/L P). Microbiologically, this stimulates primary producers like diatoms and green algae while favoring nutrient-responsive bacteria such as *Pseudomonas* and *Burkholderia*, which mineralize organic N/P.

b. Stage 2: Algal bloom formation

Elevated nutrients trigger exponential growth of phytoplankton, cyanobacteria (*Microcystis aeruginosa*, *Anabaena flos-aquae*, *Oscillatoria*), and macroalgae, forming dense blooms visible as surface scums (chlorophyll-a >10 µg/L). Cyanobacteria dominate due to N_2-fixing capabilities and many produce potent toxins.

c. Stage 3: Light penetration reduction

Bloom biomass attenuates light, inhibiting photosynthesis in submerged aquatic vegetation like *Potamogeton*. This halts O_2 production below the euphotic zone, causing submerged aquatic vegetation die-off and reduced primary productivity. Microorganisms suffer: phototrophs decline, while heterotrophs face energy deficits, amplifying reliance on organic detritus.

d. Stage 4: Algal senescence and death

Nutrient depletion, grazing, or turbulence induces bloom crash; senescent cells lyse, releasing 20-50% intracellular organics (DOC >10 mg/L). Biomass sinks as flocculates, enriching sediments and fueling benthic decomposition.

e. Stage 5: Aerobic microbial decomposition

Heterotrophic bacteria (*Flavobacterium*, *Cytophaga*) initiate aerobic catabolism: respiration spikes BOD, depleting DO as bacterial demand outpaces diffusion/reaeration.

f. Stage 6: Hypoxia and anoxia

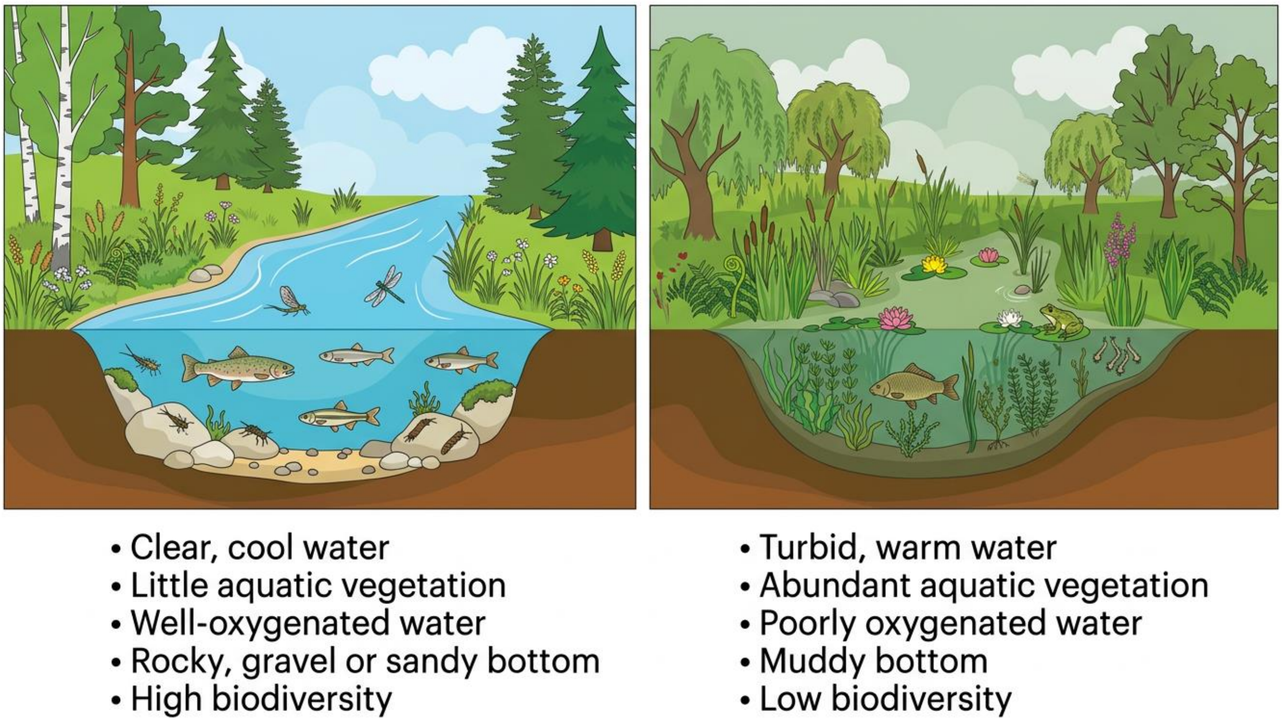
DO falls below 2 mg/L (hypoxia) or to 0 mg/L (anoxia), triggering mass mortality of aerobes. Aerobic microbial communities collapse, with 90% mortality in sensitive taxa.

g. Stage 7: Shift to anaerobic conditions

Facultative/strict anaerobes thrive: denitrifiers (*Pseudomonas denitrificans*) reduce NO_3^- to N_2; sulfate-reducers (*Desulfovibrio desulfuricans*) produce H_2S; methanogens (*Methanosaeta*) yield CH_4. Byproducts degrade habitats.

h. Stage 8: Ecosystem degradation

Biological collapse occurs, with fish kills, mortality of invertebrates, and aquatic plants increasing sharply. Biodiversity declines, and the ecosystem becomes profoundly altered. Early-stage interventions may partially reverse damage but recovery is slow and often irreversible.



Comparison of oligotrophic and eutrophic freshwater ecosystems

II.4. Aquatic biofilm

II.4.1. Definition

A biofilm is a structured, surface-associated community of microorganisms embedded in a self-produced extracellular polymeric substance (EPS) matrix, attached to biotic or abiotic surfaces, and displaying altered gene expression, physiology, and behavior compared with planktonic cells. The EPS matrix protects cells against shear forces, desiccation, disinfectants, and host defenses and creates micro-gradients of nutrients and oxygen.

II.4.2. Main stages of biofilm formation

a. Surface conditioning (pre-attachment stage)

the bare surface is coated by a "conditioning film" composed of organic and inorganic molecules from the surrounding fluid: proteins, polysaccharides, humic substances, lipids, ions, and other macromolecules.

b. Initial (reversible) attachment

- Planktonic cells approach and weakly adhere to a surface via van der Waals forces, electrostatic interactions, hydrophobic interactions, and Brownian motion.
- Flagella, pili, and fimbriae mediate surface sensing and transient adhesion; at this stage, attachment is reversible and cells can still detach under shear stress.

2. Irreversible attachment

- Adhesion becomes stable through specific adhesins and increased production of EPS at the cell–surface interface.
- Cells down-regulate motility genes (flagella) and up-regulate genes for EPS synthesis, surface adhesins, and stress responses; this is a key microbiological transition from planktonic to sessile phenotype.
- Quorum sensing systems (AHLs in Gram-negatives, oligopeptides in Gram-positives) begin to operate as local cell density increases, coordinating communal behavior.

3. Microcolony formation (early maturation)

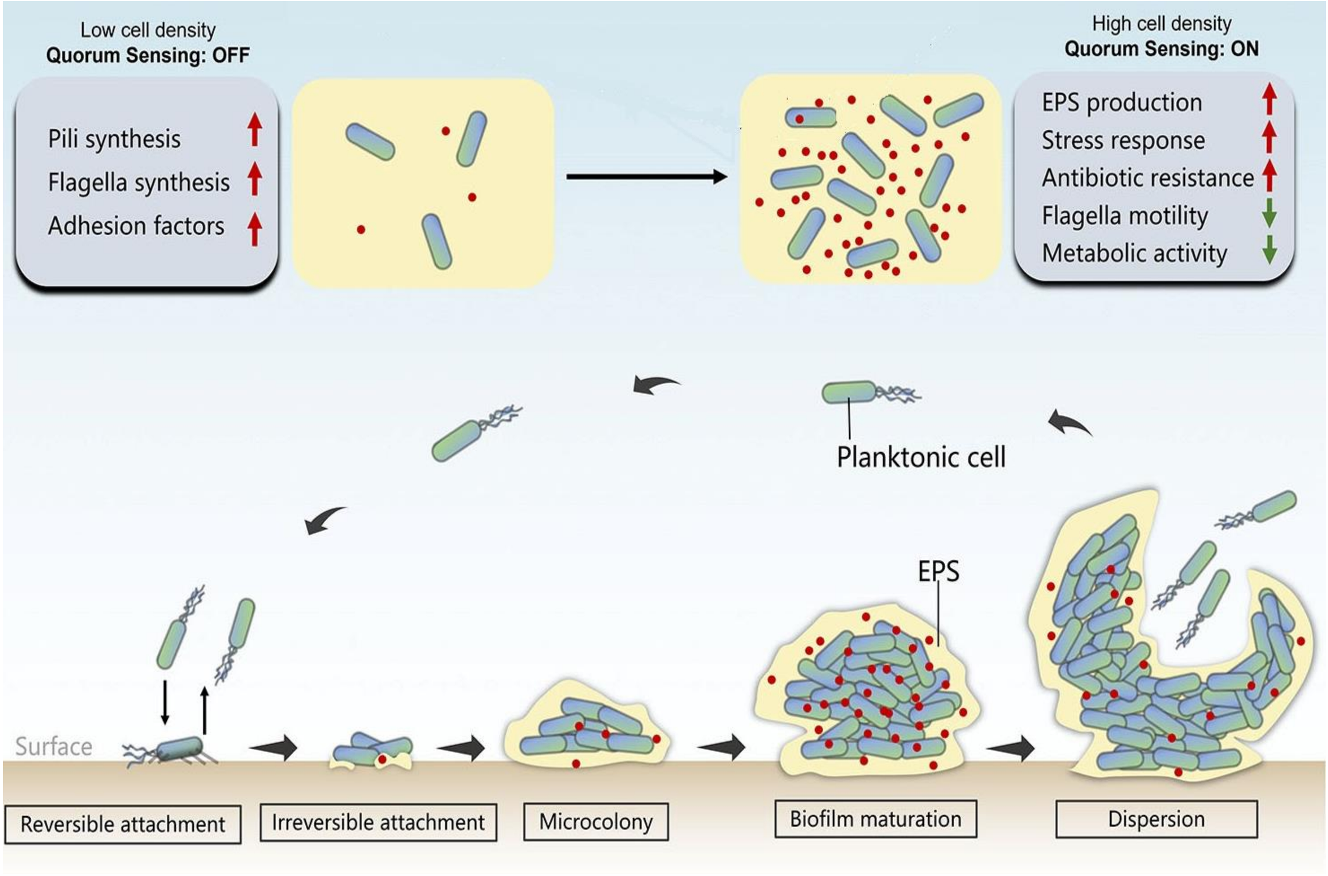
- Attached cells divide and form small clusters or microcolonies; cell–cell adhesion becomes as important as cell–surface adhesion.
- EPS production increases markedly; cells become embedded in a hydrated matrix of polysaccharides, proteins, and extracellular DNA, which acts as a scaffold and diffusion barrier.
- Microbiologically, strong spatial heterogeneity appears: gradients of oxygen, nutrients, and waste products develop over micrometers, leading to metabolic differentiation (aerobic cells at the periphery, slower or fermentative cells in deeper layers).

4. Biofilm maturation (3D structured community)

- Microcolonies expand into a three-dimensional, architecturally complex biofilm with mushroom-like or pillar-like structures and water channels that facilitate nutrient and oxygen transport.
- The community often becomes multispecies (bacteria, fungi, archaea, protozoa), with cooperative and competitive interactions (cross-feeding, syntrophy, bacteriocin production, antagonism).
- Gene expression patterns are profoundly remodeled: cells in biofilms show reduced growth rate, up-regulated efflux pumps, stress response systems, and persister cell formation, which collectively confer high tolerance to antibiotics and disinfectants.
- Quorum sensing is fully active, coordinating EPS production, virulence factor expression, and detachment processes across the population.

5. Detachment and dispersal

- In late stages, cells or clusters detach from the biofilm via erosion, sloughing, or active dispersal mechanisms; these events seed new surfaces and spread infection or contamination.
- Environmental cues such as nutrient limitation, oxygen depletion, shear stress, or changes in pH trigger intracellular signalin, activating enzymes that degrade the EPS matrix (e.g., polysaccharide lyases, nucleases) and restoring motility gene expression.
- Dispersed cells are not identical to original planktonic cells: they frequently show a distinct transcriptional profile, enhanced colonization capacity, and transiently increased virulence or antibiotic tolerance, which is microbiologically important for chronic infections and environmental spread.



Typical stages of biofilm formation and the regulatory role of quorum sensing that takes part in biofilm development. (Shan Yu et al., 2025)

III. Wastewater

III.1. Definition

Wastewater is water that has been altered by domestic, industrial, agricultural, or urban use. It contains various physical, chemical, and biological pollutants such as organic matter, nutrients (nitrogen, phosphorus), microorganisms, heavy metals, and pharmaceutical residues.

III.2. Wastewater treatment

III.2.1. Physical pretreatment

Pretreatment aims to remove coarse debris and floating materials that could disturb subsequent stages or damage equipment. It relies exclusively on physical separation processes.

- Screening: This is the first mechanical barrier, intercepting large solid objects (plastics, paper, textiles, branches) using screens or sieves with different mesh sizes (coarse, medium, fine).
- Grit removal: This process eliminates heavy mineral particles (sand, gravel) by gravitational sedimentation, thereby protecting pumps and pipes from abrasion.
- Degreasing/de-oiling: Fats and oils, which are less dense than water, are removed from the surface using injected air bubbles that promote flotation; the floating layer is then mechanically skimmed.

III.2.2. Primary treatment: sedimentation

Primary treatment aims to remove suspended solids (TSS) and a fraction of particulate organic matter. The water is directed into large settling tanks where solids sediment under gravity and form primary sludge, which is rich in organic matter. To improve the efficiency of this step, coagulants (metal salts) and flocculants (polymers) can be added to aggregate colloids and enhance clarification.

III.2.3. Secondary treatment: biological treatment

Secondary treatment, or biological treatment, relies on the activity of aerobic microorganisms that degrade dissolved organic matter under controlled conditions; it is the core of the depollution process. Heterotrophic bacteria such as *Pseudomonas*, *Bacillus*, and *Flavobacterium* use organic matter as a source of energy and carbon, converting it into CO_2, H_2O, and microbial biomass. The main technologies used include:

1. Activated sludge: a suspended-growth system in aeration tanks where bacteria form biological flocs.
2. Trickling filters (biofilters): biofilms fixed on solid media through which wastewater flows.
3. Waste stabilization ponds (lagoons): shallow natural basins where bacteria, algae, and protozoa interact.
4. Modern biological reactors: membrane bioreactors (MBR) or moving bed biofilm reactors (MBBR), which provide high performance in a compact footprint.

This treatment can achieve a 90–95% reduction in BOD_5 and remove a large proportion of microbial pathogens.

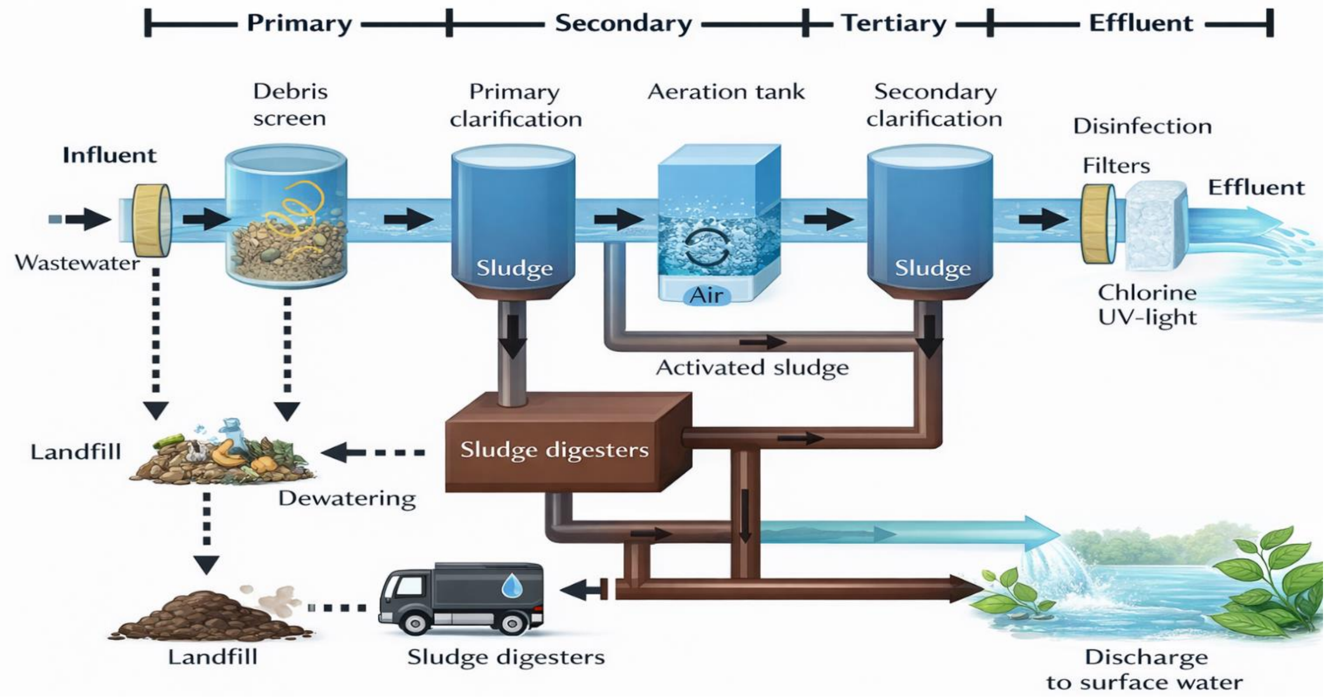
III.2.4. Tertiary treatment: advanced treatment or polishing

Tertiary treatment is an advanced finishing step applied when discharge standards are stringent or when treated water is intended for reuse. It aims to remove residual nutrients, micropollutants and to ensure final disinfection.

- Nitrogen removal:
  - Nitrification (aerobic conditions): conversion of NH_4^+ to NO_2^- and then NO_3^- by nitrifying bacteria (e.g. *Nitrosomonas*, *Nitrobacter*).
  - Denitrification (anoxic conditions): reduction of nitrates (NO_3^-) to gaseous nitrogen (N_2) by denitrifying bacteria such as *Paracoccus* and *Pseudomonas*.
- Phosphorus removal:
  - By chemical precipitation through the addition of aluminum or iron salts.

  - By biological uptake using phosphorus-accumulating organisms in enhanced biological phosphorus removal (EBPR) processes.

- Disinfection:
  - Ozonation: gaseous O_3, a powerful oxidant, eliminates pathogenic microorganisms and many micropollutants.
  - Chlorination: provides effective residual disinfection in the distribution network.
  - UV irradiation: a physical method that inactivates viruses, bacteria, and protozoa, often used as a final barrier in combination with other treatments



Wastewater treatment stages (Martín-Pozo et al. (2022)

IV. Drinking water treatment

Drinking water is water that can be consumed without risk to human health. It must be clear, free of unpleasant odor or taste, and comply with strict quality standards for microbiological, chemical, and physical parameters. It is free from pathogenic micro-organisms, toxic substances, and pollutants at harmful concentrations.

The WHO defines precise regulations to ensure the chemical and bacteriological quality of drinking water. Raw water undergoes several treatments in order to be qualified as “drinking water”:

1. **Screening**: Aims to remove large debris by filtration and then by passage through screens.
2. **Coagulation**: Destabilisation of colloidal matter by adding coagulants so that particles can aggregate.
3. **Flocculation**: Chemical agents are added to induce the formation of flocs from the aggregated colloidal particles.

4. **Sedimentation**: Removal of flocs and suspended solids by gravity.

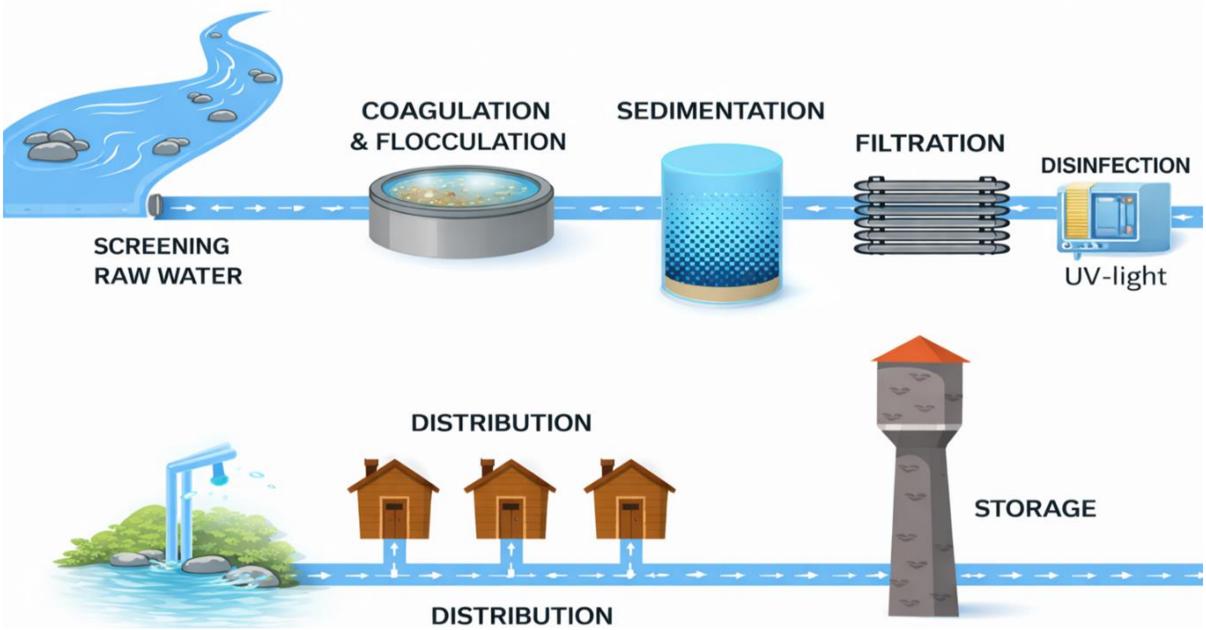
5. **Filtration**: Passage of the water through a bed of fine sand in order to remove the remaining suspended matter.

6. **Disinfection**: carried out by:

- Activated carbon filtration: used for disinfection and also contributes to the removal of natural macromolecules, compounds responsible for color, taste and odor, pesticides, dyes, and toxic metals (cadmium, mercury, etc.) present at trace levels in the water.

- Chlorination: Chlorination consists in adding chlorine compounds (such as sodium hypochlorite – bleach – or chlorine tablets) to the water, generally at strategic points in the distribution network, in order to maintain continuous disinfection and ensure the microbiological quality of drinking water. However, this method has the drawback of leaving a free chlorine residual in the water, which can alter its taste and lead to the formation of undesirable by-products.

- Ozonation: Ozonation is a water treatment process in which ozone (O_3), a gas with strong oxidizing power, is injected to eliminate pathogenic micro-organisms (bacteria, viruses, protozoa) and degrade various chemical pollutants, particularly pesticides and organic compounds. This treatment also improves the organoleptic properties of the water by reducing tastes, odors, and color. However, because ozone is an unstable gas, it must be produced on site by specific generators. In addition, it does not leave a disinfectant residual in the water, which means there is no persistence of disinfection in the distribution network.



Drinking water treatment (Mac Mahon, 2022 : Water purity and sustainable water treatment systems for developing countries)